MICROESTIMATION OF \( \alpha \)-AMINO ACIDS WITH \textit{peri}-NAPHTHENODANTAN-2,3,4-TRIONE HYDRATE

BY RADWAN MOUBASHER AND WILLIAM IBRAHIM AWAD

(From the Faculty of Science, Fouad Iut University, Cairo, Egypt)

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When free \( \alpha \)-amino acids are heated with triketohydrindene hydrate (ninhydrin) (I) in neutral or slightly acidic medium, there are formed the corresponding aldehydes with 1 carbon atom less, ammonia, carbon dioxide, and diketohydrindylidenediketohydrindamine (II) (cf. Ruhemann (1)).

\[
\begin{align*}
2 \text{II} & + \text{R-CH-COOH} \rightarrow \text{R-CHO} + \text{CO}_2 + 3\text{H}_2\text{O} \\
\text{(I)} & \text{NH}_3
\end{align*}
\]

Recently, Moubasher and Mostafa (2) found that the reaction between ninhydrin and \( \alpha \)-amino acids proceeds differently from that mentioned above, as two different compounds have been isolated, namely hydrindantin (IV) and bis-1,3-diketoindane (V), according to the accompanying scheme.

Virtanen, Laine, and Toivonen (3) have described a quantitative method for the estimation of \( \alpha \)-amino acids in protein hydrolysates based on the quantity of aldehyde liberated by means of ninhydrin. A method for the determination of free \( \alpha \)-amino acids by titration of the carbon dioxide formed in the reaction with ninhydrin has been described by Van Slyke, MacFadyen, and Hamilton (4). It was also found that isatin in glacial acetic acid and chloramine-T at pH 2.5 may be used in place of ninhydrin (cf. Van Slyke, Dillon, MacFadyen, and Hamilton (5)).
Moubasher (6) has used peri-naphthindan-2,3,4-trione hydrate (VI) as a new reagent for the quantitative determination of α-amino acids, as this reagent is able to decompose the α-amino acids quantitatively to the next lower aldehyde with 1 carbon atom less with the formation of ammonia, carbon dioxide, and dihydroxy-peri-naphthindenone (VII).

\[
(I) + R-CH-COOH \rightarrow OH + R-CHO + NH_2 + CO_2
\]

Wertlind (7) has developed a method for the microestimation of valine by its degradation with ninhydrin to isobutyraldehyde. The aldehyde is steam-distilled and then determined colorimetrically by Fabinyi's reagent (8). It is not stated in Wertlind's paper whether the Fabinyi reaction is given by the volatile aldehydes arising from other amino acids. We
have found that acetaldehyde, isovaleraldehyde, and benzaldehyde, which are formed through the degradation of alanine, aspartic acid, leucine, and phenylaminoacetic acid respectively, give Fabinyi's reaction.

In the present work it is shown that peri-naphthindan-2,3,4-trione hydrate may be used in place of ninhydrin for the degradation of alanine, valine, leucine, aspartic acid, and phenylaminoacetic acid, to their corresponding aldehydes with 1 carbon atom less according to the accompanying scheme (cf. Schönberg, Moubasher, and Mostafa (9), Moubasher (6), Moubasher and Awad (10)).

\[
\text{O} \quad \text{C} \quad \text{C} \quad \text{OH} + \text{R-CH-COOH} \rightarrow \quad \text{NH}_2
\]

(VI)

\[
\text{O} \quad \text{C} \quad \text{C} \quad \text{OH} + \text{R-CHO} + \text{NH}_2 + \text{CO}_2
\]

(VII)

In order to avoid possible loss of the distilling aldehyde, this quantitative degradation of \(\alpha\)-amino acids to the corresponding aldehydes and the steam distillation are carried out in one apparatus in place of the two used by Wertlind. The apparatus is similar to that described by Virtanen et al. (3). The procedure may be adapted to the microestimation of the aldehydes mentioned above.

**EXPERIMENTAL**

**Apparatus—**
1. Universal colorimeter (Ernst Leitz), illuminated by its ordinary electric lamp.
2. Micro pipette of 1 cc. capacity.
3. Reaction and distilling apparatus. A distillation flask (A) (10 cc. capacity) is attached to a separatory funnel (B) by a ground joint. The neck of the distilling flask is adapted with a capillary tube (C) by means of a
ground joint, through which a continuous current of carbon dioxide is passed during the reaction. \( D \) is a bulb condenser attached to the neck of the flask through a ground joint. \( E \) is a bulb fitted to the condenser by a ground joint, the end of which is dipped in a test-tube \((F)\), strongly cooled from the outside by an ice-salt mixture.

**Reagents—**

1. Citrate buffer \((5)\), pH 4.7. Grind together 17.65 gm. of \( \text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot \cdot \cdot 2\text{H}_2\text{O} \) and 8.4 gm. of \( \text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O} \) to a fine powder.

2. A solution of freshly crystallized peri-naphthindan-2,3,4-trione hydrate; 0.1 gm. per 100 cc. of distilled water.

3. \( \alpha \)-Amino acid solutions of the concentrations stated in Table I.

4. Fabinyi's reagent. \((a)\) 16 cc. of freshly distilled salicylaldehyde made up to 250 cc. with absolute alcohol. \((b)\) Exactly 10.5 N sodium hydroxide solution.

**Purification of peri-Naphthindan-2,3,4-trione Hydrate—**The compound (prepared by Errera (11)) is dissolved in a minimum amount of hot water and ascorbic acid is added to complete the precipitation of dihydroxy-peri-naphthindenone \((\text{cf. Moubasher (12)})\). The precipitate is filtered off, washed with a few cc. of hot water, suspended in a few cc. of distilled water,
and treated with a saturated solution of bromine water. The color of the substance fades to a light yellow. Sufficient distilled water is then added and the suspension is boiled until the solid dissolves. The solution is filtered while hot, and the filtrate is concentrated in the presence of 2 drops of bromine water and left to cool. Almost colorless needles are obtained, m.p. 270°.

Procedure

50 mg. of the solid buffer are placed in the flask (A). 1 cc. of the α-amino acid solution and 1 cc. of the peri-naphthindan-2,3,4-trione hydrate solution are added. Carbon dioxide is allowed to pass through for about 2 minutes and then heating is carried out until half of the solution distills off. The color of the solution is orange at first, changing to red and lastly pink. Distilled water (4 cc.) is added during the distillation through the separatory funnel (B) in order to transfer the last traces of the aldehyde formed to the receiver.

The distillate is diluted to 10 cc. with distilled water. A known amount (cf. Table I) of this solution is treated with Fabinyi's reagent (2 cc. of sodium hydroxide solution and 1 cc. of salicylaldehyde solution). The mixture is placed in a water bath at 50° for 70 minutes, the orange-red color is developed, and the solution is cooled for 10 minutes and read against a solution of known concentration of the corresponding aldehyde treated with Fabinyi's reagent under the same conditions. The color is stable for 24 hours. The reading is carried out with the Universal colorimeter.

By the application of the equation, \( \frac{R_1}{R_2} \times C_1 = C_2 \), the results recorded in Table I were obtained. \( R_1 \) = reading of the standard aldehyde solu-

### Table I

**Estimation of Amino Acids with peri-Naphthindan-2,3,4-trione Hydrate**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount of amino acid used</th>
<th>Equivalent quantity of corresponding aldehydes</th>
<th>Amount of distilled aldehyde solution (cc.)</th>
<th>Reading of standard aldehyde solution (R₁)</th>
<th>Concentration of standard aldehyde solution (C₁)</th>
<th>Reading of distilled aldehyde solution (R₂)</th>
<th>Concentration of distilled aldehyde solution (C₂)</th>
<th>Concentration in 10 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>240</td>
<td>147</td>
<td>2</td>
<td>38.8</td>
<td>37</td>
<td>31.5</td>
<td>30</td>
<td>150</td>
</tr>
<tr>
<td>Leucine</td>
<td>250</td>
<td>163.8</td>
<td>4</td>
<td>20</td>
<td>54.2</td>
<td>44</td>
<td>62.3</td>
<td>155.7</td>
</tr>
<tr>
<td>Phenylaminoacetic acid</td>
<td>200</td>
<td>154.6</td>
<td>3</td>
<td>32.0</td>
<td>41.6</td>
<td>35.4</td>
<td>46</td>
<td>153.3</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>200</td>
<td>88</td>
<td>3</td>
<td>34.6</td>
<td>41</td>
<td>17.2</td>
<td>20.4</td>
<td>68</td>
</tr>
<tr>
<td>Alanine</td>
<td>200</td>
<td>98</td>
<td>3</td>
<td>35.9</td>
<td>41</td>
<td>24.5</td>
<td>27.9</td>
<td>93</td>
</tr>
</tbody>
</table>
tion; $R_2 = \text{reading of the distilled aldehyde solution}; C_1 = \text{concentration of the standard aldehyde solution}; C_2 = \text{concentration of the distilled aldehyde solution resulting from the equation.}$

**SUMMARY**

1. *peri*-Naphthindan-2,3,4-trione hydrate is used in the microestimation of the following $\alpha$-amino acids: alanine, aspartic acid, valine, leucine, and phenylaminoacetic acid.

2. Fabinyi's reaction is given by acetaldehyde, isovaleraldehyde, and benzaldehyde and these aldehydes may be estimated colorimetrically.

**BIBLIOGRAPHY**

MICROESTIMATION OF $\alpha$-AMINO ACIDS WITH *peri* -NAPHTHINDAN-2,3,4-TRIONE HYDRATE
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