THE ACTION ON TYROSINE AND ON PHENYLAMINOACETIC ACID OF ACETIC ANHYDRIDE AND ACETONE IN THE PRESENCE OF PYRIDINE.

BY P. A. LEVENE AND ROBERT E. STEIGER.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

(Received for publication, July 6, 1927.)

There are recorded in literature several instances in which the acylation of amino acids in the presence of pyridine proceeded abnormally. We have encountered an unexpected reaction in attempting to acetylate l-tyrosine suspended in a solution of pyridine in commercial acetic anhydride (containing 90 to 95 percent of the pure anhydride). From the reaction product a crystalline substance, C_{12}H_{17}O_{4}N, melting at 122-123°C., was isolated. This compound contains at least one acetyl group (attached to the tyrosine hydroxyl) which is easily removed on boiling for a short time with 1 equivalent of 0.66 N sodium hydroxide. A compound, C_{12}H_{18}O_{6}N, is thus obtained.

On the basis of these preliminary results, the two possible structures I and II were considered for the compound C_{14}H_{17}O_{4}N.

\[
\begin{align*}
\text{I.} & \quad \begin{array}{c}
\text{CH}_2 - \text{CH} - \text{COOH} \\
\text{OCONH}
\end{array} & \quad \begin{array}{c}
\text{CH}_2 - \text{CH} - \text{COOH} \\
\text{NHCONH}
\end{array} \\
\text{II.} & \quad \begin{array}{c}
\text{CH}_2 - \text{CH} - \text{COOH} \\
\text{OCONH}
\end{array} & \quad \begin{array}{c}
\text{CH}_2 - \text{CH} - \text{COOH} \\
\text{NHCONH}
\end{array}
\end{align*}
\]

These formulæ suggested the possibility that the compound is formed by condensation of acetyltyrosine with acetone.

2 The acetone may have been present already in the commercial impure anhydride which was used, or else formed in the course of the reaction.
If the formation of the substance is actually such, then the reaction should be facilitated by the addition of acetone to the mixture used in the earlier experiments. The expectation was realized experimentally.

The same reaction was then carried out on phenylaminoacetic acid. In this case a crystalline compound, $C_{12}H_{13}O_2N$, melting between 100–101°C., was obtained. Apparently this compound is of the same structural type as the one obtained from tyrosine. In their compositions, the two reaction products differ by the same value, $C_{2}H_{4}$, from those of the amino acids from which they are derived. (In the case of tyrosine it is of course necessary to allow for the acetyl group present in the hydroxyl.)

An abundant evolution of carbon dioxide (not measured, however) was observed during the reaction with phenylaminoacetic acid at 80–90°C. Therefore, for the derivative of this acid (and possibly for the tyrosine derivative, although no observations in this direction were made on tyrosine) one may also consider the possibility of the two following structures.

Lack of time and of material prevent us from continuing this investigation at the present time. Hence an exhaustive study of the structures of the substances described and the part played by the additional acetone is postponed to a future date.

EXPERIMENTAL.

I. Action of a Mixture of Commercial Acetic Anhydride and Pyridine on l-Tyrosine. (Preliminary Experiments.)

1. 14 gm. of l-tyrosine, 60 gm. of dry pyridine, and 100 gm. of commercial acetic anhydride (90 to 95 per cent) were heated for an hour in a bath maintained at 80–90°C., the mixture being shaken from time to time. The tyrosine dissolved gradually.

---

3 Obtained from silk.
The solution was then concentrated under reduced pressure to a thick syrup to which 150 cc. of water were added. After it was stirred, then left standing for a few hours, crystallization took place. The crude product was filtered off by suction, washed with water, and dried. Yield, 11.5 gm. Recrystallization from 24 cc. of acetone gave 6 gm. of colorless material which was further purified by recrystallization from 15 cc. of acetone. Melting point, 122-123°C.

No. 60.
Found. " 63.92, " 6.66, " 5.32 (Kjeldahl).
" 5.31 (Dumas).

No. 60 recrystallized from boiling water gave No. 62, melting at 122-123°C.

No. 62.
C 63.63, H 6.56, N 5.39 (Kjeldahl).

2. Identical proportions were used as in the first experiment; the time of heating was reduced to 20 minutes and the temperature was increased to 100°C. The oil obtained by concentrating the mixture was shaken in a shaking machine with 150 cc. of water. Crystallization took place after standing overnight. 6.5 gm. of crude product thus obtained, when recrystallized from 70 cc. of boiling water in the presence of some norit, gave yellowish crystals melting at 122-123°C. The mother liquors of the first crop, when concentrated under reduced pressure, gave 4.6 gm. of crude product, thus making a total of 11.1 gm. It was fairly pure as it melted at 122-123°C, although sintering already had taken place at 115°C.

II. Action of Alkali on the Compound C₁₄H₁₇O₄N.

2.63 gm. (0.01 mol) of finely powdered product melting at 122-123°C were boiled with 15 cc. of 0.66 N sodium hydroxide (1 equivalent) until dissolved. The solution was allowed to cool somewhat and while still warm 2 cc. of 5.0 N hydrochloric acid (1 equivalent) were added, and the mixture left to crystallize. The resulting 1.8 gm. of product were recrystallized from 15 cc.
of boiling water in the presence of some norit. Melting point, 163–165°C.

No. 63.

C 64.97, H 6.85, N 6.16 (Kjeldahl).

No. 63 was then recrystallized from boiling absolute alcohol. Melting point, 163–166°C.

No. 64.

C₁₂H₁₈O₆N₉ (221.19).


" 0.49 (Van Slyke amino).

III. Action of a Mixture of Commercial Acetic Anhydride, Acetone, and Pyridine on l-Tyrosine.

37 gm. of l-tyrosine (0.2 mol), 13 gm. of acetone (0.2 mol + 1 gm. excess), 79 gm. of dry pyridine, and 200 gm. of commercial acetic anhydride (90 to 95 per cent), placed in a 1 liter round bottom Pyrex flask, were heated for an hour in a bath kept at 80–90°C. The tyrosine dissolved gradually. Occasional shaking was found necessary. The reaction mixture was then concentrated to very small volume under reduced pressure. In order to remove residual acetic anhydride, a slow, continuous current of commercial xylene was introduced by means of a capillary extending below the surface of the oily residue. The temperature of the bath was kept around 95°C.; 150 cc. of xylene were thus vaporized during this distillation. The flask, the contents of which became partly solid on cooling, was kept under reduced pressure in a desiccator over paraffin until the crystals were free from xylene. They were then dissolved in 40 gm. of pure, boiling methylethyl ketone. The solution was filtered while hot and crystallization took place on seeding. The crystals which deposited after cooling overnight at 0°C. were filtered off by suction, were washed on the filter, first with a very small amount of pure ketone, then thoroughly with anhydrous ether, and were dried in a vacuum oven at 70°C. Yield, 16.8 gm. Recrystallization from 20 gm. of methylethyl ketone gave 14.2 gm. of crystals sintering very slightly above 115°C., and melting at 122–123°C. They were
finally purified by recrystallization from 32 gm. of boiling acetone. Melting took place sharply between 122-123°C.

Nos. 80, 74.
C_{14}H_{17}O_N (263.22).

Calculated. C 63.85, H 6.51, N 5.32.
Found. No. 80. 63.93, 6.64, 5.29 (Kjeldahl).

Calculated. C 64.08, H 6.85, N 7.33.
Found No. 79. 69.44, 7.02, 7.33 (Kjeldahl).

IV. Action of a Mixture of Commercial Acetic Anhydride, Acetone, and Pyridine on Phenylaminoacetic Acid.

15 gm. of phenylaminoacetic acid\(^4\) (0.1 mol), 7 gm. of acetone (0.1 mol + 1 gm. excess), 48 gm. of dry pyridine, and 100 gm. of acetic anhydride, placed in a 500 ce. round bottom Pyrex flask, were heated for an hour in a bath kept at 80°C. and shaken occasionally. Carbon dioxide escaped at the top of the reflux condenser. The liquor was concentrated under reduced pressure to a very viscous syrup. This residue yielded a crystalline mass, either spontaneously or when stirred with a small amount of acetone. After drying, it weighed 19.7 gm. It was crystallized from 10 gm. of boiling acetone. (Filtration of the hot solution must be carried out rapidly by suction as crystallization begins immediately.) The product was washed on the filter, first with a small amount of acetone, then with anhydrous ether to effect complete removal of the yellow coloring matter. Recrystallization from an equal weight of boiling absolute alcohol gave a product which, after drying at 70°C. (vacuum oven), melted between 100-101°C.

Nos. 79, 86.
C_{13}H_{15}O_2N (191.17).

Calculated. C 69.08, H 6.85, N 7.33.
Found No. 79. 69.44, 7.02, 7.33 (Kjeldahl).

Calculated. C 69.08, H 6.85, N 7.33.
Found No. 74. 69.63, 7.24, 7.20 (Van Slyke amino).

0.08 (Van Slyke amino).


\(^6\) For combustion the substance was mixed in the boat with copper oxide.
THE ACTION ON TYROSINE AND ON PHENYLAMINOACETIC ACID OF ACETIC ANHYDRIDE AND ACETONE IN THE PRESENCE OF PYRIDINE

P. A. Levene and Robert E. Steiger