glutaric lactone was condensed with glycine with the formation of the lactone of α-acetamino-α-hydroxyglutarylglycine.

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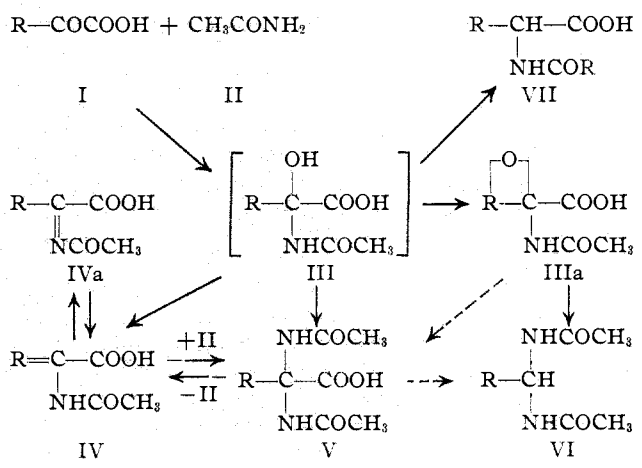
[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

The Condensation of α-Keto Acids and Acetamide[1]

BY DAVID SHEMIN AND ROBERT M. HERBST

In order to extend the dipeptide synthesis described in the preceding paper, it was necessary to prepare α,α-diacetamino derivatives from α-keto acids other than the pyruvic acid studied by Bergmann and Grafe.[2] The results obtained in the reaction of α-ketoglutaric acid with acetamide made desirable a more extensive study of this condensation, with a view to obtaining clearer insight into its mechanism.

The results obtained with the several α-keto acids studied are summarized in the following scheme



When α-ketoglutaric acid (I, R = $COOHCH_2CH_2$—) was condensed with acetamide (II) at 70°, the main product was the lactone of α-acetamino-α-hydroxyglutaric acid (IIIa, R = —$COCH_2CH_2$—). If the reaction was carried out at a higher temperature, or if IIIa was heated at a higher temperature with acetamide, a second product, γ,γ-diacetaminobutyric acid (VI, R = $COOHCH_2CH_2$—) was obtained. Whether V is an intermediate in this reaction, as indicated by the dotted arrows, or whether IIIa is decarboxylated, must remain an open question, but the opening of the lactone ring of IIIa by addition of acetamide appears to be a new reaction.

These results suggested that the formation of the diacetamino compounds proceeds in two steps, (a) addition of acetamide to the carbonyl group of the keto acid with the formation of an α-hydroxy-α-acetamino compound (III), and (b) replacement of the hydroxyl group of III by an acetamino group. This hypothesis seemed reasonable in view of the fact that benzaldehyde, on treatment with acetamide under suitable conditions, forms benzylidene-diacetamide.[3,4] However, it should be pointed out that in the preparation of α,α-diacetaminopropionic acid (V, R = CH_3—) from pyruvic acid and acetamide, a small amount of α-acetaminoacrylic acid (IV, R = CH_2=) is always formed.[2] If α-acetamino-α-hydroxypropionic acid (III, R = CH_3—) is formed as the first step in the reaction, loss of water from this intermediate readily explains the formation of α-acetaminoacrylic acid. In the case of α-ketoglutaric acid the intermediate (III) is stabilized by the formation of the lactone (IIIa). On the basis of the above hypothesis α-acetaminoacrylic acid would appear to be a by-product, but the possibility that it is an intermediate must be considered.

To test this point α-acetaminoacrylic acid (IV, R = CH_2=) was heated with acetamide. Surprisingly, almost quantitative conversion to α,α-diacetaminopropionic acid (V, R = CH_3—) resulted, so that α-acetaminoacrylic acid cannot be excluded as an intermediate. In this connection it should be recalled that α-aminoacrylic acid derivatives may be considered as tautomeric substances[2,5] capable of reacting in the forms IV or IVa. Acetamide may therefore add onto either the carbon–carbon double bond of IV or the car-

(1) This report is from a dissertation submitted by David Shemin in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187 (1930).

(3) Bulow, *Ber.*, **26**, 1972 (1893).

(4) Chattaway and Swinton, *J. Chem. Soc.*, **101**, 1206 (1912).

(5) Bergmann, Miekeley and Kann, *Z. physiol. Chem.*, **146**, 247 (1925).

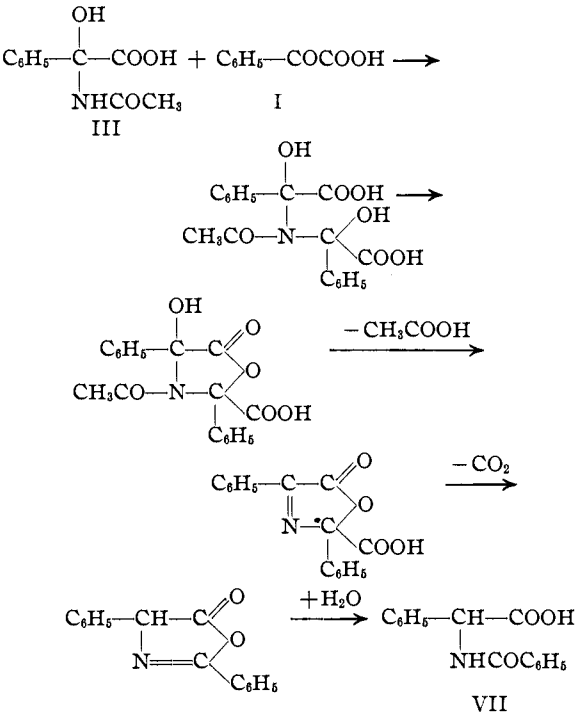
bon–nitrogen double bond of IVa.[6] Our data permit no definite conclusion as to the mode of addition of acetamide to α-acetaminoacrylic acid. The only other examples tried, cinnamic acid and α-acetaminocinnamic acid, showed no tendency to react with acetamide. However, the side chain double bond of cinnamic acid is notoriously unreactive, and the presence of the benzene ring may elicit a similar condition in the case of acetaminocinnamic acid. This is supported by the observation that when phenylpyruvic acid was heated with acetamide, α-acetaminocinnamic acid was formed in excellent yield.

The condensation of benzoylformic acid with acetamide was also studied. In this case an intermediate of type IV cannot form, although IVa is still a possibility. Actually α,α-diacetaminophenylacetic acid (V, R = C_6H_5—) was formed in good yield, together with small amounts of α-benzoylaminophenylacetic acid (VII, R = C_6H_5—) and benzylidene diacetamide (VI, R = C_6H_5—). The latter probably resulted from the interaction of acetamide with benzaldehyde, formed by decarboxylation of benzoylformic acid. Attempts to decarboxylate α,α-diacetaminophenylacetic acid were unsuccessful.

The experimental results are best explained by the assumption that the first step in the reaction between α-keto acids and acetamide is the addition of acetamide to the carbonyl group of the keto acid with the formation of an α-acetamino-α-hydroxy derivative. The second step involves replacement of the hydroxyl group by another acetamino group either directly or through an unsaturated intermediate of the Schiff base type.

The formation of the α-benzoylaminophenylacetic acid resembles that of N-phenacetylphenylalanine from α-benzoylaminocinnamic acid or phenylpyruvic acid and aqueous ammonia,[7] and that of N-acetylalanine from ammonia and pyruvic acid.[8] Du Vigneaud and Irish[9] have recently demonstrated the significance of this reaction in the biological synthesis of amino acids. The following scheme is suggested to account for the conversion of III into VII (R = C_6H_5—).

Attempts to isolate the azlactone postulated in the above scheme were unsuccessful, and it was



impossible to find evidence of a similar reaction in the case of any of the other α-keto acids studied.

Both the Erlenmeyer and the de Jong reactions can be explained by an analogous mechanism, ammonia being substituted for acetamide and water split out instead of acetic acid at the appropriate point. The formation of the amide of phenacetylphenylalanine in the Erlenmeyer reaction can be best explained by the assumption of an azlactone as a precursor, especially since Bergmann and collaborators[10] have shown that the azlactones of acylamino acids are converted into the respective amides by aqueous ammonia.

Experimental

α-Ketoglutaric Acid with Acetamide

α-Acetamino-α-hydroxyglutaric Lactone (IIIa, R = —$COCH_2CH_2$—).—α-Ketoglutaric acid (13.5 g.), prepared according to Neuberg and Ringer,[11] was treated with 6 g of acetamide at 70–75° under 10–15 mm. pressure for ten hours. The partially crystalline reaction mixture was extracted with four 50-cc. portions of hot ethyl acetate. The insoluble fraction (9.5 g.) was recrystallized from acetone–ether, from which it separated as colorless prisms which, on rapid heating, melted with decomposition at 196° (corr.). The substance was also soluble in water and alcohol, and crystallized from the latter on addition of ether. Opening of the lactone ring and esterification (see below) occur on prolonged boiling in alcoholic solution. The material was dried for analysis at 76° at 5 mm.

(6) Dr. R. E. Steiger very kindly called our attention to the latter possibility.

(7) Erlenmeyer and Kunlin, *Ann.*, **307**, 146 (1899); Erlenmeyer, *ibid.*, **337**, 205 (1904).

(8) De Jong, *Rec. trav. chim.*, **19**, 259 (1900).

(9) Du Vigneaud and Irish, *J. Biol. Chem.*, **122**, 349 (1938).

(10) Bergmann, Stern and Witte, *Ann.*, **449**, 277 (1926).

(11) Neuberg and Ringer, *Biochem. Z.*, **71**, 226 (1915).

Anal. Calcd. for $C_7H_9O_5N$: C, 44.9; H, 4.9; N, 7.5. Found: C, 45.1; H, 4.7; N, 7.5.

On direct titration with standard alkali the substance behaved like a lactone. The equivalent weight determined by back-titration after dissolving in a slight excess of alkali was 88, calculated for $C_7H_9O_5N$, 93.5. No reaction took place with bromine in aqueous solution.

On hydrolysis with normal hydrochloric acid, a 95% yield of α-ketoglutaric acid was obtained, isolated as the 2,4-dinitrophenylhydrazone, m. p. 217° (corr.) with decomposition, and showing no depression when mixed with an authentic specimen.

Glutamic Acid Hydrochloride.—α-Acetamino-α-hydroxyglutaric lactone was boiled with absolute ethyl alcohol for three hours. The resultant unsaturated ester was hydrogenated in the presence of a platinum catalyst and hydrolyzed with normal hydrochloric acid, when glutamic acid hydrochloride was obtained.

Anal. Calcd. for $C_5H_9O_4N\cdot HCl$: N, 7.6; $NH_2\cdot N$, 7.6. Found: N, 7.9; $NH_2\cdot N$, 7.7.

γ,γ-Diacetaminobutyric Acid (VI, R = $COOHCH_2$-CH_2—).—A mixture of 18 g. of α-ketoglutaric acid and 15 g. of acetamide was heated at 110 ± 5° for eight to nine hours at 10–15 mm. pressure. The reaction mixture was then dissolved in 400 cc. of cold ethyl acetate. An oil separated which crystallized on standing in the refrigerator. The crystals were filtered off and washed with cold ethyl acetate and cold absolute alcohol. Recrystallization was effected by boiling a suspension of the material in hot absolute alcohol and adding water dropwise until solution was complete. On standing in the refrigerator the substance crystallized in the form of needles, m. p. 197° (corr.), in a yield of 3.5 g. The melting point was markedly depressed when the material was mixed with α-acetamino-α-hydroxyglutaric lactone.

Anal. Calcd. for $C_8H_{14}O_4N_2$: C, 47.5; H, 6.9; N, 13.9; equiv. wt., 202. Found: C, 47.6; H, 7.0; N, 13.6; equiv. wt., 203.

γ,γ-Diacetaminobutyric acid was also formed when the lactone of α-acetamino-α-hydroxyglutaric acid (1.87 g.) was heated with acetamide (1.8 g.) at 110°. By similar methods of isolation, 900 mg. was obtained, m. p. 197° (corr.), and showing no depression when mixed with the product described above.

Anal. Calcd. for $C_8H_{14}O_4N_2$: N, 13.9. Found: N, 13.7.

The constitution was determined by hydrolysis in aqueous acid solution to the semi-aldehyde of succinic acid, isolated as the *p*-nitrophenylhydrazone, m. p. 179.5° (corr.), in a yield of 55%. The melting point showed no depression when mixed with an authentic specimen.[12]

α-Acetaminoacrylic Acid with Acetamide

α,α-Diacetaminopropionic Acid (V, R = CH_3—).—α-Acetaminoacrylic acid was prepared from α,α-diacetaminopropionic acid according to Bergmann and Grafe.[2]

Anal. Calcd. for $C_5H_7O_3N$: N, 10.9. Found: N, 10.7.

α-Acetaminoacrylic acid (4.75 g.) was heated with acetamide (10 g.) at 110–115° under 10–15 mm. pressure for three hours. The fraction insoluble in ethyl acetate was recrystallized from hot 95% ethyl alcohol. The yield of pure α,α-diacetaminopropionic acid was 4.5 g., m. p. 189–190° (corr.) with decomposition. It did not take up bromine in aqueous solution.

Anal. Calcd. for $C_7H_{12}O_4N_2$: N, 14.9. Found: N, 14.9.

α-Acetaminocinnamic Acid with Acetamide

In an attempt to condense α-acetaminocinnamic acid with acetamide under comparable conditions, 94 per cent. of the α-acetaminocinnamic acid was recovered as such.

Phenylpyruvic Acid with Acetamide

α-Acetaminocinnamic Acid (IV, R = C_6H_5CH=).—A mixture of 12 g. of phenylpyruvic acid[13] and 12 g. of acetamide was heated for three hours at 110–115° under 10–15 mm. pressure. On recrystallization from boiling water with norite, the reaction mixture yielded 7.1 g. of pure α-acetaminocinnamic acid, m. p. 193° (corr.) with decomposition.

Benzoylformic Acid with Acetamide

α,α-Diacetaminophenylacetic Acid (V, R = C_6H_5—).—Benzoylformic acid (10 g.) was heated with acetamide (10 g.) for one hour at 110–115° under 10 mm. pressure. The initially clear melt solidified during the course of heating. Crude α,α-diacetaminophenylacetic acid (9.3 g.) remained after the reaction mixture was thoroughly extracted with ether (see below) and washed with cold alcohol. On recrystallization from 70–80% alcohol α,α-diacetaminophenylacetic acid separated in the form of microscopic crystals, containing one mole of water of crystallization, m. p. 201–202° (uncorr.) with foaming. The material was dried for analysis at 110° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_{12}H_{14}O_4N_2\cdot H_2O$: H_2O, 6.7. Found: H_2O, 6.5. Calcd. for $C_{12}H_{14}O_4N_2$: C, 57.6; H, 5.6; N, 11.2. Found: C, 57.4; H, 5.6; N, 11.2.

On boiling with normal hydrochloric acid, α,α-diacetaminophenylacetic acid yielded benzoylformic acid, identified as the 2,4-dinitrophenylhydrazone, m. p. 196° (uncorr.) with decomposition, and showing no depression when mixed with an authentic specimen.[14]

α-Benzoylaminophenylacetic Acid (VII, R = C_6H_5—).—The ether-soluble fraction of the above reaction product of benzoylformic acid and acetamide, on recrystallization from hot water, gave 220 mg. of α-benzoylaminophenylacetic acid, m. p. 175° (corr.) showing no depression when mixed with an authentic sample.[15]

Benzylidene Diacetamide (VI, R = C_6H_5—).—During the reaction of benzoylformic acid with acetamide variable amounts of benzylidene diacetamide were formed. This substance was separated from the diacetaminophenylacetic acid by its ready solubility in hot absolute alcohol, from which it crystallized in the form of long needles, m. p. 250° (uncorr.) with decomposition.

(12) Dakin, *Biochem. J.*, **11**, 79 (1917).

(13) Phenylpyruvic acid can be prepared conveniently in yields of 90–96% of the theoretical by boiling α-acetaminocinnamic acid for three hours with 20 parts of normal hydrochloric acid.

(14) Corson, Sanborn, and Van Ess, THIS JOURNAL, **52**, 1623 (1930).

(15) Kossel, *Ber.*, **24**, 4145 (1891).

Anal. Calcd. for $C_{11}H_{14}O_2N_2$: N, 13.6. Found: N, 13.7.

When α,α-diacetaminophenylacetic acid was heated with acetamide under the above conditions for as long as five hours, no evidence of decarboxylation could be obtained, and only the starting material could be isolated from the reaction mixture.

Summary

1. The condensation of the following α-keto acids with acetamide has been studied: pyruvic, phenylpyruvic, α-ketoglutaric, and benzoylformic acids.

2. The condensation takes place in the following steps: (a) addition of acetamide to the carbonyl group of the keto acid with the formation of an α-acetamino-α-hydroxy acid, and (b) replacement of the hydroxyl group by a second acetamino group, either directly or by dehydration followed by addition of acetamide to the unsaturated intermediate.

3. Phenylpyruvic acid forms only α-acetaminocinnamic acid, and the latter does not add acetamide to its double bond under the experimental conditions chosen.

4. The opening of a lactone ring by addition of acetamide was observed with the lactone of α-acetamino-α-hydroxyglutaric acid.

5. In the condensation of benzoylformic acid with acetamide a secondary reaction analogous to the Erlenmeyer–de Jong reaction was observed. A mechanism is suggested to explain the formation of α-acylamino acids from α-keto acids by this reaction.

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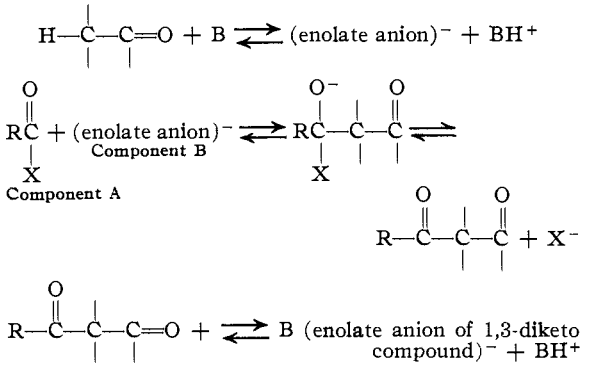
Condensations Brought about by Bases. III. The General Course of the Claisen Type of Condensation

BY CHARLES R. HAUSER

The equations proposed recently[1] for the Claisen condensation of ethyl esters may be generalized to include other carbon–carbon condensations in which a metallic enolate condenses with a compound of the general type RCOX, where X is an atom or group that is removed as an anion during the reaction. The general course for this type of condensation may be represented by the following ionic equations, in which B represents a base.



The first equation represents an acid–base reaction in which a new acid and a new base (the enolate anion) are formed. The second equation represents a carbon–carbon condensation between the enolate anion, designated component B, and a compound of the type RCOX, which is designated component A.[2] This reaction gives an intermediate organic anion, which releases X as anion, forming the 1,3-diketo compound. The last equation represents another acid–base reaction in which the 1,3-diketo compound is converted into its enolate anion. This reaction can occur, of course, only if the 1,3-diketo compound contains an enolizable hydrogen, and if a sufficiently strong base is present.

A number of apparently diverse condensations, as, for example, the condensation of two molecules of an ethyl ester in the presence of a base, and the condensation of a molecule of acetoacetic ester (as enolate) with a molecule of an acid chloride are represented by these general equations. It is proposed that any reaction that is represented by the above equations be classified as a Claisen type of condensation.

While there is little doubt that the general mechanism of the Claisen type of condensation involves the formation of a metallic enolate as an active intermediate, the detailed mechanism by which this enolate condenses with a compound

(1) (a) Hauser and Renfrow, THIS JOURNAL, **59**, 1823 (1937); (b) Renfrow and Hauser, *ibid.*, **60**, 463 (1938).

(2) In this connection see Arndt and Eistert, *Ber.*, **69**, 2383 (1936).