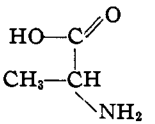
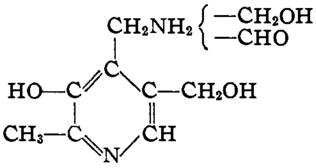
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE TULANE UNIVERSITY OF LOUISIANA]

The Condensation of Pyruvic Acid and Formamide

BY WILLIAM SHIVE[1] AND GWYN WHITE SHIVE

Since *d*(−)-alanine (I) has been postulated to be a precursor for the natural synthesis of vitamin B_6[2,3] (II), the preparation of α-formiminopropionic acid (III) was desirable in order to study its biotic properties. A study of the physical and chemical properties of the compound was also desirable in connection with the ozonolysis of 2-picoline which has been found to produce a substance that is hydrolyzed in dilute sulfuric acid to form pyruvic acid.[4] This substance is likely α-formiminopropionic acid (III) or its tautomer.


(I)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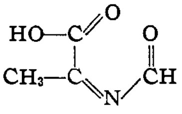

(III)

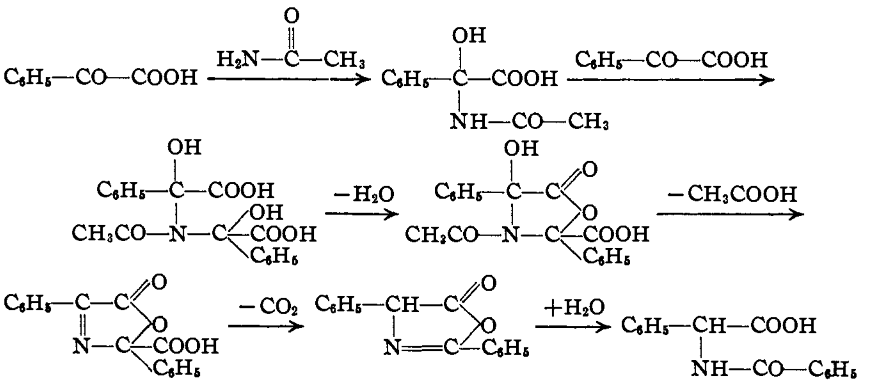
The condensation of acetamide with pyruvic acid has been shown to give a variety of products, α-acetaminoacrylic acid,[5] α,α-diacetaminopropionic acid[5] and α-hydroxy-α-acetaminopropionic acid with two molecules of acetamide in molecular combination.[6] N-Acetylalanine has not been reported as a product of this condensation.

Herbst,[6] reporting the formation of α-acetamino-α-hydroxypropionic acid as the molecular complex, stated that it was not found possible to obtain an analogous complex by the interaction of pyruvic acid with formamide.

However, results of the present investigation indicate that equal molecular proportions of formamide and pyruvic acid react rapidly at room temperature with evolution of heat. The reaction mixture soon begins to crystallize and will solidify totally to give the theoretical yield of crude α-hydroxy-α-formaminopropionic acid.

The hydroxyformaminopropionic acid is hygroscopic and unstable. On standing in a vacuum desiccator over anhydrous calcium chloride for a week or more; the melting point of the material rises and upon recrystallization from ethyl acetate, a good yield of α-formiminopropionic acid or its tautomer can be obtained. If the hydroxy compound is allowed to remain in a closed container, it liquefies after several days, and after standing for several weeks develops a pressure in the container. From the liquid material, *dl*-N-acetylalanine may be isolated. Heating the hydroxy compound to 100° under 5 mm. pressure leaves a residue from which *dl*-N-acetylalanine may be isolated. Heating the hydroxy compound with excess pyruvic acid at 90 to 100° produces the same result whereas a faster gas evolution from the mixture can be obtained by the addition of one molecular equivalent of water.

The mechanism proposed by Shemin and Herbst[7] for the same type of reaction, that of benzoylformic acid with acetamide to give α-benzoylaminophenylacetic acid, postulates the loss of water before decarboxylation which is followed by hydrolysis.



This mechanism does not account for the increased rate of carbon dioxide evolution observed in the reaction of formamide and pyruvic acid on addition of water.

α-Formiminopropionic acid does not evolve carbon dioxide on heating to 90° with pyruvic acid but reacts very rapidly on addition of one molecular proportion of water, this rate exceeding that of hydroxyformaminopropionic acid with pyruvic acid alone or even with water added. The following mechanism is proposed for this reaction.

The interaction of an imino acid with pyruvic acid has been proposed as a part of the biological mechanism of inversion and acetylation of amino acids,[8]

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[2] Snell and Guirard, *Proc. Nat. Acad. Sci.*, **29**, 66 (1943).

[3] Snell, *J. Biol. Chem.*, **158**, 497 (1945).

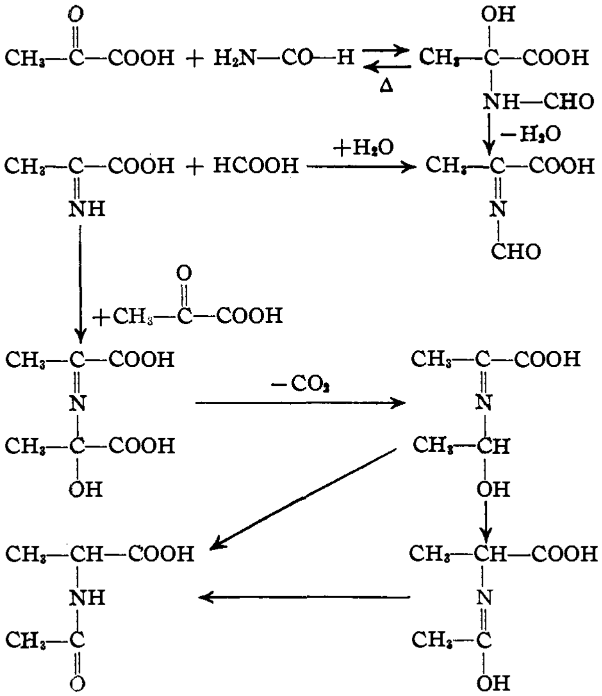
[4] Shive, Ballweber and Ackermann, unpublished work.

[5] Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187 (1930).

[6] Herbst, THIS JOURNAL, **61**, 483 (1939).

[7] Shemin and Herbst, *ibid.*, **60**, 1954 (1938).

[8] du Vigneaud, Colin, Brown, Irish, Schoenheimer and Rittenberg, *J. Biol. Chem.*, **131**, 273 (1939).



and a similar mechanism has been suggested for the formation of *dl*-N-acetylalanine from pyruvic acid and ammonia.[9] The first indicated reaction is likely one of equilibrium because pyruvic acid is found in solutions of α-hydroxy-α-formaminopropionic acid in dilute acetic acid and in aqueous ammonia.

Comparison of α-formiminopropionic acid with *dl*-alanine for its ability to replace vitamin B_6 showed the former to be less active than the latter.[10] Thus, if the biosynthesis of vitamin B_6 from alanine occurs, it does not take place through the formiminopropionic acid. The activity may be due to production of *dl*-N-acetylalanine followed by hydrolysis to *dl*-alanine during incubation of the test organism.

α-Formiminopropionic acid was found to hydrolyze in dilute sulfuric acid solution to give pyruvic acid and possessed chemical properties similar to the substance obtained on ozonolysis of 2-picoline.

Experimental

α-Hydroxy-α-formaminopropionic Acid.—One volume of formamide was thoroughly mixed with two volumes of freshly distilled pyruvic acid while cooling so that the temperature did not rise above 40°. The reaction mixture gradually crystallized until the whole mass solidified giving practically a theoretical yield of α-hydroxy-α-formaminopropionic acid. On recrystallization from chloroform, the product separated as colorless prisms, m. p. 53–55°, which were soluble in ethanol, methanol, water, ether and ethyl acetate; sparingly soluble in benzene; and practically insoluble in petroleum ether. The compound was hygroscopic and decomposed on standing, but did not decolorize bromine water.

Anal. Calcd. for $C_4H_7O_4N$: C, 36.09; H, 5.30; N, 10.52; neut. equiv., 133.1. Found: C, 35.71; H, 5.52; N, 10.61; neut. equiv., 135.7.

α-Formiminopropionic Acid.—The dehydration of α-hydroxy-α-formaminopropionic acid was accomplished by placing the compound in a small vacuum desiccator containing anhydrous calcium chloride, evacuating to less than 1 mm. pressure, and allowing the material to stand in the closed desiccator for a period of two weeks. By warming 2.69 g. of the resulting material with ethyl acetate and then cooling and filtering, 1.10 g. of crude α-formiminopropionic acid, which represents a 41.1% conversion, was obtained. The product on recrystallization from ethyl acetate gave microscopic needles melting with gas evolution at 176.2–176.6° in a Pyrex tube and at 174.0–174.6° in a soft glass tube, both melting point determinations being made simultaneously. On one occasion an apparently pure preparation obtained by the above method melted constantly at 160–161° after several recrystallizations from ethyl acetate, but with subsequent preparations only the higher melting point was observed. These preparations of α-formiminopropionic acid were not hygroscopic but did decolorize bromine water.

Anal. Calcd. for $C_4H_5O_3N$: C, 41.74; H, 4.38; N, 12.17; neut. equiv., 115.1. Found: C, 41.69; H, 4.46; N, 12.28; neut. equiv., 118.7.

By adding a small crystal of iodine to 1.01 g. of the hydroxy compound and allowing the mixture to stand for two days in a small vacuum desiccator which had been evacuated initially to less than 1 mm., there was obtained 0.24 g. of product which was isolated as described above. This yield was slightly higher than obtained without iodine under identical conditions.

Cleavage of the Condensation Products.—To test for pyruvic acid produced by cleavage of the above products, the method of Simon[11] was used. A few mg. of the compound to be tested was dissolved in 3 cc. of concentrated ammonium hydroxide, and 5 drops of a 10% solution of sodium nitroprusside were added. The development of a light green to a deep blue coloration, depending upon the concentrations, occurred with samples of pyruvic acid. With α-formiminopropionic acid, no such coloration developed, but with α-formamino-α-hydroxypropionic acid a green to blue color developed, depending upon concentrations. The former compound on standing at room temperature in 20% sulfuric acid for three hours gave a green test while on standing overnight before testing, a blue-green coloration developed.

Action of Phenylhydrazine.—Addition of 4 drops of phenylhydrazine to 150 mg. of α-formamino-α-hydroxypropionic acid dissolved in 2 cc. of 5% acetic acid caused immediate precipitation of pyruvic acid phenylhydrazone which was recrystallized from dilute ethanol and identified by mixed melting point with an authentic sample. Addition of 4 drops of phenylhydrazine to 150 mg. of the hydroxy compound dissolved in 3 cc. of anhydrous ether did not immediately cause the formation of the hydrazone, but after five minutes the pyruvic acid phenylhydrazone began to crystallize and after recrystallization from dilute ethanol was identified by mixed melting point determination with an authentic sample. Pyruvic acid treated under identical conditions began forming the hydrazone almost immediately.

***dl*-N-Acetylalanine.**—In a distilling apparatus, 1.21 g. of α-hydroxy-α-formaminopropionic acid was heated at 100° under 8 mm. pressure for six hours. On addition of the distillate to ammonium hydroxide and sodium nitroprusside, a deep blue coloration was formed. The residue was dissolved in hot ethyl acetate, cooled and filtered to obtain 0.133 g. of product which melted at 137–138° after recrystallization from ethyl acetate. A mixed melting point with an authentic sample of *dl*-N-acetylalanine prepared by the action of acetic anhydride on *dl*-alanine in acetic acid solution showed no depression.

Anal. Calcd. for $C_5H_9O_3N$: C, 45.79; H, 6.92. Found: C, 45.73; H, 7.06.

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(9) DeJong, *Rec. trav. chim.*, **19**, 259 (1900).

(10) Snell, unpublished work.

(11) Simon, *Compt. rend.*, **125**, 534 (1897).

A sample of α-formamino-α-hydroxypropionic acid was tightly stoppered in a sample bottle and allowed to stand two months. The material liquefied after several days and developed considerable pressure which was released occasionally. On dissolving the material in hot ethyl acetate and cooling, *dl*-N-acetylalanine, which was identified by mixed melting point determination, was obtained.

Heating 0.4 cc. (0.05 g.) of pyruvic acid with 0.1 cc. (0.11 g.) of formamide for two hours at 100° under atmospheric pressure, warming the reaction mixture with ethyl acetate, cooling and filtering gave 0.156 g. (49%) of *dl*-N-acetylalanine.

Rate of Formation of *dl*-N-Acetylalanine.—Heating 0.4 cc. of pyruvic acid with 0.1 cc. of formamide at 100° and measuring the volume of gas evolved gave the values in Table I under the listed conditions.

TABLE I

Vol. gas evolved at 30° (760 mm.), cc.	Time, min., with no added material Run 1	Run 2	Time, min., 0.05 cc. of dioxane added	Time, min., 0.05 cc. of water added
10	7:15	7:45	7:30	2:45
20	16:15	16:45	17:20	6:15
30	26:15	27:05	27:45	9:15
40	41:45	42:30	40:30	17:40

Heating 118.0 mg. of α-formiminopropionic acid with 88 mg. of pyruvic acid under the same conditions gave no appreciable gas evolution, whereas addition of 18 mg. of water followed by heating at 100° gave 10 cc. of gas in 4:15 minutes and 20 cc. in 11:10 minutes.

Attempted Isolation of Intermediates.—Addition of four volumes of pyruvic acid to one volume of formamide resulted in the slow formation of crystals. This mixture was warmed to 50° for several minutes and allowed to stand overnight. After warming one portion of this material slowly until the temperature at which gas evolution just began (80–90°), and then cooling, crystallization from chloroform gave a small amount of material melting at 173–174°. A mixed melting point with α-formiminopropionic acid showed no depression. A small portion of the original mixture was dissolved in chloroform and cooled to obtain a sample of material melting at 50–52°. A mixed melting point with α-formamino-α-hydroxypropionic acid showed no depression.

Summary

1. Condensation of pyruvic acid with formamide has been found to form α-hydroxy-α-formaminopropionic acid at low temperatures.

2. α-Hydroxy-α-formaminopropionic acid has been shown to be unstable, slowly decomposing at room temperature under an anhydrous atmosphere to form α-formiminopropionic acid or its tautomer; however, it has also been found that if the hydroxy compound is allowed to stand in a closed container or is heated, *dl*-N-acetylalanine is formed.

3. Some physical and chemical properties of these compounds have been reported; and a few proposals concerning the mechanisms of their formation have been made.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF CALIFORNIA, LOS ANGELES]

The Role of Neighboring Groups in Replacement Reactions. VIII. The Reaction of Stilbene Dichlorides with Silver Acetate

BY S. WINSTEIN AND DEXTER SEYMOUR

We are prompted by the work of Reulos[1] on the steric result of some reactions of stilbene halohydrins to report at this time the results of some related work which has remained interrupted by the war since 1942.

In connection with one phase of our work on the participation of neighboring groups in replacement reactions,[2] we treated the stilbene dichlorides I and II with silver acetate in acetic acid and scrutinized the products as to configuration. The necessary assignment of configurations to the stilbene dichlorides is certain from the synthesis of both optically active stilbene dichlorides by Weissberger and Bach.[3] Also, the configurations of hydrobenzoin (*meso*) and isohydrobenzoin (*dl*) VII, the esters of which are the products derived from the stilbene dichlorides, are clear from similar work by Read, Campbell and Barker.[4]

We found the *meso*-dichloride I in dry acetic acid gave rise to nearly pure *meso*-diacetate IV, while in moist acetic acid containing more than the stoichiometric amount of water it yielded nearly pure *dl*-glycol VII after saponification of the ester product, a process which we know does not affect configuration. We estimated that the products were 93% *meso* and 92% *dl*, respectively. The *dl*-dichloride II gave rise to quite similar products, which, however, appeared to be less pure stereochemically. We estimated the diacetate produced in dry acetic acid was 77% *meso* and the glycol obtained from saponification of the product from the treatment in moist acetic acid was 86% *dl*.

Zincke[5] had previously studied the reaction of the stilbene dichlorides with silver acetate in glacial acetic acid and with silver benzoate in xylene and obtained what, in his time, were very puzzling results. The two dihalides gave rise to substantially similar products, which were, however, largely *dl* from the first reaction and largely *meso* from the second. We know now from our pre-

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[1] (a) Reulos, *Compt. rend.*, **216**, 774 (1943); (b) Reulos and Le Tellier, *ibid.*, **217**, 698 (1943) (Reprint received February 2, 1945); *C. A.*, **39**, 926 (1945).

[2] For preceding paper in this series, Winstein and Henderson, THIS JOURNAL, **65**, 2196 (1943).

[3] Weissberger and Bach, *Ber.*, **64B**, 1095 (1931).

[4] Read, Campbell and Barker, *J. Chem. Soc.*, 2305 (1929).

[5] Zincke, *Ann.*, **198**, 115 (1879).