A GENERAL REACTION OF AMINO ACIDS. II.

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(Received for publication, June 21, 1928.)

The following paper contains an account of further experiments on the reaction occurring between amino acids and a mixture of pyridine and acetic anhydride. It may be recalled that it was shown in our first paper (1) that a typical $\alpha$-amino acid on being warmed with acetic anhydride and pyridine was converted into an acetylaminoacetone derivative with evolution of carbon dioxide. A $\beta$-ketonic acid was assumed to be an intermediate product.

$$\text{COOH}$$
$$\text{R} \cdot \text{CH} \cdot \text{NH}_2 \cdot \text{COOH} \rightarrow \text{R} \cdot \text{C} \cdot \text{CO} \cdot \text{CH}_3 \rightarrow \text{R} \cdot \text{CH} \cdot \text{CO} \cdot \text{CH}_3 + \text{CO}_2$$
$$\text{NH} \cdot \text{CO} \cdot \text{CH}_3 \quad \text{NH} \cdot \text{CO} \cdot \text{CH}_3$$

It was further shown that, if one or both of the hydrogen atoms of the amino group were substituted by alkyl groups, no typical reaction with ketone formation took place and that the same was true of an acid such as $\alpha$-aminohydratropic acid which contains no unsubstituted hydrogen attached to the $\alpha$-carbon atom. The bearing of these results on the mechanism of the reaction was briefly discussed. The new experiments comprise a study of the action of pyridine and acetic anhydride on a number of other types of amino acids and their derivatives. The following amino acids were found to yield substituted acetylaminoacetones which were characterized by appropriate derivatives: aspartic acid, glutamic acid, histidine, and tryptophane. On the other hand methylaspartic acid (I) gave no trace of either ketone or carbon dioxide, this result being clearly due to the absence of an unsubstituted $\alpha$-hydrogen atom adjacent to the amino group.
behavior of methylaspartic acid is analogous to that of \(\alpha\)-amino-hydratropic acid. Phenyl-\(\beta\)-alanine (II), chosen as a representative of the \(\beta\)-amino acids, gave neither ketone nor carbon dioxide but underwent simple acetylation. Serine, typical of the \(\beta\)-hydroxy-\(\alpha\)-amino acids, gave unquestionable qualitative evidence of ketone formation but the amount was small as the product appeared to be unstable. Phenylserine (III), on the other hand, gave traces of carbon dioxide but no ketone. It was largely converted into the anhydride (azlactone) of acetaminocinnamic acid (IV). The fact that \(\alpha,\beta\)-unsaturated azlactones of this type are not acted upon by pyridine and acetic anhydride was confirmed by experiments with the azlactone of benzoylaminocinnamic acid (V) prepared by condensing benzaldehyde with hippuric acid. The substance was entirely unacted upon and was recovered unchanged. On the other hand Bergmann, Stern, and Witte (2) have recently described some cyclic anhydrides—so called azlactones of the saturated \(\alpha\)-amino acids. These substances are formed by the action of acetic anhydride on amino acids and are of the type shown by the general formula (VI). Azlactones derived from leucine, phenylalanine, and aspartic acid, all react with acetic anhydride and pyridine to give carbon dioxide and the same acetylaminoketones as are furnished by the amino acids themselves. It is therefore a plausible supposition that these azlactones represent an intermediate stage in the pyridine-acetic anhydride reaction. This hypothesis offers a satisfactory explanation of the curious failure of \(\alpha\)-alkylamino acids to undergo a reaction analogous to that of the unsubstituted amino acids for the former compounds obviously cannot yield azlactones containing a hydrogen atom in the position capable of replacement by an acetyl group. On the other hand it must be admitted that as judged by the apparent evolution of carbon dioxide, the azlactones do not react noticeably more rapidly than do the amino acids themselves so that it is not improbable that they only represent one of several intermediate steps in the reaction.

A number of peculiarities are observed in the products derived from the amino acids used in the present investigation. These will be referred to in the experimental section in order to avoid repetition.
Aspartic Acid.—When finely powdered L-aspartic acid (3 gm.) is warmed with acetic anhydride (20 cc.) and pyridine (10 cc.) on the steam bath, the acid slowly goes into solution and at the end of about 3 hours 85 to 95 per cent of the theoretical amount (1 mol) of carbon dioxide has been evolved. After a short distillation with steam to remove the bulk of the acetic acid and pyridine, a solution is obtained giving the typical ketone reactions including a strong iodoform test. Prolonged steam distillation results in some decomposition and diacetyl will begin to appear in the distillate. The aqueous solution on being made acid to Congo red with sulfuric acid yields scarcely anything on extraction with ether, but on repeated shaking (5 to 6 times) with butyl alcohol, the product is readily extracted. When the solvent is removed a clear amber-like gum (3.1 gm.) is obtained which shows no inclination to crystallize. The product gives a strong iodoform reaction, a deep orange-red color with sodium nitroprusside in alkaline solution, turning magenta with acetic acid. Silver, mercury, and copper salts are all reduced on warming in alkaline solution, while silver nitrate produces no precipitate in acid or neutral solution.
On addition of an excess of phenylhydrazine (3 gm.) dissolved in 10 per cent acetic acid to an aqueous solution of the gum (1 gm.) no immediate precipitation takes place but on being warmed on the water bath carbon dioxide is freely evolved and a crystalline product separates out in fair quantity (0.9 gm.). Examination of this product quickly showed that it was not a simple hydrazone of an acetylaminoketone but was the bis-hydrazone of diacetyl. The substance melted after recrystallization from benzene at 241-243°, had all the properties of the diacetyl derivative described by Pechmann, and gave the following results on analysis.

Analysis.

C_{18}H_{18}N_{4}. Calculated. C 72.1, H 6.76, N 21.0. 

The direct formation of diacetyl itself was readily demonstrated by dissolving the original gum in dilute sulfuric acid (1:20) and distilling with steam. After a short time the distillate becomes noticeably yellow and gives all of the characteristic reactions of diacetyl.

When the gum is dissolved in dilute hydrochloric acid (10 per cent) and evaporated on the water bath a residue is obtained which contains crystals which were readily identified as ammonium chloride. The odor of diacetyl is marked during the evaporation. The non-crystalline material on being dissolved in water and extracted with ether gave a small amount of a syrupy acid agreeing in its properties with the description of β-hydroxylevulinic acid given by Wolff (3), while another portion of the syrup on being warmed on the water bath with excess of aqueous ammonia readily furnished a base, which after extraction with ether melted at 85° and gave a picrate melting at 191-192°. The base was clearly identical with tetramethylpyrazine which, as is already known, results from the action of ammonia on either diacetyl or β-hydroxylevulinic acid. The anhydrous tetramethylpyrazine gave the following results on analysis.

Analysis.

C_{8}H_{12}N_{2}. Calculated. C 70.6, H 8.83. 
The foregoing results could well be interpreted on the basis of the idea that the original non-crystalline product of the action of acetic anhydride and pyridine on aspartic acid (VII) was an acetylaminoacetone derivative (VIII) such as should result from the general reaction observed by us in many analogous cases. The fact that the substance contains no amino nitrogen and that the ratio of total nitrogen to acetyl groups is exactly 1:1 is in harmony with this view. An inspection of the formula (VIII) shows that the ketone is a derivative of levulinic acid, namely

\[ \text{COOH} \quad \text{CHNH}_2 \quad \text{CH} \cdot \text{NH} \cdot \text{CO} \cdot \text{CH}_2 \]

\[ \text{CH}_2 \quad \text{CH}_2 \]

\[ \text{COOH} \quad \text{COOH} \]

**VII.**

\[ \text{CH}_3 \]

\[ \text{COOH} \quad \text{CO} \]

\[ \text{CHNH}_2 \quad \text{CH} \cdot \text{NH} \cdot \text{CO} \cdot \text{CH}_2 \]

\[ \text{CH}_2 \quad \text{CH}_2 \]

\[ \text{COOH} \quad \text{COOH} \]

**VIII.**

\[ \text{CH}_3 \]

\[ \text{C} \cdot \text{O} \cdot \text{CO} \cdot \text{CH}_3 \]

\[ \text{CH} \cdot \text{NH} \cdot \text{CO} \cdot \text{CH}_2 \]

\[ \text{CO} \]

\[ \text{IX.} \]

\[ \text{CH}_4 \]

\[ \text{C} \]

\[ \text{C} \cdot \text{NH}_2 \]

\[ \text{CH}_2 \]

\[ \text{CO} \]

\[ \text{X.} \]

β-acetylaminolevulinic acid, and as is well known, levulinic acid and analogous γ-ketonic acids react with acetic anhydride to give substances of the type of acetyl-levulinic acid usually represented as γ-acetoxy-γ-valerolactone (4). There is reason to believe that a similar reaction takes place with the ketonic acid (VIII) under discussion and that it is converted by the further action of acetic anhydride into β-acetamino-γ-acetoxy-γ-valerolactone (IX). During the steam distillation and other operations connected with the working up of this product one of the acetyl groups is removed with regeneration of the ketone (VIII). Under certain conditions
it has been possible to convert \(\beta\)-acetylaminolevulinic acid (VIII) into a finely crystalline compound which may be designated as \(\beta\)-amino-\(\alpha\)-angelica lactone (X), although it is possible though improbable, that it is a derivative of \(\beta\)-angelica lactone. The preparation of this substance presents difficulties since the use of acids or strong alkali is ruled out owing to the ease with which the nitrogen is split off as ammonia. It has not been found possible to establish conditions which regularly result in a successful preparation and many negative results have been encountered. When \(\beta\)-acetylaminolevulinic acid is dissolved in butyl alcohol containing a trace of sulfuric acid, and evaporated and subsequently dried for a week or so in a vacuum desiccator over sulfuric acid, a gummy residue is obtained. When this is warmed with a little 5 per cent sodium carbonate solution unchanged acid is dissolved and a crystalline residue of the lactone remains which is filtered off and recrystallized from boiling water. Its properties and analysis are in accord with the structure suggested. The substance is only moderately soluble in water, either hot or cold, and its aqueous solution reacts neutral to litmus. It is readily soluble in alcohol and less so in ether. It is instantly dissolved by strong hydrochloric acid but is in part recovered unchanged on dilution and evaporation. It is not decomposed by sodium carbonate but is easily decomposed by sodium hydroxide. It does not reduce Fehling's solution but reduces permanganate in alkaline solution somewhat slowly. The aqueous solution of the substance gives no iodoform reaction when treated with iodine and sodium hydroxide in the cold, but if it is first warmed with a little caustic alkali, so as to open the lactone ring, the cooled solution gives an intense iodoform reaction. On treatment with nitrous acid the compound gives nitrogen equivalent to 8.5 to 9.5 per cent in 5 minutes.

**Analysis.**

\[
\text{C}_3\text{H}_7\text{O}_2\text{N}.
\]


**Glutamic Acid (XI).**—When glutamic acid (5 gm.) is warmed with acetic anhydride (25 cc.) and pyridine (15 cc.) on the steam bath, no carbon dioxide is evolved for about half an hour and even then the evolution is slow. After 3.5 hours the amount of carbon
dioxide amounts to only 15 to 20 per cent of the amount calculated for 1 molecular proportion. This marked difference shown by glutamic acid when compared with analogous amino acids is apparently due to its conversion into pyrrolidonecarboxylic acid by the dehydrating action of the acetic anhydride. Since pyrrolidonecarboxylic acid no longer contains a free amino group such as we have found to be essential for ketone formation from amino acids, it is incapable of further reaction and accounts for the bulk of the glutamic acid originally taken. An attempt to limit the formation of pyrrolidonecarboxylic acid by adding a little acetyl chloride to the anhydride was unsuccessful.

When the reaction mixture is steam-distilled, the residue is found to contain a moderate amount of a ketone mixed with much other material. The iodoform and nitroprusside reactions are strong, and phenylhydrazine acetate gives a precipitate which readily becomes oily. Alkaline solutions of silver, copper, and mercury salts are readily reduced. It was not found possible to isolate the ketone directly, but evidence of its presence and structure (XII) were obtained through its conversion into 2,5-dimethylpyrazine-3,6-dipropionic acid (XIII) by reactions analogous to Gabriel's pyrazine synthesis from aminoacetone.

\[
\begin{align*}
\text{XI.} & \\
& \text{CH}_3 \\
& \text{COOH} \quad \text{CO} \\
& \text{CHNH}_2 \quad \text{CH} \cdot \text{NH} \cdot \text{CO} \cdot \text{CH}_2 \\
& \text{CH}_2 \rightarrow \text{CH}_2 \\
& \text{CH}_2 \quad \text{CH}_2 \\
& \text{COOH} \quad \text{COOH} \\

\text{XII.} & \\
& \text{CH}_3 \\
& \text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{C} \cdot \text{CH}_3 \\
& \rightarrow \\
& \text{H}_3\text{C} \cdot \text{C} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH} \\

\text{XIII.} & 
\end{align*}
\]
The aqueous solution containing the acetaminoacetone derivative was made acid to Congo red and then extracted by repeated shaking with butyl alcohol. After removal of the solvent the syrupy residue was heated for an hour on the steam bath with 10 cc. of hydrochloric acid (1:1). The solution was then evaporated to remove most of the acid and then treated with excess of ammonia. After standing at room temperature for an hour the mixture was evaporated to small bulk (10 cc.). The solution which reacted acid to litmus was made more strongly acid with acetic acid and allowed to crystallize. The first crop of crystals consisted of practically pure silky needles of the pyrazine acid while subsequent crops were contaminated with glutamic acid and were discarded. The yield was only 2 to 3 per cent of the glutamic acid originally taken.

2,5-Dimethylpyrazine-3,6-dipropionic acid is a type of acid that has not hitherto been prepared. It is very sparingly soluble in cold water and only moderately soluble in boiling water. Its aqueous solution only just reddens blue litmus paper. It crystallizes from methyl alcohol, in which it is readily soluble on heating, in stout prisms while from water it separates in long needles. It is fairly soluble in ether. The acid melts at 211–213° without evolution of gas. Its saturated aqueous solution gives only a trifling brownish yellow color with ferric chloride and no immediate precipitate with either silver nitrate or mercuric chloride. When it was left standing, a finely crystalline silver salt separates in the form of heavy needles. The substance gives no diazo reaction and it contains no amino nitrogen (Van Slyke).

Analysis.

\( \text{C}_{12}\text{H}_{16}\text{O}_{4}\text{N}_{2} \). Calculated. C 57.1, H 6.35, N 11.1.

Methylaspartic Acid.—This substance (I) was prepared from acetoacetic ester in the usual way. On being heated (1 gm.) with acetic anhydride (10 cc.) and pyridine (7 cc.) on the steam bath for several hours no carbon dioxide was evolved and when the products were worked up in the usual way no trace of any aminoacetone derivative was detected. This result is obviously in accordance with what would be expected.

Serine.—The synthetic acid (1 gm.) was heated with acetic
anhydride (10 cc.) and pyridine (7 cc.). After an interval of
about 15 minutes carbon dioxide was freely evolved. After 4
hours the mixture was distilled with steam. The distillate con-
tained no volatile ketone. The residue gave a strongly positive
iodoform reaction but the size of the precipitate seemed small in
comparison with similar tests with other amino acids. Fehling's
solution was reduced on boiling while sodium nitroprusside gave
a positive but rather atypical reaction. Phenylhydrazine gave
small amounts of a sticky hydrazone, while boiling with p-nitro-
phenylhydrazine in 5 per cent sulfuric acid gave small amounts of
an osazone giving the characteristic blue color with sodium
hydroxide and alcohol. It was not found possible to prepare in
crystalline form either the original product of the reaction or a
satisfactory derivative. While the tests above enumerated clearly
indicate that some acetaminoacetone derivative, analogous to
that obtained from other amino acids, had been formed, its
amount appeared small and its stability slight. Only two experi-
ments were made with serine as the mediocre results did not seem
to justify the sacrifice of larger amounts of this amino acid.

Phenylserine.—The acid (1 gm.) was warmed on the water
bath with acetic anhydride (7 cc.) and pyridine (5 cc.) for 8 hours.
A slight evolution of carbon dioxide took place but on steam dis-
stillation neither distillate nor residue gave any reactions indica-
tive of ketone formation. Crystals separated from the non-
volatile aqueous residue which melted at 152°. They were
identified by properties and by analysis as the "azlactone"
anhydride of acetaminocinnamic acid (IV) first described by
Erlenmeyer and Früstuck (5). On being dissolved in warm
sodium hydroxide and precipitated with hydrochloric acid,
acetaminocinnamic acid melting at 190° was obtained.

Phenyl-β-Alanine.—This amino acid (1 gm.) was treated exactly
as described above in the case of phenylserine. No carbon dioxide
was evolved and no ketone formation took place. The aqueous
residue gave a large yield of acetyl-phenyl-β-alanine, which after
crystallizing from acetic acid melted at 161–162° as described by
Posner (6).

Histidine.—Experiments were made with either the free his-
tidine base or the dihydrochloride without disclosing any dif-
ference. On being warmed (1 gm.) on the water bath with
acetic anhydride (7 cc.) and pyridine (5 cc.), carbon dioxide was evolved freely after about 10 minutes and in the course of 2 hours 93.5 to 97 per cent of the amount of carbon dioxide calculated for 1 molecular proportion was obtained. After steam distillation the residue was found to give intense iodoform and nitroprusside reactions and to reduce alkaline solutions of copper, silver, and mercury salts. Phenylhydrazine acetate gave an unattractive hydrazone slowly separating in minute needles. Semicarbazide and aminoguanidine gave no crystalline derivatives. Since the acetylaminoketone appeared to crystallize with difficulty it was decided to remove the acetyl group by evaporation with 10 per cent hydrochloric acid. The concentrated solution on standing deposited stout colorless prisms melting at 205–206° which gave results on analysis in satisfactory agreement with the expected dihydrochloride of (4)-imidazolyl-(3)-amino-butanone (2) (XIV).

\[
\begin{align*}
\text{CH} &= \text{C} - \text{CH}_2 - \text{CH} - \text{CO} - \text{CH}_3 \\
\text{N} & \quad \text{NH} \quad \text{NH}_2 \\
\text{C} & \quad \text{H}
\end{align*}
\]

\text{XIV.}

\text{Analysis.}

\text{C}_6\text{H}_{11}\text{ON}_2\cdot2\text{HCl.}

\text{Calculated.} \quad \text{C 37.2, H 5.75, N 18.6, amino N 6.19, Cl 31.4.}

\text{Found.} \quad \text{" 37.0, " 6.01, " 18.7, " 5.95, " 31.7.}

The yield of crystalline hydrochloride amounts to about 60 per cent of the theoretical amount but much additional product remains in the mother liquor. It gives the reactions described above typical of aminoacetone derivatives and also an intense diazo reaction while on treatment with alkali yields a pyrazine derivative which will be further examined. It will be noticed that the base only differs from \(\beta\)-iminoacetethylamine by the substitution of a hydrogen atom by an acetyl group but unlike the latter substance it was found by Dr. C. Lieb scarcely to affect the blood pressure when doses of from 1 to 20 mg. were injected intravenously into a cat.

\text{Tryptophane.—This amino acid (1 gm.) was treated as usual with acetic anhydride (6 cc.) and pyridine (4 cc.). The reaction}
was fairly vigorous and carbon dioxide was evolved freely. On steam distillation a little tar separated and was removed by filtration. On concentration of the aqueous solution, a clear yellow oil, moderately soluble in water, was obtained. It did not crystallize. Bromine water gives a thick yellow precipitate while iodine and sodium hydroxide give a deep wine-red color with only a small separation of iodoform. Fehling’s solution is reduced on boiling with development of an indole-like odor. The glyoxylic reaction is quite unlike tryptophane; at first a red color develops which turns to olive-green. On addition of an excess of phenylhydrazine in 5 per cent acetic acid to the solution, a thick yellow hydrazone separates out which is sparingly soluble in water but very soluble in organic solvents, with the exception of petroleum ether. It may be crystallized from a little benzene by addition of petroleum ether. The hydrazone on analysis gave results corresponding fairly closely with those calculated for the hydrazone of an acetyl derivative of the anticipated ketone (XV).

\[
\text{C} - \text{CH}_2 \cdot \text{CH(NH} \cdot \text{CO} \cdot \text{CH}_2) \cdot \text{CO} \cdot \text{CH}_3
\]

\[
\text{C}_6\text{H}_4 \quad \text{CH} \\
\text{N} \\
\text{H}
\]

XV.

Analysis.

\[
\text{C}_{29}\text{H}_{24}\text{O}_2\text{N}_4. \quad \text{Calculated: C 70.2, H 6.39, N 14.9.} \\
\text{Found: } \text{C 69.9, H 6.65, N 14.5.}
\]

The experiments with tryptophane must only be regarded as of a preliminary character.

Azlactones.—The azlactones of leucine, phenylalanine, and aspartic acid were prepared by the method given by Bergmann, Stern, and Witte (2). They behaved with pyridine and acetic anhydride precisely like the amino acids from which they were prepared and furnished the same acetylaminooacetone derivatives, the characterization of which has already been described. The details of these experiments therefore need no minute description. On the other hand the azlactone of benzoylaminoacinnamic acid gave neither carbon dioxide nor ketone as was to be anticipated.
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