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Studies in isotopic labelling of amino acids and polypeptides

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STUDIES IN ISOTOPIC LABELLING

OF

AMINO ACIDS AND POLYPEPTIDES

A Thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

by

STEPHEN MURRAY

Supervisor - Mr. W. Hannan

Wollongong University College
The University of New South Wales
May, 1973
808071
The aromatic protons of amino acids and small peptides have been exchanged with deuterium under conditions which produced no significant exchange of the non-aromatic protons. A heterogeneous catalyst system was found to be suitable for the deuterium exchange of biologically active molecules, and platinum used in a 1:1 molar ratio with the labelled compound, was the most selective of the Group VIII metal catalysts.

Substantial exchange of the aromatic protons of phenylalanine, tyrosine, tryptophan and histidine occurred when the reaction was allowed to proceed for 1 day, at 70°C. A similar deuterium distribution was found in the aromatic amino acid residues of a number of model peptides under the same reaction conditions. Substantial incorporation into the diketopiperazine ring of labelled cyclo-dipeptides was found when iridium was used to catalyse the reaction. The optical purity and any peptide bonds present in the labelled compounds were preserved throughout the exchange reactions.
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G. ACKNOWLEDGEMENTS 79
Proteins occupy a central position in the architecture and function of living matter. They are intimately connected with all phases of chemical and physical activity that constitutes the life of a cell. A protein is a polymer derived from amino acids which are linked together by amide bonds. The order in which the amino acids are arranged is called the primary structure of the protein. The biological activity of a protein not only depends on the order of amino acids but also on the three dimensional arrangement of the molecule in space. This is usually referred to as the conformation and is dependent on the presence of hydrogen bonding within chains (secondary structure) and hydrogen bonding between different chains of the protein molecule (tertiary structure).

In order to study the effect of the secondary and tertiary structures on biological activity it is important to identify those amino acids which are readily accessible to the substrate molecules. The best method of determining the primary structure of a protein is by chemical methods and the conformation by X-ray analysis. This is extremely time consuming since proteins are very complex molecules and the large amount of X-ray data is difficult to evaluate and to interpret. From the biological point of view a further disadvantage of X-ray analysis is that it is concerned with the crystalline protein only and thus the X-ray data may not be relevant to the biologically active protein which is only active in an aqueous medium.

There have been numerous publications in recent years that emphasise the potential of nuclear magnetic resonance (N.M.R.) investigations on the study of conformation of amino acids, peptides and proteins. The application of proton N.M.R. to proteins however is limited because of the large number of protons present in a protein molecule. This fact combined with a relatively small spread in chemical
\[ \text{Where } M = \text{metal surface} \]
Figure 1

\[
\begin{align*}
R_1 & - \frac{R_2}{C} - M + \frac{H}{M} \\
H & - \frac{C}{R_1} - \frac{R_1}{R_2} + 2M
\end{align*}
\]
shifts and large intrinsic line widths makes interpretation very difficult. Specific deuteriation of proteins results in much simpler N.M.R. proton spectra and allows more detailed studies to be carried out. This provides promise with regards to the resolution of specific resonances in the spectra of peptides and proteins.

If the proton exchange could be limited to specific amino acids (i.e. neutral, basic or acidic amino acids) or to easily accessible residues, that is amino acids which are situated near the active site of the protein molecule, or amino acids situated on the surface of the protein, then an N.M.R. spectrum would yield valuable information related to the conformation of a biologically active protein.

The aim of this investigation was to selectively deuterium label model amino acids and peptides in their aromatic side chains under conditions which would leave the secondary and tertiary structure of a biologically active protein molecule intact.

Based on the work of Garnett and Calf it seems clear that such exchange reactions might be carried out using Group VIII metal catalysts and deuterium oxide, as the source of deuterium. Since the aim was to label a biologically active protein molecule, the exchange conditions had to be mild enough to preserve

(a) the steric purity of the component amino acids
(b) the peptide bonds
(c) the conformation

Previous work on the catalytic labelling of optically active molecules, such as lactic acid at 130°C has shown that substantial racemisation may accompany deuterium exchange. This has been explained by the mechanism shown in figure (1).

Whilst the degree of racemisation under these exchange
conditions may be less severe in a protein molecule, where the asymmetric centres of the individual amino acids are less liable to be exposed to the metal surface, it is clear that milder reaction conditions must be used to minimise the danger of racemisation.

Since peptides and proteins are polymers of amino acids linked by peptide bonds (-CONH-) which are sensitive to hydrolysis in both acidic and basic media, it is essential that labelling must be carried out under conditions which will leave the peptide bonds intact.

Finally one has to be careful that the deuterium labelling does not result in conformational changes of the protein molecule (denaturing). In fact proteins are often denatured by (a) heat (b) pH changes (c) reagents which tend to rupture hydrogen bonding. Keeping the above restrictions in mind, a literature survey of catalytic labelling procedures potentially suitable for peptides and proteins was made.

The literature suggests that aromatic molecules such as benzene or aromatic polycyclics are most conveniently exchanged under mild conditions if Group VIII metals are used as heterogeneous catalysts. Although acid induced exchange has been shown to greatly facilitate deuterium incorporation into the aromatic ring, the required hydrogen ion concentration was too large for it to be safe to use with peptides and proteins. This suggests that a suitable starting point for the deuterium labelling of aromatic amino acids, peptides and proteins would be a heterogeneous catalytic system using Group VIII metals in a neutral aqueous medium.
DISCUSSION
It is convenient to classify three types of protons in an aromatic amino acid. These are (a) labile (b) aromatic and (c) non-aromatic protons.

(a) Labile protons: The labile protons are bonded to strongly electronegative functions such as the carboxylic acid and the amine group of an amino acid. The protons exchange rapidly and equilibrium deuterium incorporation occurs in most aqueous solutions without the aid of a catalyst.

\[
\text{\ce{\text{\OCH2-CH-COOH}} + D_2O \rightarrow \text{\ce{\text{\OCH2-CH-COOD + HDO}}}}
\]

Back-exchange of these deuterons is also rapid in aqueous solutions.

(b) Aromatic protons: Garnett and Sollich have proposed that the dissociative \( \pi \)-complex substitution mechanism predominates in aromatic proton exchange reactions which are carried out in deuterium oxide. They postulate that the aromatic molecule is adsorbed on the metal surface by donation of \( \pi \)-electrons into the empty d-orbitals of the metal catalyst. There is also a significant donation of metal d-electrons into vacant anti-bonding orbitals of the molecule.
Figure 2

Figure 3
The adsorbed π-complexed aromatic (1) then reacts with a metal radical (active site) by a substitution process. During this reaction the molecule rotates through 90° and changes from horizontal adsorption to vertical σ-bonded chemisorption. (figure 2)

Rotation of the ring is necessary since "edge on" or "vertical" π-complexing is prevented by the orbital symmetry and by the steric hinderance of the aromatic protons. While σ-bonded the aromatic compound undergoes a second substitution reaction at the carbon-metal bond with a chemisorbed deuterium atom and returns to the π-bonded state. (figure 3)

The aromatic molecule may then either desorb, as a mono-deuterated species, or undergo further substitution, mainly in the meta and para positions. Decreased exchange rates in the ortho positions of alkyl benzenes have been observed and these have been attributed to steric effects, because large and sterically crowded substituents (t-butyl) tend to decrease the rate of exchange more than smaller substituents (methyl).

(c) Non-aromatic protons: It is generally recognised that non-aromatic protons exchange by a dissociative mechanism proposed by Farkas and Farkas. This mechanism involves the chemisorption of the molecule through carbon-hydrogen bond rupture. (figure 4)

Modified π-complexed mechanisms have been proposed for the exchange of molecules which contain allylic protons. These mechanisms have involved π-allylic intermediates.
Figure 4
Pour methods for the preparation of Group VIII metal catalysts are currently favoured. These involve the use of oxides or chlorides followed by exposure to:

(a) hydrogen at various temperatures 33,18,19
(b) potassium or sodium borohydride 34,35,4
(c) other organic reducing agents 36
(d) radiation induced (U.V., X-rays) reduction 4,37,38

Since sodium borohydride reduced catalysts have been shown to give a high yield of ring deuteration, this method was used exclusively for the preparation of reduced Group VIII metal catalysts.4

In order to evaluate the degree and position of deuterium exchange which have taken place in an amino acid or peptide labelling experiment, it was necessary to develop a suitable analytical procedure. The literature suggests that N.M.R. 39 or mass spectroscopy 40,41 are most suitable for this purpose. Since an N.M.R. instrument was not readily available to us, mass spectrometric techniques were developed for the analysis of the reaction products. Since amino acids and peptides are not sufficiently volatile for direct mass spectrometric study, it was necessary to modify these compounds to give volatile derivatives. This was achieved by converting the free carboxylic acid function of the products to ethyl esters 42 by treatment with thionyl chloride-ethanol, followed by condensation of the amino function with either pivaldehyde (3) or acetyl acetone (4) in a neutral medium. (figure 5)

It was then possible to gas chromatograph the amino acid derivatives, and examine the degree of deuterium
R - CH₂ - CH - NH₂\textsubscript{2} \textsubscript{COOH} \xrightarrow{\text{SOCl₂}} R - CH₂ - CH - NH₂\textsubscript{2} \textsubscript{HCl}

Amino acid ethyl ester hydrochloride

\[ R - CH₂ - CH - NH₂\textsubscript{2} \textsubscript{HCl} + CH₃ - C - CH = 0 \xrightarrow{\text{OH}^-} \text{ion-exchange beads} \]

Pivaldehyde (3)

Acetyl acetone (4)

\[ R - CH₂ - CH - NH₂\textsubscript{2} \textsubscript{HCl} + CH₃ - C - CH₂ - C - CH₃ \xrightarrow{\text{ion-exchange beads}} \text{OH}^- \]

Figure 5
incorporation of the eluent by mass spectrometry. Peptides were either hydrolysed to monomers which could be examined in turn by G.L.C. - I.S. or converted to the acetyl acetone ethyl ester derivatives of the peptides and then analysed directly by mass spectrometry.

In this system the volatile sample molecules pass into the ionisation chamber where they are bombarded by electrons of controlled energy. An impacting electron of energy barely exceeding the ionisation potential (usually 8 - 14 eV), of the molecule, removes an electron to form a positively charged radical ion, referred to as the molecular ion. At an increased electron energy (up to 70 eV) bond fission occurs in one or more steps, or is accompanied by a rearrangement of the molecular or fragment ion(s). Multiple ionisation and ion - molecule collisions are less frequent occurrences. Amino acids and peptides which have similar side chains tend to produce similar mass spectrometric patterns.

The amino acids which were labelled in this study tended to fall into two distinct groups (a) the aromatic and heterocyclic; and (b) the non - aromatic or neutral amino acids.

Phenylalanine is the simplest aromatic amino acid and was used to establish conditions under which only the aromatic protons would exchange. The factors which affect the rate of deuterium incorporation were (a) the metal catalyst (b) the amount of catalyst used (c) the temperature of the exchange reaction.

The pivaldehyde ethyl ester derivative of phenylalanine (5) was prepared for mass spectrometric deuterium analysis. The dominant signals in the mass spectrum were produced by fragments formed from bond rupture about the Cα atom.

\[
\text{H}_3\text{C}\text{CH}_2 - \text{CH} - \text{N} = \text{CH} - \text{C} - \text{CH}_3
\]

\[
\text{COOC}_2\text{H}_5\quad \text{CH}_3
\]

(5)
Table 1 Effect of catalyst on the rate of deuterium incorporation into phenylalanine.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Time</th>
<th>Temp</th>
<th>Deut. Dist.</th>
<th>Deuterium Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>m/e</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>170 171 172</td>
<td>Deut.Inc. 188 189 190</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(atom %)</td>
<td>m/e</td>
</tr>
<tr>
<td>Nickel</td>
<td>1 day</td>
<td>70°C</td>
<td>60 40 40</td>
<td>40 6 9 32 28 16 10</td>
</tr>
<tr>
<td>Palladium</td>
<td>1 day</td>
<td>70°C</td>
<td>97 3 3</td>
<td>0 0 47 32 10 5</td>
</tr>
<tr>
<td>Iridium</td>
<td>1 day</td>
<td>70°C</td>
<td>100 0 0</td>
<td>0 0 11 77 7 5</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>38°C</td>
<td>98 2 2</td>
<td>2 25 28 25 19 2</td>
</tr>
<tr>
<td>Platinum</td>
<td>1 day</td>
<td>70°C</td>
<td>99 1 1</td>
<td>1 0 0 10 57 11 11</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>38°C</td>
<td>99 1 1</td>
<td>1 0 0 9 80 10 38</td>
</tr>
</tbody>
</table>

phenylalanine $6 \times 10^{-5}$ moles; catalyst $6 \times 10^{-5}$ moles; deuterium oxide 0.05 moles.
The loss of the ester formation (- 73 m.u.) produces an ion usually referred to as the "amine" fragment and it is present in the mass spectrum of many amino acid derivatives. Rupture of the $\text{C}_\alpha - \text{C}_\beta$ bond is typical of aromatic and heterocyclic amino acids and it may arise by two common fragmentation pathways. These are (a) the side chain may be eliminated as the cation and in the case of phenylalanine a signal at m/e 91 is observed; more commonly (b) the phenylalanine side chain can be eliminated by homolytic cleavage of the $\text{C}_\alpha - \text{C}_\beta$ bond, as a radical resulting in a large signal at m/e 170 ($\text{M}^+ - \text{CH}_2\text{-O}$).

The amine fragment, m/e 188, contains eight protons from the phenylalanine molecule and so a 37.5% deuterium incorporation is consistent with the exchange of three protons. Since the meta and para protons are most readily exchanged, the results in Table I suggest that both platinum and iridium proceed almost to equilibrium in these three positions. At lower temperatures platinum appears to be the more effective catalyst.

As shown in Table II an increase in the amount of catalyst results in a greater deuterium incorporation and this is due to the increased number of active sites in the reaction medium. A platinum to phenylalanine ratio of 1:1 to 1:3 is necessary to produce rapid exchange of three of the aromatic protons at 70°C.

The reaction temperature also affects the rate of exchange of both aromatic and non-aromatic protons. It is necessary to select a suitable reaction temperature at which only the aromatic protons would exchange at a significant rate. The results in Table III, show that at 120°C there is significant exchange of the non-aromatic protons. The extent of this exchange decreases at lower temperatures so that at 50°C and 70°C there is still substantial deuterium incorporation in
<table>
<thead>
<tr>
<th>Pt / Phe molar ratio</th>
<th>Deut. Dist.</th>
<th>Deuterium Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m/e</td>
<td>Deut. Inc. (atom %)</td>
</tr>
<tr>
<td></td>
<td>170 171</td>
<td>188 189 190 191 192 193</td>
</tr>
<tr>
<td>1:7</td>
<td>99 1</td>
<td>1 0 18 30 47 5</td>
</tr>
<tr>
<td>1:5</td>
<td>99 1</td>
<td>1 0 18 28 45 9</td>
</tr>
<tr>
<td>1:3</td>
<td>98 2</td>
<td>0 0 10 71 12 8</td>
</tr>
<tr>
<td>1:1</td>
<td>99 1</td>
<td>0 0 10 67 11 11</td>
</tr>
</tbody>
</table>

Phenylalanine 6 x 10^{-5} moles; deuterium oxide 0.05 moles; time 1 day; temperature 70°C.
Table III  Effect of temperature on rate of exchange of phenylalanine.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Deut. Dist.</th>
<th>Deuterium Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m/e</td>
<td>Deut. Inc.</td>
</tr>
<tr>
<td></td>
<td>170 171 (atom %)</td>
<td>188 189 190 191 192 193 194 195 196 (atom %)</td>
</tr>
<tr>
<td>120°C</td>
<td>82 18</td>
<td>18 0 0 1 5 28 29 25 13</td>
</tr>
<tr>
<td>100°C</td>
<td>96 4</td>
<td>0 0 0 21 28 37 15</td>
</tr>
<tr>
<td>70°C</td>
<td>99 1</td>
<td>0 0 11 67 11 11</td>
</tr>
<tr>
<td>50°C</td>
<td>96 4</td>
<td>0 0 14 80 7</td>
</tr>
<tr>
<td>38°C</td>
<td>98 2</td>
<td>39 28 13 16 4</td>
</tr>
</tbody>
</table>

phenylalanine $6 \times 10^{-5}$ moles; platinum $6 \times 10^{-5}$ moles; deuterium oxide 0.05 moles; time 1 day.
Table IV  Effect of time on the extent of deuterium incorporation into phenylalanine

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m/e</td>
<td>(atom %)</td>
<td>m/e</td>
</tr>
<tr>
<td></td>
<td>170 171</td>
<td></td>
<td>188 189 190 191 192 193 194 195</td>
</tr>
<tr>
<td>1 day</td>
<td>96 4</td>
<td>14 80 7</td>
<td>37</td>
</tr>
<tr>
<td>3 days</td>
<td>98 2</td>
<td>6 69 10 7 5 3</td>
<td>43</td>
</tr>
<tr>
<td>5 days</td>
<td>99 1</td>
<td>5 64 11 9 6 5</td>
<td>46</td>
</tr>
<tr>
<td>21 days</td>
<td>98 2</td>
<td>23 25 46 6</td>
<td>54</td>
</tr>
<tr>
<td>28 days</td>
<td>98 2</td>
<td>9 23 58 9</td>
<td>59</td>
</tr>
</tbody>
</table>

Phenylalanine 6 x 10^{-5} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles; temperature 50°C.
the aromatic ring but no significant exchange of the aliphatic protons. At temperatures greater than 70°C there is increased deuterium incorporation in the ortho positions and some exchange of the allylic protons also takes place.

Table IV shows that the deuterium incorporation increases substantially as the reaction is allowed to proceed for a greater length of time. This increase is found particularly in the aromatic and allylic protons.

Since it may be desirable to selectively deuterium label an aromatic amino acid residue in a protein molecule it is important to establish whether other aromatic amino acids exchange under similar conditions. For example tyrosine, which is a para hydroxy substituted phenylalanine is of interest as it is a constituent of many proteins. The effect of the para hydroxy substituent in tyrosine on the rate of deuterium incorporation is difficult to assess because this substituent unfortunately affects the solubility of the amino acid.

The pivalaldehyde ethyl ester derivative of tyrosine cannot be gas chromatographed satisfactorily and therefore the acetyl acetone ethyl ester (6) was prepared for direct mass spectrometric analysis via the solid probe inlet system.

\[
\text{HO} - \text{CH}_2 - \text{CH} - \text{NH} - \text{C} = \text{CH} - \text{C} - \text{CH}_3 \\
\text{COOC}_2\text{H}_5 - \text{CH}_3 - 0
\]  

(6)

The amine fragment m/e 218 (M+ - COOC₂H₅) produces a prominent peak in the spectrum and may be used to assess the deuterium incorporation into the molecule. Rupture of the Cα - Cβ bond produces the m/e 107 peak (CH₂-\(\text{O}^-\))OH \((7)\) almost exclusively as homolytic cleavage of the Cα - Cβ bond to produce the \(\left[ \text{CH} - \text{COOC}_2\text{H}_5 \right]^-\) fragment at m/e 184.

\[
\text{CH}_3 - \text{C} = \text{CH} - \text{C} - \text{CH}_3 \\
\text{NH}
\]  

(8)
which, although it is the predominant process in the case of phenylalanine, does not occur because of the predominant formation of the ion at m/e 107 (7). The molecular ion, at m/e 291, is also prominent in the mass spectrum of ACA - L - tyr OEt, and this allows one to readily establish the total deuterium incorporation in the molecule. *44 - 47*

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>Catalyst</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>218</td>
<td>219</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Pt</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Pd</td>
<td>54</td>
<td>5</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Ir</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>tyrosine 5x 10^-5 moles; catalyst 6 x 10^-5 moles; deuterium oxide 0.05 moles. Deut. Inc. in aromatic ring at equilibrium = 67%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table V shows that platinum and iridium catalysts are again preferred over palladium as the isolated tyrosine contained significant amounts of di- and tetra- deuterated species. The extent of the deuterium exchange was decreased markedly when a higher concentration of tyrosine was used (Table V and VI). This is because most of the tyrosine was not in solution at the higher concentration (0.5M) and therefore did not exchange with deuterium. This is confirmed when the solubility of the tyrosine was increased, by raising the pH of the reaction mixture, the deuterium incorporation also increased.
**Table VI** Effect of solubility on deuterium incorporation into tyrosine.

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>pH</th>
<th>m/e</th>
<th>Deut.Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>218</td>
<td>219</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>neut.</td>
<td>63</td>
<td>10</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>neut.</td>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>3</td>
<td>48</td>
<td>11</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>10</td>
<td>49</td>
<td>8</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>12</td>
<td>42</td>
<td>8</td>
</tr>
</tbody>
</table>

Tyrosine $5 \times 10^{-4}$ moles; catalyst Pt $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles; Deut. Inc. in aromatic ring at equilibrium = 67%

It can be predicted that a para substituent should be less strongly chemisorbed on the catalyst because the $\pi$ - electron cloud is distorted and therefore the $\pi$ - electrons are less free to bond with the catalyst. The adsorption process may be modified through additional charge-transfer interactions of the substituent lone pair with the catalyst surface. This has been shown to be the case when para fluoro phenylalanine was exchanged. (Table VII)

**Table VII** Deuterium exchange of para Fluoro Phenylalanine

<table>
<thead>
<tr>
<th>Sample</th>
<th>m/e</th>
<th>Deut.Inc. (atom %)</th>
<th>m/e</th>
<th>Deut.Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>170</td>
<td>171</td>
<td>206</td>
<td>207</td>
</tr>
<tr>
<td>A</td>
<td>99</td>
<td>1</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>B</td>
<td>97</td>
<td>3</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

Sample A : reference
Sample B : Time 2 days; Temperature 70°C;
platinum $6 \times 10^{-5}$ moles;
para fluoro phenylalanine $6 \times 10^{-5}$ moles;
deuterium oxide $0.05$ moles.
Conversely if a para substituent of low ionisation potential (e.g. para nitro) was used (Table VIII), deuterium incorporation is less because the molecule becomes so strongly chemisorbed that there is a loss of activity on the catalyst surface and a displacement of the chemisorbed water.48

Table VIII  Deuterium exchange of para Nitro Phenylalanine

<table>
<thead>
<tr>
<th>Sample</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>170</td>
<td>97</td>
<td>233</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>3</td>
<td>234</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>95</td>
<td>5</td>
<td>235</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>5</td>
<td>236</td>
<td>7</td>
</tr>
</tbody>
</table>

Sample A: reference
Sample B: time 3 days; temperature 70°C;
platinum 6 x 10^{-5} moles;
para nitro phenylalanine 6 x 10^{-5} moles
deuterium oxide 0.05 moles

The data in Tables VII and VIII was obtained by converting the labelled para fluoro or para nitro phenylalanine to their pivaldehyde ethyl ester derivatives (9). The amine fragments

\[
\begin{align*}
(X - \text{C}_0 - \text{CH}_2 - \text{CH} - \text{N} = \text{CH} - \text{C} - \text{CH}_3)^+ \quad \text{and the ester} \\
\text{fragments (M}^+ - X - \text{C}_0 - \text{CH}_2) \quad \text{were used to assess the position} \\
\text{of the exchange.}
\end{align*}
\]

\[
X = \text{F} \quad \text{a} = \text{m/e } 206 \quad \text{b} = \text{m/e } 170
\]

\[
X = \text{NO}_2 \quad \text{a} = \text{m/e } 233 \quad \text{b} = \text{m/e } 170
\]

The aromatic amino acid tryptophan is also a common constituent of proteins; its structure contains the

\[
\text{NH}_\text{meity}
\]
Deuterium incorporation was studied via the acetyl acetone ethyl ester derivative (10) which was introduced directly into the mass spectrometer by way of the solid probe inlet system.

\[
\text{Deuterium incorporation in the aromatic ring was determined by examining the species at m/e 130 (CH}_2\text{).}
\]

Since the molecular ion signal at m/e 314 was small no accurate measurement of the total deuterium uptake could be made. As is the case for tyrosine, rupture of the \( \text{C} \alpha - \text{C} \beta \) bond does not produce the ester fragment at m/e 184 (\( \text{CH} - \text{NH} - \text{C} = \text{CH} - \text{C} - \text{CH}_3 \)) because of the stability of the alternative ion at m/e 130.

\[
\text{Table IX. Deuterium exchange of Tryptophan}
\]

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>Deuterium Distribution</th>
<th>Deut.Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m/e</td>
<td>130</td>
</tr>
<tr>
<td>Refrence</td>
<td></td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>1 day 50°C</td>
<td></td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td>1 day 90°C</td>
<td></td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>1 day 120°C</td>
<td></td>
<td>10</td>
<td>21</td>
</tr>
</tbody>
</table>

tryptophan \( 5 \times 10^{-5} \) moles; platinum \( 6 \times 10^{-5} \) moles; deuterium oxide 0.05 moles

Deut.Inc. in aromatic ring at equilibrium = 71%

Table IX shows that there is considerable exchange in the aromatic ring of tryptophan. Substantial exchange of two protons at 50°C and of three protons at higher temperatures was found. Previous experiments involving tryptophan
## Table X

**Deuterium exchange of Glycine**

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>98 99 100 101</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>98 2</td>
<td>1</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>94 4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Deuterium exchange of Alanine**

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>112 113 114 115 116</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>98 2</td>
<td>0.5</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>86 8 6</td>
<td>5</td>
</tr>
<tr>
<td>1 day</td>
<td>90°C</td>
<td>60 21 11 6 2</td>
<td>17</td>
</tr>
</tbody>
</table>

**Deuterium exchange of Leucine**

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>154 155 156 157 158</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>84 16</td>
<td>2</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>80 10 6 4</td>
<td>4</td>
</tr>
</tbody>
</table>

Glycine $1.1 \times 10^{-4}$ moles; alanine $0.97 \times 10^{-4}$ moles; leucine $0.70 \times 10^{-4}$ moles; platinum $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles.
exchanged with tritiated trifluoro acetic acid have shown that all five protons in the aromatic ring do exchange. It was also noted that only two or three of these exchanged deuterons are stable to back exchange by protons from a neutral aqueous system.

The neutral "non-aromatic" amino acids have an aliphatic side chain and a number of these compounds were exchanged under conditions similar to those which produced deuterium incorporation into the aromatic amino acids. For analysis of the deuterium content, the pivaldehyde ethyl ester derivatives (11) of these amino acids were prepared.

\[
\begin{align*}
\text{Glycine} & : & R - \text{CH} - N = \text{CH} - \overline{\text{C}} - \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{Alanine} & : & \text{COOC}_2\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{Leucine} & : & \text{CH}_3
\end{align*}
\]

\[
(11)
\]

Table X shows that the aliphatic protons of the neutral amino acids exchange at a slower rate than do the protons of the aromatic amino acids. These aliphatic protons exchange by a classical dissociative mechanism which requires a higher activation energy than the \( \pi \)-electron complex mechanism. Alanine exchanged at 90°C incorporates significant amounts of deuterium in a number of positions of the aliphatic side chain.

Proline, which is an imino amino acid, does not form a pivaldehyde ethyl ester derivative. The deuterium incorporation analyses were carried out on the acetyl acetone ethyl ester derivative. (12)

\[
\begin{align*}
\text{CH}_2 - \text{CH}_2
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2, \text{CH} - \text{COOC}_2\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 - \text{C} = \text{CH} - \overline{\text{C}} - \text{CH}_3
\end{align*}
\]

(12)
The $M^+$ signal, at m/e 225, in the mass spectrum of ACA-L-pro OEt is prominent and may be used to assess the extent of the deuterium incorporation.

Table XI  Deuterium exchange of Proline

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td></td>
<td>225 226 227 228 229</td>
<td>94 3 3 1</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td></td>
<td>92 8 8 1 1</td>
</tr>
</tbody>
</table>

Proline $0.87 \times 10^{-4}$ moles; platinum $6 \times 10^{-5}$ moles; Deuterium oxide $0.05$ moles.

Table XI shows that the deuterium exchange in proline follows the same pattern found in the neutral amino acids i.e. the aliphatic protons exchange very slowly.

Ornithine contains two amine groups and belongs to the series of basic amino acids. To establish the degree of deuterium incorporation, the labelled ornithine sample was converted to the pivaldehyde ethyl ester derivative (13) and injected into the G.L.C.-M.S. system.

$$\begin{align*}
\text{CH}_3 - 
\text{CH} - 
\text{CH} = 
\text{N} - (\text{CH}_2)_3 - 
\text{CH} - 
\text{N} = 
\text{CH} - 
\text{C} - 
\text{CH}_3
\end{align*}$$

$$\begin{align*}
\text{CH}_3 
\text{COOC}_2\text{H}_5
\end{align*}$$

(13)

Its mass spectrum showed an amine fragment ($M^+ - \text{COOC}_2\text{H}_5$) at m/e 223 which was used to assess the deuterium incorporation into the molecule. (Table XII)

Table XII  Deuterium exchange of Ornithine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>m/e</th>
<th>Deut. Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td></td>
<td>223 224 225 226 227</td>
<td>97 3 0.5</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td></td>
<td>94 6 1 1</td>
</tr>
</tbody>
</table>

Ornithine $0.71 \times 10^{-4}$ moles; platinum $6 \times 10^{-5}$ moles; Deuterium oxide $0.05$ moles.
As expected, deuterium incorporation was low due to the absence of aromatic protons in the molecule.

Histidine is also of interest as its side chain contains an imidazole ring \( \text{N} \equiv \text{N} \). For its mass spectrometric analysis the acetyl acetone ethyl ester derivative (14) proved to be most convenient. The mass spectrum of the acetyl acetone ethyl ester showed a prominent \( M^+ \) signal at m/e 265 which could be used to assess the total deuterium incorporation into the molecule.

\[
\begin{array}{c}
\text{N} \equiv \text{N} \quad \text{CH}_2 - \text{CH} - \text{NH} - \text{C} = \text{CH} - \text{C} - \text{CH}_3 \\
\text{COOC}_2\text{H}_5 \quad \text{CH}_3 \quad 0
\end{array}
\] (14)

In addition rupture of the \( C_\alpha - C_\beta \) bond resulted in two different ions; \( (\text{N} \equiv \text{N} - \text{CH}_2)^+ \) m/e 81 and the ester fragment at m/e 184 \( (\text{CH} - \text{NH} - \text{C} = \text{CH} - \text{C} - \text{CH}_3)^+ \), which yielded the deuterium incorporation into the aromatic ring (m/e 81) and the aliphatic proton (m/e 184).

<table>
<thead>
<tr>
<th>Table XIII</th>
<th>Deuterium exchange of Histidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Temp</td>
<td>m/e</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Reference 3 days 70° C</td>
<td>184 185</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Histidine \( 0.64 \times 10^{-4} \) moles; platinum \( 6 \times 10^{-5} \) moles; deuterium oxide \( 0.05 \) moles.

The results in Table XIII show that substantial exchange occurs in the aromatic ring while the aliphatic proton remains untouched under the reaction conditions.

The dipeptides are the simplest "protein" model compounds available which allow one to study the stability of the peptide bond under the above reaction conditions. They are also of value when assessing the extent of deuterium
incorporation into an aromatic amino acid in the presence of a neutral or basic amino acid. For our study the most convenient model compounds were phenylalanine dipeptides but tyrosine and tryptophan containing peptides have been examined.

The stability of the peptide bond under the catalytic labelling conditions was most readily examined by running the reaction mixture on T.L.C. and visualising the products by spraying with ninhydrin to produce a blue spot for both peptides and any liberated amino acids. In all the dipeptides studied the peptide bond was found to be stable under the exchange reaction conditions.

The position and extent of deuterium incorporation into the phenylalanine peptides was most conveniently examined by hydrolysing the peptide in 6N HCl and converting the liberated amino acids to their pivaldehyde ethyl ester derivatives. After G.L.C. the components were examined separately by mass spectroscopy. In the case of tyrosine and tryptophan containing peptides the deuterated species were converted directly to their acetyl acetone ethyl ester derivatives and these were examined directly by mass spectroscopy.

Glycyl - L - phenylalanine is the simplest phenylalanine dipeptide and the rate of deuterium incorporation was found to be similar to that of phenylalanine. The presence of the glycy1 residue does not hinder the chemisorption of the aromatic ring of gly - L - phe to the catalyst, as the rate of exchange was roughly equal to that of phenylalanine.

The labelled dipeptide was readily hydrolysed and the extent of deuterium exchange was determined from the mass spectra of the components; piv - gly OEt and piv - phe OEt. Incorporation of deuterium into the aromatic ring of phenylalanine was estimated from the amine fragment (m/e 188)
Table XIV

Deuterium exchange of the phenylalanyl residue of Glycyl - L - Phenylalanine

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m/e</td>
<td>(atom %)</td>
<td>m/e</td>
<td>(atom %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>170 171</td>
<td></td>
<td>188 189 190 191 192 193 194 195 196</td>
<td></td>
</tr>
<tr>
<td>12 hrs</td>
<td>85°C</td>
<td>98 2</td>
<td>2</td>
<td>3 5 7 13 4 2</td>
<td>38</td>
</tr>
<tr>
<td>17 hrs</td>
<td>85°C</td>
<td>98 2</td>
<td>2</td>
<td>5 4 14 9 2 1</td>
<td>35</td>
</tr>
<tr>
<td>21 hrs</td>
<td>85°C</td>
<td>96 4</td>
<td>4</td>
<td>1 1 10 19 22 5 1 1</td>
<td>47</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>99 1</td>
<td>1</td>
<td>3 10 13 66 7 1</td>
<td>33</td>
</tr>
<tr>
<td>3 days</td>
<td>70°C</td>
<td>98 2</td>
<td>2</td>
<td>2 9 12 65 10 2</td>
<td>35</td>
</tr>
<tr>
<td>6 days</td>
<td>70°C</td>
<td>95 5</td>
<td>5</td>
<td>3 6 12 66 8 4 1</td>
<td>36</td>
</tr>
<tr>
<td>4 days</td>
<td>38°C</td>
<td>99 1</td>
<td>1</td>
<td>61 18 11 10</td>
<td>9</td>
</tr>
</tbody>
</table>

**gly - L - phe** $0.45 \times 10^{-4}$ moles; platinum $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles.
and the exchange of the non-aromatic proton was determined from the ester fragment (m/e 170). Deuterium incorporation into glycine was estimated from the amine fragment (m/e 98).

Table XIV shows that the aromatic protons exchange rapidly in three positions at 70°C or above. At 38°C the rate of exchange of the aromatic protons is very much slower. The non-aromatic proton of phenylalanine contained in the m/e 170 fragment does not show deuterium incorporation even at 85°C.

The aliphatic protons of glycine exchange significantly at 85°C but the rate of exchange was very much slower at 70°C and negligible at 38°C (Table XV).

Table XV. Deuterium exchange of the glycyl residue of Glycyl - L - Phenylalanine.

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>m/e</th>
<th>Deut.Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>12 hrs</td>
<td>85°C</td>
<td>86</td>
<td>12</td>
</tr>
<tr>
<td>17 hrs</td>
<td>85°C</td>
<td>85</td>
<td>13</td>
</tr>
<tr>
<td>21 hrs</td>
<td>85°C</td>
<td>80</td>
<td>16</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>95</td>
<td>4</td>
</tr>
<tr>
<td>3 days</td>
<td>70°C</td>
<td>90</td>
<td>8</td>
</tr>
<tr>
<td>6 days</td>
<td>70°C</td>
<td>80</td>
<td>16</td>
</tr>
<tr>
<td>4 days</td>
<td>38°C</td>
<td>96</td>
<td>4</td>
</tr>
</tbody>
</table>

gly - L - phe 0.45 x 10^-4 moles; platinum 6 x 10^-5 moles; deuterium oxide 0.05 moles.

In order to determine if back exchange of the incorporated deuterium atoms occurred under the hydrolysis conditions a gly - L - phe sample (exchanged at 70°C for 2 days) was analysed via the acetyl acetone ethyl ester derivative to avoid the necessity of hydrolysing the dipeptide. The amount of deuterium incorporated in the aromatic ring was assessed from the m/e 91 peak and was found to be consistent with the
Table XVI  Deuterium exchange of the phenylalanyl residue of 
L-Alanyl-L-Phenylalanine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>170</td>
<td>171</td>
<td>188</td>
<td>189</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>98</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>99</td>
<td>1</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>6 days</td>
<td>70°C</td>
<td>99</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Deut. Inc. (atom %)

L-ala-L-phe 0.42 x 10^{-4} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles.

Table XVII  Deuterium exchange of the alanyl residue of 
L-Alanyl-L-Phenylalanine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>112</td>
<td>113 114 115 116</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>87</td>
<td>9      4</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>88</td>
<td>7      6</td>
</tr>
<tr>
<td>6 days</td>
<td>70°C</td>
<td>47</td>
<td>19     15     18  1</td>
</tr>
</tbody>
</table>

L-ala-L-phe 0.42 x 10^{-4} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles.
value determined from the pivaldehyde ethyl ester derivative of the hydrolysed molecule (percentage deuterium incorporation 33%).

Labelling experiments with L-alanyl-L-phenylalanine paralleled the results obtained with gly-L-phe. The mass spectrometric analysis of L-ala-L-phe was carried out on the pivaldehyde ethyl ester derivatives of the amino acids liberated by hydrolysis of the dipeptide. The deuterium incorporation into the aliphatic protons of alanine was determined from the amine fragment at m/e 112.

\[
\begin{align*}
\text{(CH}_3\text{-CH-N=CH-CH}_3\text{)}^+ \\
\text{CH}_3
\end{align*}
\]

Table XVI shows that there is almost complete exchange of three aromatic protons after one day at 70°C. The deuterium exchange of the non-aromatic proton of phenylalanine, as determined from the m/e 170 signal, was negligible even after six days. This proton was situated near the centre of the molecule and therefore chemisorption was severely sterically hindered.

The rate of exchange of the aliphatic protons of alanine was much slower than the exchange of the aromatic protons in phenylalanine (Table XVII), but the extent of deuterium incorporation into the alanyl residue was somewhat enhanced when compared with gly-L-phe.

A study of L-prolyl-L-phenylalanine under these conditions is of interest as the imino ring may prevent chemisorption of the peptide due to steric hindrance. Unfortunately the dipeptide is only sparingly soluble in the reaction medium and the results may be influenced by this fact.

Since proline does not form a pivaldehyde ethyl ester derivative it was necessary to examine the acetyl acetone
Table XVIII  Deuterium exchange of L-Prolyl-L-Phenylalanine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>Deut. Inc.</th>
<th>Deuterium Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m/e</td>
<td>Deut. Inc.</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>152 153</td>
<td>(atom %)</td>
</tr>
<tr>
<td>2 days</td>
<td>70°C</td>
<td>96 4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>76 24</td>
<td>3</td>
</tr>
</tbody>
</table>

L-pro-L-phe 0.38 x 10^{-4} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles.

Table XIX  Deuterium exchange of Glycyl-L-Tryptophan

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>Deut. Inc.</th>
<th>Deuterium Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m/e</td>
<td>Deut. Inc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>242 243 244</td>
<td>(atom %)</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>90 4 6</td>
<td>5</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>78 13 9</td>
<td>10</td>
</tr>
<tr>
<td>3 days</td>
<td>70°C</td>
<td>76 15 9</td>
<td>11</td>
</tr>
<tr>
<td>6 days</td>
<td>70°C</td>
<td>74 18 8</td>
<td>11</td>
</tr>
<tr>
<td>4 days</td>
<td>38°C</td>
<td>94 4 2</td>
<td>3</td>
</tr>
</tbody>
</table>

gly-L-try 0.38 x 10^{-4} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles.
ethyl ester derivative of the dipeptide for direct mass spectrometric analysis. Its mass spectrum shows a prominent molecular ion \( M^+ \) (m/e 372) which may be used to assess the total deuterium incorporation into the molecule and a major fragment ion at m/e 152.

\[
\text{CH}_2 - \text{CH}_2 \xrightarrow{\text{NH - C = CH - C - CH}_3} \text{CH}_2 \xrightarrow{\text{CH}_3} \text{CH} = \text{CH} - \text{C - CH}_3 + \]

which was used to assess the extent of deuterium incorporation into the aliphatic amino acid residue.

Table XVIII shows that there is substantial incorporation into three positions of the molecule. The exchange pattern of the m/e 152 peak indicated that there was very little incorporation into the prolyl residue. The large amount of undeuterated dipeptide in the product is due to the reduced solubility of the peptide in the reaction medium.

Glycyl - L - tryptophan was used to assess the effect of an aliphatic residue on the deuterium incorporation into tryptophan. The acetyl acetone ethyl ester derivative (15) of gly - L - try was used for mass spectrometric analysis.

\[
\text{CH}_3 - \text{C - CH} = \text{C - NH} - \text{CH}_2 - \text{C - NH} - \text{CH} - \text{CH}_2 - \text{COOC}_2\text{H}_5
\]

The mass spectrum of ACA - gly - L - try OEt showed two fragments which were of value for assessing the deuterium incorporation into various parts of the molecule. The m/e 130 fragment (CH\(_2\) - )\(^+\) is typical of any tryptophan compound and was used to determine the extent of deuterium incorporation into the aromatic protons. Homolytic cleavage of the C\(_\alpha\) - C\(_\beta\) bond, which is accompanied by incorporation of an extra hydrogen atom, produced the second
### Table XX  Deuterium exchange of L - Tryptophyl - L - Leucine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>n/e</th>
<th>Deut.Inc.</th>
<th>Deut.Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(atom %)</td>
<td>(atom %)</td>
</tr>
<tr>
<td>268</td>
<td>269</td>
<td>300</td>
<td>130</td>
<td>131</td>
</tr>
<tr>
<td>7 days</td>
<td>70°C</td>
<td>89</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

L - try - L - leu 0.34 x 10^{-4} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles.

### Table XIII  Deuterium exchange of Glycyl - L - Tyrosine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut.Inc.</th>
<th>m/e</th>
<th>Deut.Inc.</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td>(atom %)</td>
<td></td>
<td>(atom %)</td>
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<tr>
<td>3 days</td>
<td>70°C</td>
<td>243</td>
<td>11</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>7 days</td>
<td>70°C</td>
<td>244</td>
<td>9</td>
<td>13</td>
<td>26</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>110</td>
<td>111</td>
<td>112</td>
</tr>
</tbody>
</table>

Gly - L - tyr 0.41 x 10^{-4} moles; platinum 6 x 10^{-5} moles; deuterium oxide 0.05 moles.
fragment at m/e 242

\[
\left( \text{CH}_3 - \text{C} - \text{CH} = \text{C} - \text{NH} - \text{CH}_2 - \text{C} - \text{NH} - \text{CH}_2 \right)^+ \]

This fragment was used to assess the deuterium exchange of the aliphatic protons.

Substantial exchange occurred in at least two positions of the aromatic ring at 70°C, although little exchange was found at 38°C (Table XIX). The aliphatic protons exchanged very slowly at both temperatures.

L-Tryptophyl-L-leucine was used as a model compound to show the effect of a large aliphatic side chain on the rate of exchange. The peptide was converted to ACA-L-try-L-leu OEt for mass spectrometric deuterium analysis and the fragmentation pattern of this compound was found to be similar to that of gly-L-try. The m/e 130 fragment was again used to assess the deuterium incorporation into the aromatic ring and the fragment ion at m/e 298,

\[
\left( \text{CH}_3 - \text{C} - \text{CH} = \text{C} - \text{NH} - \text{CH}_2 - \text{C} - \text{NH} - \text{CH} - \text{CH}_3 \right)^+ \]

was used to determine the amount of exchange in the aliphatic protons.

As found previously there was substantial exchange of two aromatic protons with no significant exchange of the aliphatic protons at 70°C. (Table XX)

Glycyl-L-tyrosine was used as a model compound to investigate the effect of a glycyl residue on the deuterium incorporation of tyrosine. For analysis, the acetyl acetone ethyl ester (16) was prepared.

\[
\text{CH}_3 - \text{C} - \text{CH} = \text{C} - \text{CH}_2 - \text{C} - \text{NH} - \text{CH} - \text{CH}_2 - \text{O} - \text{OH} \quad (16) \]
### Table XXII
Deuterium exchange of L-Tyrosyl-Glycine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
<th>Deut. Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>242</td>
<td>243</td>
<td>244</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
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<td>1</td>
</tr>
<tr>
<td>7 days</td>
<td>70°C</td>
<td>62</td>
<td>28</td>
<td>8</td>
</tr>
</tbody>
</table>

L-tyr-gly $0.41 \times 10^{-4}$ moles; platinum $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles.

### Table XXIII
Deuterium exchange of L-Tyrosyl-L-Alanine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
<th>Deut. Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>256</td>
<td>257</td>
<td>258</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>95</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>64</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>3 days</td>
<td>70°C</td>
<td>95</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>7 days</td>
<td>70°C</td>
<td>77</td>
<td>17</td>
<td>7</td>
</tr>
</tbody>
</table>

L-tyr-L-alal $0.38 \times 10^{-4}$ moles; platinum $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles.
The dominant fragment in the mass spectrum of ACA - gly - L - tyr OEt was found at m/e 107 (CH$_2$ - O - OH)$^+$ and from this peak the extent of deuterium incorporation into the aromatic ring was assessed. The signal at m/e 242 (CH$_3$ - C - CH = C - NH - CH$_2$ - C - NH - CH$_2$ - COOC$_2$H$_5$)$^+$ was produced by homolytic cleavage of the C$_2$ - C$_3$ bond, accompanied by the uptake of an additional hydrogen atom and was used to assess the deuterium exchange of the aliphatic protons.

No significant exchange of the aliphatic protons by deuterium was found when the reaction was allowed to proceed for 3 days at 70°C although considerable deuterium exchange of the aliphatic and allylic protons did occur when seven days were allowed for the reaction. Extensive exchange of the four aromatic protons was also observed at 70°C (Table XXI).

The deuterium exchange of L - tyrosyl - glycine was also studied. The mass spectrum of ACA - L - tyr - gly OEt (17) was used to determine the extent of deuterium incorporation and although it differed from the mass spectrum of ACA - gly - L - tyr OEt, the fragments at m/e 107 and m/e 242 were still present.

CH$_3$ - C - CH = C - NH - CH - C - NH - CH$_2$ - COOC$_2$H$_5$

Table XXII shows that the rate of exchange in L - tyr - gly is similar to the rate of exchange of gly - L tyr (Table XXI) and so the arrangement of the amino acids in the peptide has no effect on the rate of deuterium exchange.
\[ R_1 - \text{CH} - \text{C} = \text{O} + R_2 \]
\[ O = \text{C} - \text{CH} + \]

(18)

Figure 7.
\[ \text{CH} - \text{C} = \text{O} \]

\[ \text{N} \text{H} \quad \text{N} \text{H} \]

\[ \text{C} - \text{CH} - \text{R}_2 \]

\[ \text{R}_1 - \text{CH} - \text{C} = \text{O} + \text{R}_2^+ \]

\[ \text{N} \text{H} \quad \text{N} \text{H} \]

\[ \text{O} = \text{C} - \text{CH} \cdot \quad (19) \]
The extent of deuterium incorporation in L-tyrosyl-L-alanine was similar to that found in L-tyr-gly. The mass spectrum of ACA-L-tyr-L-alalOEt showed an ion at m/e 107 from which the extent of deuterium incorporation in the aromatic ring was determined. A signal at m/e 256
\[
\left( \text{CH}_3 - \text{C} - \text{CH}_2 - \text{C} - \text{NH} - \text{CH}_2 - \text{C} - \text{NH} - \text{CH} - \text{COOC}_2\text{H}_5 \right)^+ 
\]
which was due to homolytic cleavage of the C\text{\textsubscript{a}} - C\text{\textsubscript{b}} bond followed by a hydrogen atom uptake, yielded the extent of the aliphatic proton exchange.

Substantial exchange of the aromatic protons occurred rapidly at 70°C (Table XXIII). Exchange of the allylic and other aliphatic protons becomes significant only when the reaction was allowed to proceed for at least seven days.

Other suitable model compounds for this study are the cyclo dipeptides which can be considered as being formed by internal condensation of two amino acids to produce a cyclic compound. This ring system, containing two nitrogen atoms in the 1,3 positions is known as azine, so these compounds are derivatives of 2,4 diketoazine and are also referred to as diketopiperazines. The cyclo dipeptides are sufficiently volatile under reduced pressure to be examined directly by mass spectrometry. The mass spectra of these compounds contain fragments formed by rupture of the C\text{\textsubscript{a}} - C\text{\textsubscript{b}} bonds. The characteristic ions (18) and (19) (figure 7) allow the deuterium incorporation into the side chains to be estimated.

Most cyclo dipeptides also contain a fragment at m/e 113
\[
\left[ \begin{array}{c}
\text{CH}_2 - \text{C} = \text{O} \\
\text{NH} & \text{NH} \\
0 & \text{C} - \text{CH}
\end{array} \right]^+ 
\]
deuterium incorporation into the diketopiperazine ring.

Cyclo - D - phenylalanyl - L - phenylalanine was exchanged under the reaction conditions used previously. This compound contained two aromatic side chains and two aliphatic protons are found in the diketopiperazine ring.
Table XXIV  Deuterium exchange of cyclo - D - Phenylalanyl - L - Phenylalanine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>Catalyst</th>
<th>m/e 91</th>
<th>92</th>
<th>93</th>
<th>94</th>
<th>95</th>
<th>96</th>
<th>97</th>
<th>98</th>
<th>99</th>
<th>Deut.Inc. (atom %)</th>
<th>m/e 203</th>
<th>204</th>
<th>205</th>
<th>206</th>
<th>207</th>
<th>208</th>
<th>209</th>
<th>210</th>
<th>Deut.Inc. (atom %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1 day</td>
<td>70°C</td>
<td>Pt</td>
<td>21</td>
<td>14</td>
<td>12</td>
<td>14</td>
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<td>13</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Ir</td>
<td>3</td>
<td>14</td>
<td>11</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>61</td>
<td>28</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>38°C</td>
<td>Pt</td>
<td>6</td>
<td>20</td>
<td>13</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>40</td>
<td>20</td>
<td>13</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 days</td>
<td>38°C</td>
<td>Pt</td>
<td>13</td>
<td>18</td>
<td>13</td>
<td>12</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

cyclo - D - phenylalanyl - L - phenylalanine 6 x 10^-5 moles; catalyst 6 x 10^-5 moles; deuterium oxide 0.05 moles.
The extent of deuterium incorporation into the aromatic rings was assessed from the signal at m/e 91 (CH$_2$ - O) or from the signal at m/e 203 (H$^+$ - O - CH$_2$). Comparison of the exchange patterns obtained from these two signals also gives an indication of the extent of deuterium incorporation into the diketopiperazine ring. This is important as in this case the ion at m/e 113 is of low intensity.

Table XXIV shows platinum to be the more effective catalyst at 70°C. The rate of exchange was decreased markedly when the reaction temperature was lowered to 38°C although substantial exchange did occur when the reaction was allowed to proceed for 21 days. The greatest deuterium exchange occurred in the aromatic and allylic protons (up to seven protons exchanged). In cyclo-D-phe-L-phe the aromatic rings are coplanar and may be chemisorbed simultaneously thereby increasing the strength of the chemisorption. When this occurs, the diketopiperazine ring is held above the catalyst surface and so exchange of its protons cannot take place.

Cyclo-L-leucyl-D-phenylalanine contains an aliphatic (R$_1$ = CH$_3$ - CH - CH$_2$ -) and an aromatic side chain CH$_3$ (R$_2$ = CH$_2$ - O) attached to the diketopiperazine ring. The extent of the deuterium incorporation into the various parts of the molecule may be determined from the fragment ions:

- m/e 91: O - CH$_2$ +
- m/e 113: CH$_2$ - C = O
- m/e 169: CH$_3$ - CH - CH - C = O

Table XXV shows that platinum was the more selective catalyst and the exchange of the aromatic protons was almost to equilibrium at 70°C. Comparison of the exchange
Table XXV  Deuterium exchange of cyclo - L - Leucyl - D - Phenylalanine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>Catalyst</th>
<th>m/e</th>
<th>Deut. Inc.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
<th>m/e</th>
<th>Deut. Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>90</td>
<td>91</td>
<td>2</td>
<td>97</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>70°C</td>
<td>20</td>
<td>71</td>
<td>62</td>
<td>90</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>70°C</td>
<td>80</td>
<td>15</td>
<td>4</td>
<td>37</td>
<td>37</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyclo - L - leu - D - pha 0.38 x 10^{-4} moles; catalyst 6 x 10^{-5} moles; deuterium oxide 0.05 moles.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
patterns of the m/e 113 and m/e 169 fragments indicates that incorporation had occurred in the diketopiperazine ring but not in the aliphatic side chain. The iridium catalyst appeared to enhance incorporation in the diketopiperazine ring in preference to either the aromatic or aliphatic side chains.

The mass spectrum of cyclo-L-tryptophyl-L-alanine (20) is also simple to interpret as it contains the characteristic tryptophan fragment at m/e 130 (CH₂-[\text{NH}]-[\text{C}=\text{C}])⁺ as its base peak.

The alternative cleavage of the Cα−Cβ bond is not favoured and the signal at m/e 127 \(\left[\text{CH}_3-\text{CH}-\text{C}=\text{O}\right]^+\) is of very low abundance. The total deuterium incorporation into the molecule was determined from the molecular ion peak at m/e 257.

\[
\text{CH}_3-\text{CH}-\text{C}=\text{O} \\
\text{NH} \quad \text{NH} \\
\text{O} = \text{C} - \text{CH} - \text{CH}_2 - \text{[NH]} \\
\] (20)

Table XXVI shows that platinum was again the best catalyst producing substantial exchange of four aromatic protons at 70°C. The exchange of the aromatic protons was very much slower at 38°C with significant incorporation only after 21 days.

The simplest tyrosine cyclo dipeptide is cyclo-glycyl-L-tyrosine. In this case greater deuterium incorporation was found than in the closely related glycyl-L-tyrosine. The mass spectrum of the compound contains three signals which could be used to determine the position and extent of the deuterium incorporation. The deuterium exchange of the
<table>
<thead>
<tr>
<th>Time</th>
<th>Temp</th>
<th>Catalyst</th>
<th>m/e</th>
<th>Deut.Inc.</th>
<th>m/e</th>
<th>Deut.Inc.</th>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Pt</td>
<td>130</td>
<td>95</td>
<td>131</td>
<td>5</td>
</tr>
<tr>
<td>3 days</td>
<td>70°C</td>
<td>Pt</td>
<td>130</td>
<td>33</td>
<td>131</td>
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<td>70°C</td>
<td>Ir</td>
<td>130</td>
<td>34</td>
<td>131</td>
<td>20</td>
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<tr>
<td>4 days</td>
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<td>Pt</td>
<td>130</td>
<td>85</td>
<td>131</td>
<td>20</td>
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<td>21 days</td>
<td>38°C</td>
<td>Pt</td>
<td>130</td>
<td>54</td>
<td>131</td>
<td>15</td>
</tr>
</tbody>
</table>

**Table XXVI**  Deuterium exchange of cyclo-L-Tryptophyl-L-Alanine.

cyclo - L - try - L - ala $0.39 \times 10^{-4}$ moles; catalyst $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles.
### Table XXVII  Deuterium exchange of cyclo - Glycyl - L - Tyrosine.

| Sample | m/e 220 | m/e 221 | m/e 222 | m/e 223 | m/e 224 | m/e 225 | m/e 226 | m/e 227 | m/e 228 | Deut.Inc. m/e 114 | Deut.Inc. m/e 115 | Deut.Inc. m/e 116 | Deut.Inc. m/e 117 | Deut.Inc. m/e 118 | Deut.Inc. m/e 107 | Deut.Inc. m/e 109 | Deut.Inc. m/e 110 | Deut.Inc. m/e 111 | Deut.Inc. m/e 112 | Deut.Inc. m/e 113 | Deut.Inc. (atom %) |
|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| A      | 91      | 9       | 3       | 7       | 19      | 31      | 23      | 12      | 3        | 2             | 0.7            | 95             | 5               | 1               | 97             | 3               | 16             | 5               | 19             | 5              | 2               | 41             |
| B      | 3       | 7       | 19      | 31      | 23      | 12      | 3        | 2       | 0        | 0             | 27             | 67             | 27              | 6               | 10             | 3               | 5              | 29             | 36             | 19             | 5              | 2               | 41             |
| C      | 0       | 0       | 11      | 17      | 24      | 23      | 16      | 0       | 0        | 0             | 45             | 12             | 32              | 17              | 5               | 38             | 5               | 3              | 24             | 31             | 17             | 5              | 54             |
| D      | 16      | 25      | 28      | 19      | 12      | 19      | 87      | 13      | 0        | 0             | 0              | 0              | 11              | 17              | 24              | 16             | 9              | 4              | 3              | 2              | 2              | 2              | 10             |
| E      | 0       | 0       | 11      | 17      | 24      | 16      | 9        | 4       | 3        | 2              | 0              | 0              | 11              | 17              | 24              | 16             | 9              | 4              | 3              | 2              | 2              | 2              | 10             |

Cyclo - Glycyl - L - Tyr 0.41 x 10^-4 moles; catalyst 6 x 10^-5 moles; deuterium oxide 0.05 moles.

- Sample A: reference
- B: 70°C, 1 day, platinum catalyst
- C: 70°C, 3 days, platinum catalyst
- D: 70°C, 1 day, iridium catalyst
- E: 55°C, 4 days, platinum catalyst.
### Table XXVIII  Deuterium exchange of cyclo-\( L \)-Tyrosyl-\( L \)-Valine.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Reference</th>
<th>Pt</th>
<th>Ir</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/e</td>
<td>m/e</td>
<td>m/e</td>
<td>m/e</td>
</tr>
<tr>
<td>156</td>
<td>157</td>
<td>158</td>
<td>159</td>
</tr>
<tr>
<td>Deut. Inc. (atom %)</td>
<td>Deut. Inc. (atom %)</td>
<td>Deut. Inc. (atom %)</td>
<td>Deut. Inc. (atom %)</td>
</tr>
<tr>
<td>94</td>
<td>6</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>89</td>
<td>15</td>
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<td>2</td>
</tr>
<tr>
<td>31</td>
<td>40</td>
<td>23</td>
<td>10</td>
</tr>
</tbody>
</table>

Time 1 day; temperature 70°C; cyclo-\( L \)-tyr-\( L \)-val 0.34 x 10^{-4} moles; catalyst 6 x 10^{-5} moles; deuterium oxide 0.05 moles.

### Table XXIX  Deuterium exchange of cyclo-\( L \)-Alanyl-\( L \)-Hexahydrophenylglycine

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp.</th>
<th>Catalyst</th>
<th>Deuterium Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Pt</td>
<td>55</td>
</tr>
<tr>
<td>3 days</td>
<td>70°C</td>
<td>Pt</td>
<td>36</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>Ir</td>
<td>0</td>
</tr>
</tbody>
</table>

cyclo-\( L \)-ala-\( L \)-hexahydrophenylglycine 0.47 x 10^{-4} moles; catalyst 6 x 10^{-5} moles; deuterium oxide 0.05 moles.
aromatic protons was determined from the signal at m/e 107 \((\text{H}_2\text{O} - \text{O} - \text{CH}_2)^{+}\) and the deuterium incorporation in the diketopiperazine ring was assessed from the ion at m/e 114 \(\left[\begin{array}{c}
\text{CH}_2 - \text{C} = \text{O} \\
\text{NH} \quad \text{NH} \\
\text{O} = \text{C} - \text{CH}_2
\end{array}\right]^{+}\). The total deuterium incorporation was determined from the molecular ion signal at m/e 220.

Table XXVII indicates that substantial exchange of the aromatic protons occurred when platinum was used, both at 38°C and at 70°C. Extensive incorporation into the diketopiperazine ring also occurred when the compound was exchanged for 3 days at 70°C. As in the case of cyclo-L-Leu-D-Phe, the iridium catalyst again favoured some exchange of the protons of the diketopiperazine ring before the aromatic protons.

The mass spectrum of cyclo-L-Tyr-L-Val contains three peaks which were used to determine the position and extent of the deuterium incorporation into the molecule. Deuterium exchange of the aromatic protons was assessed from the ion at m/e 107 and the signal at m/e 113 was used to determine the incorporation into the diketopiperazine ring. Comparison of the exchange patterns in the vicinity of the m/e 113 and the m/e 156 peaks \(\left[\begin{array}{c}
\text{CH}_3 - \text{CH} - \text{CH} - \text{C} = \text{O} \\
\text{CH}_3 \quad \text{NH} \quad \text{NH} \\
\text{O} = \text{C} - \text{CH}_2
\end{array}\right]^{+}\) gives an estimate of the degree of incorporation into the valine residue.

Table XXVIII shows that the platinum catalyst produced extensive exchange of the aromatic protons and the iridium catalyst enhanced the exchange of the protons of the diketopiperazine ring.

Cyclo-L-Ala-L-Hexahydrophenylglycine contains two aliphatic side chains and it was used to test the
stability of the aliphatic protons under the exchange conditions. The total deuterium incorporation was determined from the molecular ion (m/e 210) \[\boxed{\text{CH}_2 - \text{C} = 0}\] and proton exchange of the diketopiperazine ring was estimated from the intensity of the fragment ion at m/e 127 \[\boxed{\text{CH}_2 - \text{C} = 0}\].

Significant exchange occurred in two positions of the diketopiperazine ring when a platinum catalyst was used at 70°C, but an iridium catalyst was found to be even more active under the same conditions (Table XXIX). Substantial deuterium incorporation in the aliphatic ring did not occur with either catalyst even after 3 days.

An experiment with cyclo-L-alanyl-L-hexahydrophenylalanine gave similar results. The total deuterium incorporation was assessed from the molecular ion signal at m/e 224 \[\boxed{\text{CH}_2 - \text{C} = 0}\] and the deuterium incorporation into the diketopiperazine ring was determined from the peak at m/e 128 \[\boxed{\text{CH}_2 - \text{C} = 0}\].

Only very small amounts of deuterium were incorporated into the peptide, primarily in the diketopiperazine ring (Table XXX).

To check the effect of a second aliphatic residue on the exchange pattern of an aromatic amino acid, L-leucyl-glycyl-L-phenylalanine was examined under the same exchange conditions. The extent of deuterium incorporation
was assessed by acid hydrolysis of the deuterated peptide followed by derivatisation to the pivaldehyde ethyl ester components and G.L.C.-M.S. analysis.

Table XXX Deuterium exchange of cyclo-L-Alanyl-L-Hexahydrophenylalanine

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Temp</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>reference</td>
<td>70°C</td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
</tr>
</tbody>
</table>

Cyclo - L - ala - L - hexahydrophene 0.44 x 10^-4 moles; platinum 6 x 10^-5 moles; deuterium oxide 0.05 moles.

The extent of deuterium exchange of the aromatic protons (24%) of the phenylalanine residue in the tripeptide was smaller than the exchange of these protons in the free amino acid (37%) or in phenylalanine dipeptides (33%) under the same reaction conditions. The exchange of the aliphatic protons of the two neutral amino acid residues was negligible at 70°C. (Table XXXI)

Since steric purity is essential for the activity of a biologically functioning peptide, it was important to establish whether the proposed catalytic exchange procedure proceeded with retention of optical purity. With this in view we examined the optical purity of labelled phenylalanine by polarimetry.

As expected the results showed that the optical purity of the phenylalanine sample was related to the exchange of the proton at the asymmetric carbon atom. In addition the steric purity of a deuterated sample of L-phenylalanine (85°C) was shown to be 98.8 ± 0.1% pure by G.L.C. of the T.F.A. - L - pro - L - phe OMe derivative. The exchange at the asymmetric carbon atom of this sample, was found by mass spectrometry to be less than 1%.
### Table XXI  
**Deuterium exchange of L-Leucyl-Glycyl-L-Phenylalanine**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td><strong>Temp.</strong></td>
<td><strong>m/e</strong></td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
<td>170 171</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leucine</th>
<th>Deut.Dist.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td><strong>Temp.</strong></td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glycine</th>
<th>Deut.Dist.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td><strong>Temp.</strong></td>
</tr>
<tr>
<td>1 day</td>
<td>70°C</td>
</tr>
</tbody>
</table>

L-leu-gly-L-phe $0.29 \times 10^{-4}$ moles;  
platinum $6 \times 10^{-5}$ moles; deuterium oxide $0.05$ moles.
Table XXXII  Optical purity of labelled phenylalanine

<table>
<thead>
<tr>
<th>Exchange Temp</th>
<th>Optical Purity</th>
<th>% Deut.Inc. m/e 188*</th>
<th>% Deut.Inc. m/e 170**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>-33.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>50°C</td>
<td>-30</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>70°C</td>
<td>-30</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>90°C</td>
<td>-34.5</td>
<td>54</td>
<td>4</td>
</tr>
<tr>
<td>120°C</td>
<td>-14.4</td>
<td>76</td>
<td>18</td>
</tr>
</tbody>
</table>

* ion at m/e 188 contains the aromatic protons
** ion at m/e 170 contains the asymmetric carbon

The relationship between the optical rotation of tryptophan and the temperature of the exchange reaction followed a similar pattern to that of phenylalanine. Above 90°C racemisation of the L-tryptophan samples became significant (Table XXXIII) but below this temperature the optical purity can be maintained.

Table XXXIII  Optical purity of L-tryptophan

<table>
<thead>
<tr>
<th>Reaction Temperature</th>
<th>Optical Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>-30.8°</td>
</tr>
<tr>
<td>50°</td>
<td>-28°</td>
</tr>
<tr>
<td>90°</td>
<td>-28°</td>
</tr>
<tr>
<td>120°</td>
<td>-13°</td>
</tr>
</tbody>
</table>

The steric purity of a labelled tryptophan sample (exchanged at 85°C for 24 hrs) was determined by G.L.C. from the N-trifluoroacetyl 2-butyl ester. The sample was found to consist of 99.6% L-tryptophan.

Since the steric purity of some phenylalanine containing dipeptides can be readily determined by N.M.R. spectroscopy we examined a deuterated dipeptide L-alanyl-L-phenylalanine by this technique. The method depends on the position of the methyl doublet in the N.M.R. spectrum and for each diastereoisomer there is a different position. The presence of an aromatic amino acid in the dipeptide greatly
enhances the shift of the methyl doublet.

The optical purity of the deuterium labelled L - alanyl L - phenylalanine (85°C, 1 day) was determined by comparing the N.M.R. spectrum of this compound with the N.M.R. spectrum of D,L - ala - D,L - phe.\textsuperscript{54,55} The absence of the signals at 1.2 and 1.3 ppm in figure 8, indicated greater than 99% optical purity.

Similarly the steric purity of diketopiperazines derived from two asymmetric amino acids can be readily determined by chromatographic techniques. An examination of seven diketopiperazines deuterated under varying conditions (from 1 day at 70°C to 21 days at 38°C) by T.L.C. showed that less than 1% of the other diastereoisomer was present in the exchange sample.

The results summarised in this thesis suggest that catalytic labelling of small peptides containing aromatic amino acids such as phenylalanine, tyrosine, tryptophan and histidine is possible under conditions which leaves the peptide bond and steric purity of the residues intact.

A decapeptide, containing two phenylalanine residues, Gramicidin S, was exchanged under the standard reaction conditions (1 day, 70°C) using a platinum catalyst. The conformational purity of the peptide was checked by T.L.C., before the labelled Gramicidin S was hydrolysed to its constituent amino acids. The pivaldehyde ethyl ester derivatives of the liberated amino acids were prepared for mass spectrometric deuterium analysis.

The extent of deuterium incorporation into the aromatic ring of the liberated phenylalanine was found to be insignificant (approximately 1%). The slow rate of exchange of the aromatic protons of Gramicidin S may have been due to the position of these protons in the molecule. The secondary
structure of this peptide has been determined by Ovchinnikov and co-workers\textsuperscript{56} from the preliminary X-ray analysis carried out by Schmidt, Hodgkin and Oughton.\textsuperscript{57} The aromatic rings of the phenylalanyl residues were found to be on the internal surface of the molecule and therefore could not be adsorbed on the surface of the heterogeneous catalyst.

Although experiments with Gramicidin S do not clearly show the rate of exchange expected of larger peptides, it is felt that other peptides with more favourable conformations would be labelled with deuterium under the exchange conditions.
EXPERIMENTAL
GENERAL

Optical rotations were measured on a Hilger instrument using either a 0.5 dm or a 1 dm polarimetry tube.

G.L.C. separations were carried out using a Varian 600C gas chromatograph, fitted with a hydrogen flame detector. The column used was a 7' x 1/8" stainless steel tube packed with 5% OV17 on chromosorb W (silanized). Helium was used as the carrier gas.

Mass spectra were recorded on an E.A.I. QUAD 300 D low resolution mass spectrometer under the following conditions:
- Ionisation chamber temperature 250°C
- Emission current 4 amps
- Ion energy 5 volts
- Ionising voltage 12 eV

The compounds were introduced into the mass spectrometer either by the solid probe inlet system or by the G.L.C. The most significant mass spectrometric signals were reported with their peak heights expressed as a percentage of the peak height of the largest signal in the spectrum.

Thin layer chromatography (T.L.C.) plates were prepared from "Merck - Kieselgel G. according to the method of Stahl". Ninhydrin was used to detect free amino groups, while cyclo-dipeptides were detected by the chlorination method.
REACTION TUBE FOR EXCHANGE REACTION

Vacuum Line

Tap "t"

7.5 cm

Preconstricted and sealed here

12.5 cm

Reagents

Figure 9
EXCHANGE REACTION PROCEDURES

In a typical experiment sodium borohydride (400 mg) was added slowly to a suspension of platinum oxide (100 mg) in water (50 mg). Hydrolysis of the excess borohydride was completed by warming the suspension at 70°C for ten minutes. The suspension of the reduced catalyst was then centrifuged and the solution decanted. The reduced catalyst was washed free from excess salts with water, centrifuging and decanting the water after each washing. The soluble chlorides of certain metals (iridium and nickel) were used in the preparation of the reduced catalyst. The chlorides (containing the equivalent of 100 mg of the metal) were dissolved in water (50 ml) prior to activation with sodium borohydride.

10 mg of the reduced catalyst were weighed into the pyrex glass reaction tube together with 10 mg of the amino acid or peptide and 1 ml of deuterium oxide. The reaction mixture was frozen in an acetone-dry ice bath and evacuated to a pressure of 0.005 torr. After the usual degassing procedures (allowing the sample to attain room temperature under vacuum with "t" closed (figure 9), freezing again and evacuating) the tube was sealed at the point where it had been preconstricted to a capillary.

After the reaction was stopped the tube was cooled and then opened. The labelled compound was completely dissolved in additional solvent, and the metal catalyst removed by filtration. The labelled amino acids were purified on an ion exchange resin column (Zeo-Carb 225) after which the compound was dried and examined for steric purity followed by analysis of the deuterium incorporation.

STERIC PURITY OF AMINO ACIDS AND PEPTIDES

The optical rotations of labelled phenylalanine and
tryptophan samples were determined from 0.04 N solutions, contained in a 0.5 dm, 5 ml polarimetry tube. The steric purity of phenylalanine was also determined by G.L.C. of the T.F.A.-L-pro-L-phe ONe derivative on a 5' by 1/8" column packed with 5% SE30 on chromosorbed W. The column was held isothermally at 140°C and helium at a flow rate of 2.0 ml/min, was used as the carrier gas. The steric purity of a labelled tryptophan sample was determined on the same column, from the N-trifluoroacetyl-2-buty1 ester. The optical rotation of tyrosine was determined from a 4 N hydrochloric acid solution in a 1 dm, 1 ml polarimetry tube.

The steric purity of L-ala-L-phe was determined by N.M.R. spectroscopy using a Varian A - 60 spectrometer operating at 60 Mc/s with tetramethyl silane (T.M.S.) as an internal reference.

The diastereoisomers formed in the exchange of the diketopiperazines were separated by T.L.C. using either isopropylether - chloroform - acetic acid (6:3:1) or chloroform - methanol - acetic acid (14:2:1) solvent systems. To visualise the diastereoisomers the thoroughly dried silica gel plates were sprayed with a freshly diluted 10 - 15 % solution of commercial clorax bleach in water and left to dry in a ventilated hood at room temperature for exactly 30 minutes, then sprayed with ethanol and after an additional 10 minutes, sprayed with a 1:1 mixture of 1% potassium iodide and freshly prepared solution of o-tolidine in 10% acetic acid - water solvent. This method can also detect free amino acids liberated by rupture of the peptide bonds, to a concentration of 0.5 - 1 moles. 59, 60

The free amino acids formed by hydrolysis of the peptide bond in the deuterium exchange of dipeptides were separated
by T.I.C. using a butanol–acetic acid–water (2:1:1) solvent system. The silica gel plates were dried and the products visualised by spraying with ninhydrin and evaporating the solvent at 70°C.

**PREPARATION OF MASS SPECTROMETRIC DERIVATIVES.**

The initial step in the preparation of the mass spectrometric derivatives involved the formation of the hydrochloride ethyl ester of the amino acid or peptide. In a typical experiment phenylalanine (100 mg) was refluxed with an ethanol–thionyl chloride reagent (2 ml) for 30 minutes. (This reagent was unstable above 5°C and was formed by adding thionyl chloride (1 ml) dropwise to ethanol (10 ml) cooled in dry ice.) The solvent was removed under vacuum and the hydrochloride ethyl ester washed a number of times with ethanol to remove any traces of acid present.

**GENERAL PROCEDURE FOR THE PREPARATION OF PIVALDEHYDE ETHYL ESTERS.**

The hydrochloride ethyl ester of the amino acid was dissolved in ethanol and the solution was brought to a pH of 7 by the addition of ion-exchange beads (A.G.I. – X8, Bio–Rad Cal, in the bicarbonate form). Pivaldehyde (0.1 ml) was added and traces of water produced by this reaction were removed by the addition of molecular sieve (3A).

- **Piv – Phe OEt**
  - Molecular formula: \( C_{16}H_{23}O_2N \)
  - M.Wt.: 261
  - 
    - m/e 204 \([M^+ – tC_4H_9O_2(5\%)]\)
    - 188 \([M^+ – C_3H_5O_2(28\%)]\)
    - 170 \([M^+ – C_6H_5CH_2(100\%)]\)
    - 142 (15%) , 120 (28%), 91 (51%), 91 \([C_6H_5CH_2(23\%)]\)
Piv - p - Nitro - Phe OEt
Molecular formula C_{16}H_{22}O_{4}N_{2} M.Wt. 306
m/e 306 [M^+ (5%)], 233 [M^+ - C_{3}H_{5}O_{2} (21%)], 221 (24%), 193 (23%), 170 [M^+ - CH_{2}C_{6}H_{4}NO_{2} (100%)], 137 (37%), 106 (100%).

Piv - p - Fluoro - Phe OEt
Molecular formula C_{10}H_{22}O_{2}NF M.Wt. 279
m/e 279 [M^+ (14%)], 250 [M^+ - C_{3}H_{5}O_{2} (44%)], 170 [M^+ - CH_{2}C_{6}H_{4}F (100%)], 96 (15%).

Piv - Ala OEt
Molecular formula C_{10}H_{19}O_{2}N M.Wt. 185
m/e 156 [M^+ - C_{2}H_{5} (4%)], 112 [M^+ - C_{3}H_{5}O_{2} (100%)], 96 (12%), 86 (40%).

Piv - Gly OEt
Molecular formula C_{9}H_{17}O_{2}N M.Wt. 171
m/e 156 [M^+ - CH_{3} (16%)], 142 [M^+ - C_{2}H_{5} (17%)], 114 [M^+ - tC_{4}H_{9} (12%)], 98 [M^+ - C_{3}H_{5}O_{2} (100%)], 86 (89%).

Piv - Leu OEt
Molecular formula C_{13}H_{25}O_{2}N M.Wt. 227
m/e 170 [M^+ - tC_{4}H_{9} (8%)], 154 [M^+ - C_{3}H_{5}O_{2} (30%)], 138 (27%), 105 (37%), 96 (100%), 82 (90%).

Piv - Orn OEt
Molecular formula C_{17}H_{32}O_{2}N_{2} M.Wt. 296
m/e 239 [M^+ - tC_{4}H_{9} (9%)], 223 [M^+ - C_{3}H_{5}O_{2} (5%)], 196 (29%), 168 (31%), 154 (100%), 140 (67%).
Phenylalanine containing dipeptides were hydrolysed by refluxing for 12 hrs. in 4N hydrochloric acid. The acid was remove under vacuum and the liberated amino acids were washed a number of times with ethanol and dried to remove traces of water and hydrochloric acid prior to the preparation of the pivaldehyde ethyl ester derivatives.

**GENERAL PROCEDURE FOR THE PREPARATION OF ACETYL ACETONE ETHYL ESTERS**

The hydrochloride ethyl ester of the amino acid was again prepared and dissolved in ethanol. The pH of this solution was brought to 7 by the addition of ion-exchange beads (A.G.I. - X8, Bio - Rad Cal., in the bicarbonate form). A few drops of acetyl acetone were added followed by a stick of molecular sieve (3A, Matheson, N.J.) to remove traces of water. The reaction mixture was allowed to stand overnight before the supernatant liquid was withdrawn with a syringe and the solvent evaporated in vacuo. The sample was then introduced into the mass spectrometer by way of the solid probe inlet system.

**ACA - Tyr OEt**  
Molecular formula $C_{16}H_{21}O_4N$  
M.Wt. 291

m/e 291 [ $M^+$ (20%)], 218 [ $M^+$ - $C_3H_5O$ (20%)],
184 [ $M^+$ - $CH_2C_6H_4OH$ (100%)], 141 (36%),
170 [ $CH_2C_6H_4OH$ (78%)].

**ACA - Try OEt**  
Molecular formula $C_{18}H_{22}O_3N_2$  
M.Wt. 314

m/e 229 (2%), 182 (1.5%), 156 (6.5%),
130 [ $CH_2$ - $\text{NH}$ (100%)], 100 (30%).
ACA - Pro OEt  Molecular formula $\text{C}_{12}\text{H}_{19}\text{O}_3\text{N}$  M.Wt.  225

m/e 225 $[\text{M}^+ \text{ (100%)}]$, 196 $[\text{M}^+ - \text{C}_2\text{H}_5\text{(64%)}]$

194 (4%), 182 $[\text{M}^+ - \text{C}_2\text{H}_3\text{O} \text{ (64%)}]$

152 $[\text{M}^+ - \text{C}_3\text{H}_5\text{O}_2 \text{ (57%)}]$

ACA - His OEt  Molecular formula $\text{C}_{13}\text{H}_{19}\text{O}_3\text{N}_3$  M.Wt.  265

m/e 265 $[\text{M}^+ \text{ (19%)}]$, 192 $[\text{M}^+ - \text{C}_3\text{H}_5\text{O}_2 \text{ (37%)}]$

184 $[\text{M}^+ - \text{CH}_2\text{N} \text{ (82%)}]$, 167 (62%), 142 (31%)

130 (100%), 84 $[\text{CH}_2\text{NH} \text{ (94%)}]$

The acetyl acetone ethyl ester derivatives of the dipeptides of tyrosine and tryptophan were prepared in the same way as the derivatives of the amino acids except super-dry ethanol was used because the peptide bond was susceptible to hydrolysis under these conditions. The super-dry ethanol was prepared by reacting clean dry magnesium (5g) and iodine (0.5g) with 75ml of 99% ethanol. The mixture was warmed until the iodine had disappeared and all the magnesium had been converted to the ethylate. 900ml of the absolute alcohol were then added and the mixture was refluxed for 30 minutes. The ethanol (99.95%) was distilled off directly into the vessel in which it was stored.

ACA - Gly - Tyr OEt

Molecular formula $\text{C}_{18}\text{H}_{24}\text{O}_5\text{N}_2$  M.Wt.  348

m/e 348 $[\text{M}^+ \text{ (2%)}]$, 330 $[\text{M}^+ - \text{H}_2\text{O} \text{ (10%)}]$

257 (7%), 242 $[\text{M}^+ + \text{H} - \text{CH}_2\cdot\text{C}_6\text{H}_4\cdot\text{OH} \text{ (2%)}]$

192 (53%), 139 (100%)

107 $[\text{CH}_2\cdot\text{C}_6\text{H}_4\cdot\text{OH} \text{ (36%)}]$. 

ACA - Tyr - Gly OEt

Molecular formula $C_{18}H_{24}O_{5}N_2$  M.Wt.  348

m/e 348 $[M^+ (1.5\%) ]$, 290 (9\%), 249 (5\%),
242 $[M^+ + H - CH_2.C_6H_4.OH (12\%)]$,
199 (18\%), 184 (100\%), 176 (13\%),
107 $[CH_2.C_6H_4.OH (80\%)]$.

ACA - Gly - Try OEt

Molecular formula $C_{20}H_{25}O_4N_3$  M.Wt.  371

m/e 371 $[M^+ (45\%)]$, 257 (6\%),
242 $[M^+ + H - CH_2\text{-}NH\text{-}C_6H_4.OH (65\%)]$,
215 (100\%), 195 (12\%), 157 (61\%),
130 $[CH_2\text{-}NH\text{-}C_6H_4.OH (99\%)]$.

ACA - Try - Leu OEt

Molecular formula $C_{24}H_{33}O_4N_3$  M.Wt.  427

m/e 427 $[M^+ (4\%)]$, 385 (8\%), 370 $[M^+ - C_4H_9 (20\%)]$, 328 (32\%),
298 $[M^+ + H - CH_2\text{-}NH\text{-}C_6H_4.OH (12\%)]$.

MASS SPECTRA OF CYCLO DIPEPTIDES

The cyclo dipeptides were sufficiently volatile to be introduced directly into the mass spectrometer by way of the solid probe system.

Cyclo - Phe - Phe

Molecular formula $C_{18}H_{18}O_2N_2$  M.Wt.  294

m/e 294 $[M^+ (3\%)]$, 235 (21\%), 203 $[M^+ - CH_2.C_6H_5 (6\%)]$, 175 (15\%), 120 (100\%),
91 $[CH_2.C_6H_5 (18\%)]$. 
Cyclo — Leu — Phe

Molecular formula $C_{15}H_{20}O_{2}N_{2}$  M.Wt.  260

$m/e$ 260 $[M^+ (3\%)]$, 204 $[M^+ + H - C_4H_9 (13\%)]$, 169 $[M^+ - CH_2C_6H_5 (20\%)]$, 113 $[M^+ + H - C_4H_9 - CH_2C_6H_5 (30\%)]$, 91 $[CH_2C_6H_5 (100\%)]$.

Cyclo — Gly — Tyr

Molecular formula $C_{11}H_{12}O_3N_2$  M.Wt.  220

$m/e$ 220 $[M^+ (8\%)]$, 114 $[M^+ + H - CH_2C_6H_4OH (20\%)]$, 107 $[CH_2C_6H_4OH (100\%)]$.

Cyclo — Tyr — Val

Molecular formula $C_{14}H_{18}O_3N_2$  M.Wt.  262

$m/e$ 262 $[M^+ (2.5\%)]$, 156 $[M^+ + H - CH_2C_6H_4OH (88\%)]$, 113 $[M^+ + H - C_3H_7 - CH_2C_6H_4OH (38\%)]$, 107 $[CH_2C_6H_4OH (100\%)]$.

Cyclo — Tyr — Ala

Molecular formula $C_{12}H_{14}O_3N_2$  M.Wt.  234

$m/e$ 234 $[M^+ (2\%)]$, 128 $[M^+ + H - CH_2C_6H_4OH (72\%)]$, 113 $[M^+ + H - CH_2C_6H_4OH - CH_3 (8\%)]$, 107 $[CH_2C_6H_4OH (100\%)]$, 85 (5\%), 57 (6\%).
Cyclo-Try-Ala

Molecular formula $\text{C}_{14}\text{H}_{15}\text{O}_{2}\text{N}_{3}$  M.Wt. 257
m/e $[130 \text{ CH}_2 - \text{NH}]_{100\%}$,
118 (3.5%), 98 (14%), 83 (13%), 57 (70%).

Cyclo-Ala-Hexahydrophenylalany

Molecular formula $\text{C}_{11}\text{H}_{18}\text{O}_{2}\text{N}_{2}$  M.Wt. 210
m/e 210 $[\text{M}^+ (3\%)$], 139 (5%),
127 $[\text{M}^+ - \text{C}_6\text{H}_{11} (20\%)]$,
113 $[\text{M}^+ + \text{H} - \text{C}_6\text{H}_{11} - \text{CH}_3 (100\%)]$,
83 $[\text{C}_6\text{H}_{11} (10\%)]$, 67 (8%), 57 (10%).

CORRECTION FOR NATURALLY OCCURRING ISOTOPES

In the mass spectra of all carbon compounds there is an isotope peak, due to the presence of naturally occurring isotopes in particular carbon - 13 and deuterium, one mass unit above each fragment ion signal. To calculate the extent of deuterium incorporation in a particular fragment, an isotope correction formula was applied:
\[
\text{Height of } m + 1 \text{ peak} = nX \times 100
\]
\[
\text{Height of } m \text{ peak} = 1 - X
\]
\[= 1.12 \text{ n }\%
\]
Where
\[m = \text{fragment ion peak}\]
\[m + 1 = \text{isotope peak one mass unit from the fragment ion peak}\]
\[n = \text{number of carbon atoms in the fragment}\]
\[X = \text{fraction of carbon occurring as carbon - } ^{13}\]

For multiple exchange of deuterium into a fragment, the average deuterium content \(\phi\), is calculated from the following equation

\[
\phi = \frac{1}{n} \sum_{i=1}^{i=n} i \cdot di
\]

where \(di\) is the percentage of aromatic molecules (of \(n\) hydrogen atoms) containing \(i\) deuterium atoms.

These formulae were used in the following computer program to calculate the extent of deuterium incorporation in a particular fragment ion.
C C PROGRAMME ISOTOPE-CORRECTION S. MURRAY

DIMENSION D(30), NR (30), P(30), NH(30)

1 FORMAT (5H PEAK8X1HO, 9(5X11))
2 FORMAT (5H PEAK, 4X, 10(4X, 12))
3 FORMAT (10H CORR PC 10F6.2 //)
4 FORMAT (7H PHI = F10.2, 10X9H THETA = F10.2/13H PERCENTAGE D = F10.3//)
5 FORMAT (50H NAME OF SYSTEM)

10 FORMAT (/)

FM = 0.01105

900 READ 5
PUNCH 5
PUNCH 10

C INPUT NO. OF REPLACEABLE HYDROGENS

READ, NC, ND
CN = NC
FAC = CN*FM
N = ND + 1
READ, (NH(I), I = 1, N
DO 40 I = 1, N

40 D(I) = NH(I)
CD(1) = NH(1)
DO 150 I = 1, ND
J = I + 1

50 CD(J) = D(J) - FAC* CD(I)
IF(CD(J))51, 51, 150
51 CD(J) = 0
150 CONTINUE
SUM = 0
DO 55 I = 1, N

55 SUM = SUM + CD(J)
DO 58 I = 1, 30

58 P(I) = 0
DO 60 I = 1,N
   60 P(I) = (CD(I)/SUM)*100.
PUNCH 1, (J, J = 1, 9)
PUNCH 3, (P(I), I = 1, 10)
PUNCH 10
IF(N - 10)100, 100, 80
   80 PUNCH 2, (J, J = 10, 19)
PUNCH 3, (P(I), I = 11, 20)
PUNCH 10
IF(N - 20)100, 100, 90
   90 PUNCH 2, (J, J = 20, 29)
PUNCH 3, (P(I), I = 21, 30)
PUNCH 10
100 PHI = 0.
   DO 65 I = 2, N
       AJ = I - 1
       65 PHI = PHI + P(I)*AJ
THETA = 0.
   DO 70 I = 1, ND
       J = I - 1
       BJ = (ND - J)
       70 THETA = THETA + P(I) * BJ
PD = (PHI/(PHI + THETA))*100.
PUNCH 4, PHI; THETA, PD
PUNCH 10
GOTO 9000
END
ABBREVIATIONS

ala : alanine
gly : glycine
his : histidine
leu : leucine
orn : ornithine
phe : phenylalanine
pro : proline
try : tryptophan
tyr : tyrosine
val : valine

T.F.A. : trifluoroacetic acid
Piv. : pivaldehyde
ACA : acetyl acetone

M.S. : mass spectrometry
G.L.C. : gas-liquid chromatography
T.L.C. : thin layer chromatography
N.M.R. : nuclear magnetic resonance
ppm : parts per million
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Table XXIII Deuterium exchange of L - tyrosyl - L - alanine
Table XXIV Deuterium exchange of cyclo - L - leucyl-D - phenylalanine
Table XXV Deuterium exchange of cyclo - D - phenylalanyl - L - phenylalanine
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Table XXXII Optical purity of labelled phenylalanine
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BIBLIOGRAPHY


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