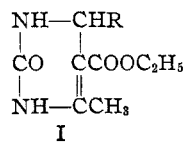
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

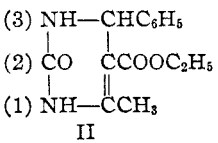
Researches on Pyrimidines. CXXXVI. The Mechanism of Formation of Tetrahydropyrimidines by the Biginelli Reaction[1]

BY KARL FOLKERS[2] AND TREAT B. JOHNSON

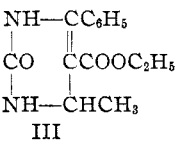
The reaction of numerous aldehydes with urea and a β-keto ester to give a tetrahydropyrimidine was discovered by Biginelli.[3] Structure I, as formulated by Biginelli, may be used to represent these tetrahydropyrimidines, in which R is the grouping joined to —CHO of the particular aryl, alkyl or arylalkyl aldehyde employed in conjunction with urea and ethyl acetoacetate. Accordingly, when benzaldehyde was used, Biginelli obtained a pyrimidine which he considered to be represented by structure II, namely, 2-keto-4-phenyl-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine, and he studied this member of his series in greatest detail. In their short study of this reaction, Biginelli's structure II was apparently accepted by Hinkel and Hey;[4] and in a recent extended study[5] of the condensation, no contradictory constitutional evidence was found.



I



II

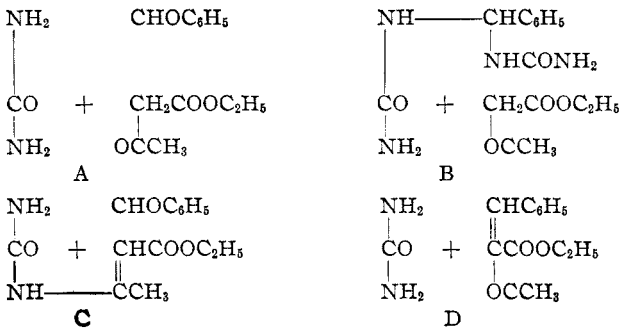


III

Since a conception of mechanism which was based on experiments designed for the purpose was greatly needed, the authors have studied further the formation of pyrimidine II in an effort to obtain mechanism formations which would be of value in their further application of the condensation. The data obtained and their interpretations are summarized in this paper. As a basis, the Biginelli pyrimidine formula, II, was accepted. Evidence in favor of it with regard to the position of the double bond was the preparation of the isomeric pyrimidine, III, and the hydrogenation of pyrimidine III[6] and pyrimidine II[7] to the same 4-cyclohexylhexahydropyrimidine derivative, and then the saponification of this reduced ester to the 5-pyrimidine carboxylic acid. Very recently, Bergmann and Johnson[8] have confirmed the pyrimidine structure, I, by a new and different synthesis of 2-keto-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine of which these pyrimidines may be considered 4-substituted derivatives.

(1) Presented, in part, at the Washington Meeting of the American Chemical Society, March, 1933.
(2) E. R. Squibb and Sons Research Fellow in Organic Chemistry.
(3) Biginelli, *Ber.*, **24**, 1317 (1891); *Gazz. chim. ital.*, **23**, 360 (1893).
(4) Hinkel and Hey, *Rec. trav. chim.*, **48**, 1280 (1929).
(5) Folkers, Harwood and Johnson, THIS JOURNAL, **54**, 3751 (1932).
(6) Folkers and Johnson, *ibid.*, **55**, 1140 (1933).
(7) Folkers and Johnson, *ibid.*, **55**, 2886 (1933).
(8) Results that are to be published soon from this Laboratory.

In his original work, Biginelli showed, as his constitutional proof, that pyrimidine II could be obtained experimentally in four different ways. The systems of reactants representing these four methods are: (A) urea, benzaldehyde and ethyl acetoacetate; (B) benzal-bisurea and ethyl acetoacetate; (C) ethyl β-carbamidocrotonate and benzaldehyde; and (D) urea and ethyl α-benzalacetoacetate. Systems B, C and D represented the three primary bimolecular reactions that were possible from system A.



Since it seemed improbable that the three components of system A reacted simultaneously to give the pyrimidine, queries that followed were: which reactants (B, C or D) were formed from A and that lead to pyrimidine II and at what rate and to what extent? Which system by itself leads to the pyrimidine most readily and to the greatest extent? Are the true systems leading to the pyrimidine not system B, C or D, but precursors or derived products of them, etc.?

In conjunction with the reactants, the catalytic effects had also to be considered. It has been well demonstrated before, and by this paper, that this condensation proceeds exceedingly slowly, if at all, unless catalyzed by acid, and it has been indicated[5] that when the pyrimidine was

TABLE I[a]

EFFECT OF CATALYST ON THE RATE OF FORMATION OF PYRIMIDINE II

Catalyst	Amount	Percentage yield[c]
....	.....	(0.38)[b]
Iodine	<0.3 g.	33.1
Iodine	.3 g.	56.1
Sulfuric acid (concd.)	4 drops[d]	67.0
Hydrochloric acid (concd.)	2 drops	53.8
Hydrochloric acid (concd.)	4 drops	67.0
Hydrochloric acid (concd.)	8 drops	74.6

[a] Based on runs of 0.05 mole each of urea and benzaldehyde and 0.075 mole of ethyl acetoacetate dissolved in 20 ml. of abs. ethanol and refluxed for two hours. [b] Obtained by pouring solution into 300 ml. of water and allowing twenty-four hours for crystallization. [c] Yields represent the amount of pyrimidine separating from reacted solution. [d] 5-ml. pipet.

obtained without the use of a definite catalyst, the derived acetic acid was the catalytic factor. Further data on the effect of catalysts on the formation of pyrimidine II are given in Table I. The reacted solution with iodine as catalyst was distinctly acid to litmus, so the acid formed was probably the prime catalytic agent. Quite evidently, within limits, the yield was proportional to the amount of catalyst present. When ten drops of piperidine were used as catalyst, there was no formation of pyrimidine II, but benzaldehyde and the ethyl acetoacetate reacted to give a 39.8% yield of the β_1-form of 1-methyl-2,4-dicarbethoxy-3-phenyl-5-keto-cyclohexanol.[9] Although the chief interests of Table II were the comparative system yields of the pyrimidine, it clearly demonstrated, again for system A and anew for systems B and C, that the yield was proportional to the amount within limits of the catalyst present.

The reaction between citral, urea and ethyl acetoacetate gave two pyrimidines, one of m. p. 150–150.8° and the other of m. p. 109.5–110.5°.[5] On hydrogenation with a platinum catalyst, these isomers each absorbed very easily 2 moles of hydrogen to give the same product, namely, 2-keto-4-(2,5-dimethyl-heptyl)-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine. Therefore, the citral employed contained at least two of the known citral isomers, probably the diastereoisomers, each of which gave a pyrimidine, and since the isomerism was in the side chain and not the pyrimidine nucleus, further study of the isomers was irrelevant.

Experimental

There are given in Table II the yields of pyrimidine II as obtained with varying amounts of catalyst by the four systems of reactants under comparable conditions. These same data are presented in part in the curves of Fig. 1 for better visualization and discussion. The quantities of reactants for the systems were: (A) 0.025 mole each of urea, redistilled benzaldehyde and acid-free ethyl acetoacetate; (AA) 0.05 mole of urea and 0.025 mole each of benzaldehyde and ethyl acetoacetate; (B) 0.025 mole each of benzal-bisurea[10] and ethyl acetoacetate; (C) 0.025 mole each of ethyl β-carbamidocrotonate[11] and benzaldehyde; (D) 0.025 mole each of urea and ethyl α-benzalacetoacetate.[12]

The reactants in each run were dissolved in 35 ml. of absolute ethanol. This amount of solvent was not sufficient in all runs to keep the mixture homogeneous. However, it was not desirable to increase the amount of solvent, even to overcome this criticism. Concentrated sulfuric acid was used as the catalyst because of its low volatility and its low water content. It did not seem necessary to try to eliminate the last traces of water from the initial solution because of the water formed in the actual condensation. Each run was refluxed for two hours and then allowed to stand at 25° for twelve to fifteen hours for crystallization. This period of reaction was arbitrary and affixed so that data on the reaction rates would be obtained. Therefore, the yields as secured with the lower

(9) M. p. 151–152° (corr.). Hantzsch gave m. p. 152–153° [*Ber.*, **18**, 2583 (1885)].

(10) Schiff, *Ann.*, **151**, 192 (1869).

(11) Behrend, *Ann.*, **229**, 5 (1885); Donleavy, unpublished results of this Laboratory. The crude product obtained for the preparation of 6-methyl-uracil was recrystallized from dilute ethanol.

(12) Knoevenagel, *Ber.*, **29**, 172 (1896); **31**, 730; Claisen and Matthews, *ibid.*, **218**, 178 (1883).

TABLE II

COMPARATIVE YIELDS OF PYRIMIDINE II FROM THE SYSTEMS

Catalyst, concd. sulfuric acid, drops	Percentage yields from systems A	AA	B	C	D[d]	Catalyst, concd. sulfuric acid, drops	Percentage yields from systems A	AA	B	C	D[d]
0	0[a]	..	..	0[a]	..	12	66.1	70.7	76.1	69.2	0[c]
1	13.9	21.5	..	40.0	0	16	..	..	..	64.6	..
2	29.2	..	46.1	47.7	..	20	66.1	73.8	79.2	66.1	0
3	40.0	50.8	54.6	53.8	0	30	..	..	78.4	..	..
3	55.4[b]	..	..	..	..	35	..	..	..	..	0
6	53.8	..	71.5	..	..	50	..	..	..	..	0

[a] On pouring the reacted solution into 300 ml. of water and allowing to stand for twenty-four hours, there were no crystals of the pyrimidine present. This testified to the scarcity of water in the absolute ethanol and the lack of free acid in the ethyl acetoacetate. [b] Solution refluxed for seven hours instead of two hours. [c] The yield was also zero when four drops of water were added. [d] Yields of 1.5–3.8% of pure pyrimidine were obtained by pouring the solution into 300 ml. of water, etc.

concentrations of catalyst are those from interrupted reactions. The yields of Table II represent that amount of pyrimidine crystallizing out of the original solution. Actually these are the minimum yields, for there is a small amount remaining in solution. However, the systems are best compared on these yields. The solubility of pyrimidine II in 95% ethanol at 25° is about 0.35 g. per 35 ml. In each case the filtrate and the 5 ml. of wash alcohol were poured into 300 ml. of water to precipitate the last of the pyrimidine with a small amount of gummy material. After fifteen hours this solid was filtered and dried. The percentage of pyrimidine in this material decreased as the amount of catalyst for the run was increased, and varied in quantity as follows: (A) 0.6–1.2 g.; (AA) 0.5–0.9 g.; (B) 0.5–0.9 g.; (C) 0.9–1.0 g.; and for (D) the amount was only 0.1–0.25 g. of very pure pyrimidine. For accuracy, the benzaldehyde and ethyl acetoacetate were measured out from burets, and the ethyl α-benzalacetoacetate was measured from a calibrated tube. The urea was weighed to the hundredths and the benzal-bisurea and ethyl β-carbamidocrotonate to the tenths. The absolute ethanol was measured from pipets, and the drops of sulfuric acid were counted from a full 5-ml. pipet.

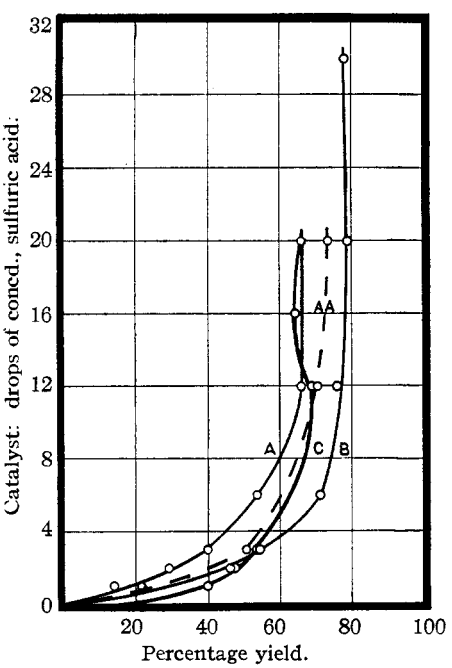


Fig. 1.—Comparative yields and rate of formation of pyrimidine II from the systems.

2-Keto-4-(2,5-dimethylheptyl)-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine.—Three and eight-tenths grams each of the pyrimidine of m. p. 109.5–110.5° and m. p. 150–150.8° (obtained by the interaction of citral, urea and ethyl acetoacetate) were reduced separately by dissolv-

ing in 80 ml. of glacial acetic acid and shaking under three atmospheres hydrogen pressure for four minutes in the presence of 0.2 g. of platinum catalyst. Each pyrimidine absorbed two moles of hydrogen. The solutions were concentrated under diminished pressure and the residues poured into 100 ml. of water. The oils quickly solidified, and, after two crystallizations from aqueous ethanol, melted (and mixed m. p.) at 131–134°.

Anal. Calcd. for $C_{17}H_{30}N_2O_3$: C, 65.75; H, 9.74. Found: (micro) C, 65.68; H, 9.71.

Discussion of Results

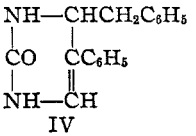
System A.—When only one or two drops of acid were used in the reaction between the three components, there was observed at the starting of refluxing a few white particles, probably benzal-bisurea,[13] which soon disappeared. The formation of this compound was the only indication of mechanism obtained from the three component system.

System B.—The reaction between benzal-bisurea and ethyl acetoacetate in the higher acid concentrations gave the highest yields of pyrimidine of any system. In this system there was a second molecular quantity of urea which was initially present in benzal-bisurea. Probably this second urea molecule functioned on the product side of the reaction. Benzal-bisurea was quite insoluble in hot absolute ethanol, and it was not appreciably affected when an alcohol suspension containing acid was refluxed. However, when ethyl acetoacetate was added to the solution, the insoluble benzal-bisurea disappeared and the pyrimidine precipitated, with a velocity proportional to the amount of acid used. If benzal-bisurea had broken down in some manner to give urea and benzaldehyde, then the increased yields of system B might have been due partially or wholly to a mass effect of this excess urea, for it was known that the yield was increased by the mass effect of excess ethyl acetoacetate. System AA was identical with A except for the use of a second molecular quantity of urea, and the increase in yield of system AA with respect to A was a measure of this mass effect. Since this increase in yield was only a part of the increase of B over A, it was concluded that system B constituted a definite step in the mechanism.

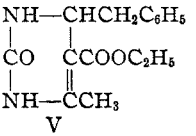
System C.—The yields of pyrimidine from the interaction of ethyl β-carbamidocrotonate and benzaldehyde were much greater than those of system A for low concentration of acid, but were almost identical for the higher concentrations of acid. This observation suggested that the mechanism of pyrimidine formation was the same for systems A and C for the higher concentrations of acid. Such a suggestion implied that at these higher concentrations of acid the ethyl β-carbamidocrotonate underwent hydrolysis into urea and ethyl acetoacetate, or, in other words, system C changed into system A. Evidence for such hydrolysis of ethyl β-carbamidocrotonate was found in the two following facts.

(13) Hinkel and Hey expressed their belief that the first action in this condensation consisted in the formation of benzal-bisurea.

First, it was recently discovered[14] that phenylacetaldehyde would react with urea under the same experimental conditions used for the preparation of pyrimidine II to form a new type of pyrimidine derivative, namely, 2-keto-4-benzyl-5-phenyl-1,2,3,4-tetrahydropyrimidine, IV. When phenyl-



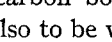
IV



V

acetaldehyde reacted with ethyl β-carbamidocrotonate in the same quality absolute ethanol and under the high concentration of acid used for system C, pyrimidine IV was isolated again in good yield. The urea needed for this condensation could come only by the primary hydrolysis of the ethyl β-carbamidocrotonate.

Second, when 0.025 mole each of benzal-bisurea and ethyl β-carbamidocrotonate reacted for two hours in 35 ml. of ethanol with 20 drops of catalyst, there was obtained an 81.5% yield of pyrimidine II. The formation of pyrimidine II, by system B in this case, showed the presence of ethyl acetoacetate which could come only by the hydrolysis of ethyl β-carbamidocrotonate.

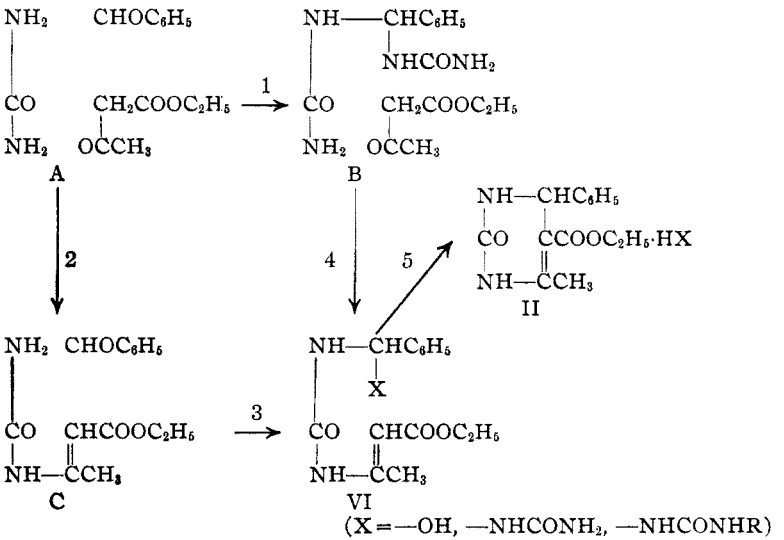
Even though such a small quantity of water was present, probably the hydrolysis of ethyl β-carbamidocrotonate differed, with respect to the reactant and medium, only in degree from the reported hydrolysis of ethyl β-aminocrotonate into the ammonium salt and ethyl acetoacetate by an acidic solution at 25°.[15] The nitrogen–carbon bond of the  group in certain cyclic ureides is known also to be weak in acid solution.

Strong evidence that ethyl β-carbamidocrotonate did react directly with benzaldehyde is found in the much higher yields of pyrimidine II from system C in contrast with A for the low concentration of acid, and the fact that in the above-mentioned reaction between ethyl β-carbamidocrotonate and phenylacetaldehyde there also was isolated the 4-benzylpyrimidine derivative, V.

System D.—It was obvious from the exceedingly small yields of pyrimidine obtained by the interaction of urea and ethyl α-benzalacetoacetate that this system did not function directly in the three component process. This small yield of pyrimidine would require further study before statements regarding its formation would be warranted.

Mechanism Formulations.—From these results it appeared quite definite that pyrimidine II could be formed by the direct interaction of benzal-bisurea and ethyl acetoacetate (system B), and ethyl β-carbamidocrotonate and benzaldehyde (system C), and that these two systems were possibly related according to the following scheme:

(14) Folkers and Johnson, THIS JOURNAL, **55**, 3361 (1933).
(15) Collie, *J. Chem. Soc.*, **71**, 303 (1898).

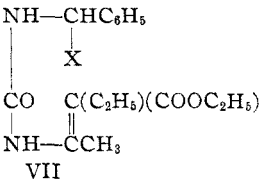


System B, or C, or both, might have first formed from system A. Then either, or both, of these systems, on reacting with the proper remaining component (ethyl acetoacetate for B and benzaldehyde for C) and then undergoing cyclization, formed pyrimidine II. The catalyzed mechanism of any individual step is as yet problematical. For this reason it was desirable to substitute "X" for the radical functioning in structure VI. The extent to which any of these steps proceeded or predominated would be expected to depend upon those factors which influence the relative rates of competitive reactions. There was support for this hypothesis. Step 4, or the formation of the 1,6-linkage, was a union well known in the chemistry of acyclic and cyclic ureides for its ease of formation. The experimental conditions for the preparation of benzal-bisurea indicated the ease of formation of the 3,4-linkage (step 3). Step 5 appeared as the slowest, or the rate-determining, step. Curve B (representing system B) of Fig. 1 is a measure of the combined rates of steps 4 and 5 and curve C of the combined rates of steps 3 and 5; and since steps 4 and 3 are conceived to be fast as compared with 5, then both curves should be an actual measure of step 5. This relationship was borne out by the near quality of yields for 1 to 3 drops of catalyst, as shown by curves B and C. The divergence of curve C for greater amounts of catalyst may be accounted for by the hydrolysis of the ethyl β-carbamidocrotonate. Steps 3 and 5 are somewhat related to the discussion of the condensation of secondary amines with aldehydes and naphthols.[16]

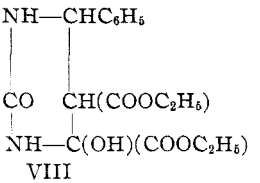
Biginelli and the present authors were unable to isolate a pyrimidine from the system ethyl α-ethylacetoacetate, urea and benzaldehyde. This

(16) Littman and Brode, THIS JOURNAL, **52**, 1655 (1930).

hypothesis would explain such a failure, for the structure VII would have no hydrogen atom available for cyclization.



VII



VIII

By the interaction of ethyl oxalacetate, benzaldehyde and urea, Biginelli obtained the 6-hydroxypyrimidine, VIII, and from this, by dehydration, the tetrahydropyrimidine derivative. This would indicate for step 4 of the hypothesis the addition of the urea molecule to that of the β-keto ester (probably the keto form), followed by dehydration. That the hydroxyl group of the structure $-NHCONHC(OH)(CO_2C_2H_5)$ is stabilized by the negative $-COOC_2H_5$ group is not unexpected. For example, ethylidene urea[17] results from the interaction of acetaldehyde and urea, but if chloral and urea interact, the intermediate product, β-trichloro-α-hydroxyethylurea, $Cl_3CCHOHNHCONH_2$, is isolated in good yields[18] and can then be converted to trichloroethylideneurea.

Summary

From a detailed study of the mechanism of formation of 2-keto-4-phenyl-5-carbethoxy-6-methyl-1,2,3,4-tetrahydropyrimidine from urea, benzaldehyde and ethyl acetoacetate by the Biginelli reaction, it has been concluded that the urea reacted first with benzaldehyde to form benzal-bisurea, or with the ethyl acetoacetate to form ethyl β-carbamidocrotonate. Then, one, or both, of these intermediates further reacted with the proper remaining component (ethyl acetoacetate and benzaldehyde, respectively), and by a final cyclization reaction the pyrimidine was formed. Evidence to support this formulation has been given. Direct interaction between urea and ethyl α-benzalacetoacetate did not appear to function directly in this mechanism.

It has been shown that ethyl β-carbamidocrotonate hydrolyzed with ease into urea and ethyl acetoacetate under the experimental conditions used in this study.

The two previously reported pyrimidine isomers, obtained by the condensation of citral, urea and ethyl acetoacetate, have been shown to have the cause of the isomerism in the heptadienyl side chain, and not the pyrimidine nucleus.

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PUBLISHED SEPTEMBER 5, 1933

(17) Schiff, *Ann.*, **151**, 204 (1869).
(18) Coppen and Titherly, *J. Chem. Soc.*, **105**, 32 (1914).