A SIMPLE APPARATUS AND PROCEDURE FOR DETERMINATION OF AMINO ACIDS BY THE NINHYDRIN REACTION*

BY BERT E. CHRISTENSEN, EDWARD S. WEST, AND KEENE P. DIMICK

(From the Department of Chemistry, Oregon State College, Corvallis, and the Department of Biochemistry, University of Oregon Medical School, Portland)

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Considerable work has recently been published on the ninhydrin reaction with amino acids (2, 4, 5). This reaction appears to be specific for carboxyl groups of α-amino acids, while other organic acids such as lactic, acetic, and citric are unaffected (4). Because ninhydrin does not react with urea, peptides, other primary amines, and ammonia, Van Slyke and coworkers report this reagent to be more specific for amino acids in biological material than the classical nitrous acid reaction (4).

The utilization of ninhydrin for the quantitative determination of amino acids has been attempted in several ways. Earlier work was confined to colorimetric or photometric procedures which were subject to rather serious limitations (2).

Later Ruhemann (3) and Grassmann and von Arnim (1) have shown that the color-forming reaction of ninhydrin with amino acids is accompanied with the evolution of CO₂.

Both Van Slyke (4) and Dillon and Mason (2) have devised methods for the determination of amino acids based on the measurements of the CO₂ evolved. These investigators have adapted the manometric procedures as developed by Van Slyke (6, 7) and others for the determination of liberated carbon dioxide. This necessitates the use of equipment which may not be readily available in many laboratories.

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Recently Christensen has developed a very simple apparatus for the measurement of evolved carbon dioxide which has been successfully applied in several ways (8). Since the apparatus appears to lend itself perfectly to the determination of amino acids by the ninhydrin reaction, this investigation was undertaken.

Method

Apparatus—The apparatus used in this investigation is described in a previous article (8).

Solutions—(1) Barium hydroxide; (2) HCl; (3) thymol blue; (4) glycerol; (5) ninhydrin solution containing 30 mg. of ninhydrin per cc.; (6) saturated KH$_2$PO$_4$.

Procedure

The absorption vessel B (cf. (8) Fig. 1) is evacuated to approximately 30 mm. and filled with air drawn through a soda lime tower. It is then charged with approximately 2.5 ml. of 0.05 N Ba(OH)$_2$, accurately measured with a protected automatic pipette (8) and again evacuated. This operation should be done as fast as possible. In order to prevent sticking, the ground glass joints are lubricated with glycerol.

1 ml. of water is placed in the U-tube connecting the reaction vessel with the absorption flask. 1 to 4 mg. of amino acid in 2 ml. of water are introduced into the reaction vessel which is then connected to the absorption flask through the U-tube. 1 ml. of ninhydrin solution and 1 ml. of saturated phosphate solution are introduced into the cup of the reaction vessel which is then connected to a large soda lime tower.

The reaction vessel is then brought to a temperature of 110–115° and maintained there for 15 minutes by means of a phosphoric acid bath. During the heating sufficient (CO$_2$-free) air is slowly drawn through the system (by opening slightly the stop-cock of the absorption flask) to maintain the reaction vessel slightly below atmospheric pressure. After heating, the system is permitted to come to atmospheric pressure by opening the stop-cock of the absorption flask so that the flow of air is even and well controlled.

The absorption flask is then disconnected and set aside for 15 minutes. 3 ml. of acetone are now added to the flask and the excess Ba(OH)$_2$ titrated with approximately 0.03 N HCl from a
5 ml. microburette. During the titration the contents of the flask are protected from atmospheric CO$_2$ by a rubber dam held over the mouth of the flask with a rubber band. The tip of the burette is admitted through a pinhole cut in the center of the cover.

**Results**

*Blank Determinations*—Owing to small amounts of contaminating CO$_2$ (from reagents and residual air) blank runs are necessary to standardize the procedure. 4.60 ml. of HCl were required to neutralize 2.5 ml. of Ba(OH)$_2$. In blank runs 4.54, 4.54, and 4.52 ml. of acid were required.

**Analysis of Amino Acids**—In order to check the accuracy of the method, samples of pure amino acids were analyzed by this method and by the micro-Kjeldahl procedure. As indicated in Table I, alanine, serine, and tryptophane gave

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Weight of sample mg.</th>
<th>Per cent recovery Ninhydrin</th>
<th>Per cent recovery Kjeldahl</th>
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</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>1.848</td>
<td>95.3</td>
<td>101.5</td>
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<tr>
<td>Aspartic acid</td>
<td>1.930</td>
<td>100.2</td>
<td>99.9</td>
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<tr>
<td>Cysteine</td>
<td>2.615</td>
<td>99.5</td>
<td>99.8</td>
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<tr>
<td>Leucine</td>
<td>2.783</td>
<td>100.5</td>
<td>99.8</td>
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<tr>
<td>Norleucine</td>
<td>2.555</td>
<td>100.7</td>
<td>99.5</td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>2.365</td>
<td>100.9</td>
<td>100.3</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.620</td>
<td>100.8</td>
<td>100.3</td>
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<td>Glutamic acid*</td>
<td>3.600</td>
<td>100.7</td>
<td>99.9</td>
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<tr>
<td>Valine</td>
<td>2.470</td>
<td>101.0</td>
<td>99.9</td>
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<tr>
<td>Serine</td>
<td>1.763</td>
<td>100.4</td>
<td>100.1</td>
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<td>Alanine</td>
<td>1.965</td>
<td>99.4</td>
<td>99.9</td>
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<tr>
<td>Isovaline</td>
<td>3.450</td>
<td>1.0</td>
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<tr>
<td>Tyrosine</td>
<td>4.470</td>
<td>100.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Lysine dihydrochloride†</td>
<td>3.940</td>
<td>112.5</td>
<td>110.5</td>
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<tr>
<td>Cystine</td>
<td>3.630</td>
<td>98.2</td>
<td>96.7</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>1.115</td>
<td>100.1</td>
<td></td>
</tr>
<tr>
<td>α-Aminocaprylic acid</td>
<td>3.100</td>
<td>100.1</td>
<td></td>
</tr>
</tbody>
</table>

* Heated for 5 minutes.
† Calculated on the basis of pure lysine dihydrochloride.

TABLE I

*Comparison of Results by Ninhydrin and Micro-Kjeldahl Methods*

- Calculated on the basis of pure lysine dihydrochloride.
- *Heated for 5 minutes.*
Determination of Amino Acids

theoretical results. Mason reports low results (2) with these three amino acids when run under slightly different conditions.

In two respects the observations of Van Slyke were confirmed. Lysine gave variable results (depending on the acidity). Glutamic acid gave high results if the reaction was allowed to run the usual time.

SUMMARY

1. A simple procedure is described for the determination of amino acids by reaction with ninhydrin.
2. The time required for a complete determination is 20 minutes.
3. Agreement with Kjeldahl determinations of amino acids was within 1 per cent.

BIBLIOGRAPHY

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