[CONTRIBUTIONS FROM THE SHEFFIELD LABORATORY OF YALE UNIVERSITY.]

RESEARCHES ON PYRIMIDINES: THIOCYTOSINE-5-CARBOXYLIC ACID.

[FIFTY-SECOND PAPER.]

BY TREAT B. JOHNSON AND JOSEPH A. AMBLER.

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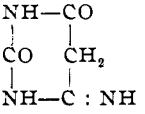
Ethyl cyanoacetate, $CNCH_2COOC_2H_5$, condenses with thiourea and guanidine[1] in the presence of alcohol and sodium ethylate, and cyanoacetic acid with urea in presence of phosphorus oxychloride, giving the pyrimidines—4-iminobarbituric I, 2-thio-4-iminobarbituric II, and 2,4-diiminobarbituric acids III. These iminopyrimidines undergo hydrolysis easily with dilute acids, giving ammonia and the same pyrimidines as are obtained by condensation of diethyl malonate with urea, thiourea, and guanidine, *viz.*: barbituric IV, thiobarbituric V, and 2-iminobarbituric acids VI:[2]



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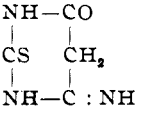

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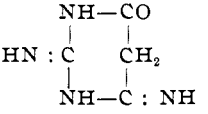

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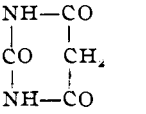

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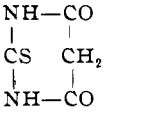

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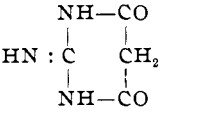


VI.

Notwithstanding the reactivity of these two esters towards urea, thiourea and guanidine, attempts to obtain mercaptopyrimidines by condensing them with the simple pseudothioureas have been unsuccessful. Wheeler[3] examined the behavior of the strongly acid esters—ethyl acetylcyanoacetate[4] VII, triethyl oxalomalonate[5] VIII, and diethyl oxaloacetate[6] IX—towards pseudoethylthiourea and observed, in every case, that the esters did not react to give pyrimidines but formed stable addition products or salts with the pseudourea. On the other hand, Johnson[7] observed that the higher homolog of diethyl oxalacetate or diethyl oxalopropionate,[8] X, condenses smoothly with pseudoethylthiourea, giving 2-ethylmercapto-4-carbethoxy-5-methyl-6-oxypyrimidine:

[1] Traube, *Ber.*, **33**, 1371, 3035; *Ann.*, **331**, 64.

[2] Michael, *J. prakt. Chem.*, [2] **35**, 456; **49**, 36; Traube, *Ber.*, **26**, 2553.

[3] *Am. Chem. J.*, **38**, 358.

[4] Haller, *Ann. chim. phys.*, [6] **17**, 207.

[5] Bouveault, *Bull. soc. chim.*, [3] **19**, 78.

[6] Wislicenus, *Ann.*, **246**, 317.

[7] *J. Biol. Chem.*, **3**, 299.

[8] Wislicenus and Kiesewetter, *Ber.*, **31**, 194.

$CH_3COCH(CN)COOC_2H_5$ VII. $C_2H_5OOCCOCH(COOC_2H_5)_2$ VIII.

$C_2H_5OOCCOCH_2COOC_2H_5$ IX. $C_2H_5OOCOCH(CH_3)COOC_2H_5$ X.

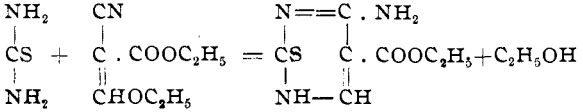
Although diethyl malonate and ethyl cyanoacetate do not condense with pseudothioureas, on the other hand their corresponding ethoxymethylene derivatives, *viz.:* diethyl ethoxymethylenemalonate,[1] XI, and ethyl α-cyano-β-ethoxyacrylate,[2] XII, react smoothly, giving pyrimidines:

$C_2H_5OCH : C(COOC_2H_5)_2$ XI. $C_2H_5OCH : C(CN)COOC_2H_5$ XII.

Wheeler, Johnson and Johns, for example, condensed the malonate, XI, with pseudoethylthiourea and obtained 2-ethylmercapto-5-carbethoxy-6-oxypyrimidine.

Johnson[3] later investigated the action of ethyl α-cyano-β-ethoxyacrylate, XII, on pseudoethylthiourea and made the interesting observation that this ester reacts, in two ways, with the pseudourea, giving a mixture of 2-ethylmercapto-5-cyano-6-oxypyrimidine, XIV, and 2-ethylmercapto-5-carbethoxy-6-aminopyrimidine, XV. The formation of the latter pyrimidine was analogous to that of 2-thio-4-iminobarbituric acid from thiourea and ethyl cyanoacetate.

In continuing our study of the reactivity of ethyl α-cyano-β-ethoxyacrylate, XII, it seemed of interest to examin the behavior of this ester towards thiourea. We now find that it reacts with thiourea in a manner analogous to ethyl cyanoacetate, giving an aminopyrimidine. In fact, it condenses, apparently, in one manner only, giving an excellent yield of 2-thio-4-carbethoxy-6-aminopyrimidine, XIII. We obtained no evidence of the formation of 2-thio-5-cyano-6-oxypyrimidine, XVI. The reaction is expressed by the following equation:



XIII.

2-Thio-5-carbethoxy-6-aminopyrimidine, XIII, is converted quantitatively into thiocytosine-5-carboxylic acid, XVIII, by saponification with alkali. This pyrimidine crystallizes from water with one molecule of water while the corresponding cytosine acid[4] separates from water in an anhydrous condition. Especially interesting was the behavior of the

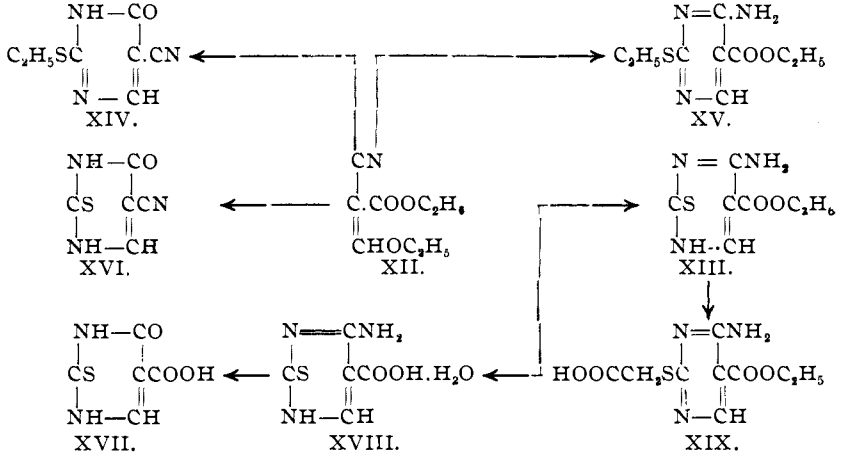
[1] Claisen, *Ann.*, **297**, 75.

[2] De Bollemont, *Compt. rend.*, 128, 1338; 129, 5. *Bull. soc. chim.*, [3] 25, 18, 28, 39.

[3] *Am. Chem. J.*, 42, 506.

[4] Wheeler and Johns, *Am. Chem. J.*, 38, 599.

thiopyrimidine, XVIII, on hydrolysis. Wheeler and Johns[1] observed that the corresponding cytosine derivative was decomposed by heating with 20 per cent. sulfuric acid, giving uracil-5-carboxylic acid. We now find that the sulfur pyrimidine behaves in a similar manner and is converted, quantitatively, under the same conditions, into 2-thio-6-oxypyrimidine-5-carboxylic acid, XVII. It is interesting to note here the firmness with which the sulfur is bound in this pyrimidine. An attempt also to desulfurize 2-thio-5-carbethoxy-6-aminopyrimidine, XIII, by digestion, in aqueous solution, with chloroacetic acid[2] was unsuccessful. They reacted, giving a stable mercaptopyrimidine, *viz.*: 5-carbethoxy-6-aminopyrimidine-2-thioglycolic acid, XIX. These various transformations are represented by the following structural formulas:

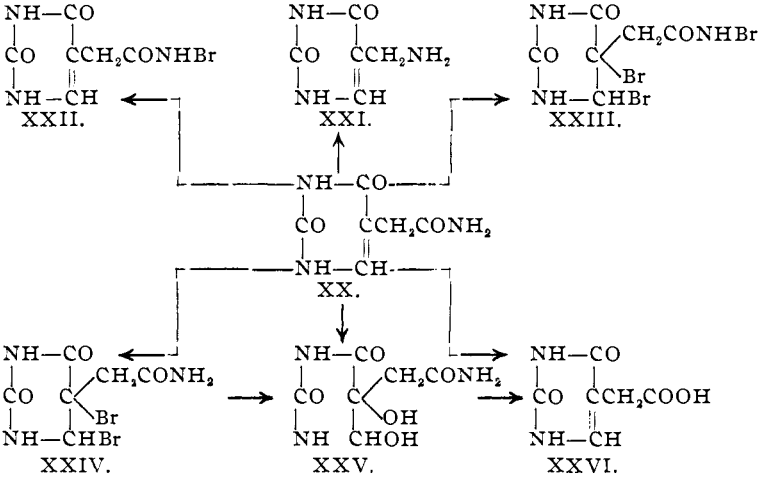


In the course of work on thymine compounds now in progress in this laboratory, it was desirable to determin whether thymylamine, XXI, could be obtained from 2,6-dioxypyrimidine-5-acetamide,[3] XX, by the application of the Hofmann rearrangement. Wheeler and Johns[4] have previously attempted to apply this reaction in the pyrimidine series and used in their experiments 2-ethylmercapto-6-aminopyrimidine-5-carboxamide. They observed, however, that no Hofmann rearrangement took place with this amide by treatment with bromine and alkali, and showed that the bromine did not attack the amide group, as might be expected, but added apparently to the double bond in the 4,5-positions, giving a hexahydropyrimidine. When their addition product was treated with alkali it was converted into cytosine-5-carboxamide, 2-ethylmer-

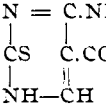
[1] *Loc. cit.*
[2] Wheeler and Liddle, *Am. Chem. J.*, **40**, 547.
[3] Johnson, THIS JOURNAL, **33**, 745, 758.
[4] *Am. Chem. J.*, 40, 233.

capto-6-aminopyrimidine or cytosine-5-carboxylic acid, depending on the conditions employed.

We now find that 2,6-dioxypyrimidine-5-acetamide, XX, reacts with bromine in a manner similar to 2-ethylmercapto-6-aminopyrimidine-5-carboxamide and adds the halogen at the double bond of the pyrimidine ring, giving 2,6 - dioxy - 4,5 - dibromohexahydropyrimidine - 5 - acetamide, XXIV. We obtained no evidence of the formation of bromoamides having the constitutions represented by formulas XXII and XXIII. When the dibromide, XXIV, was warmed gently with alkali it was transformed into a substance having the composition and properties of 2,6-dioxy-4,5-dihydroxyhexahydropyrimidine-5-acetamide, XXV. This transformation, however, was not smooth and the yield of the hexahydro compound was small. The constitution of this dihydroxypyrimidine, XXV, was established by the facts that ammonia was evolved when it was digested with alkali, and that it underwent reduction with hydriodic acid, giving 2,6-dioxypyrimidine-5-acetic acid,[1] XXVI, and ammonium iodide. This pyrimidine acid is not reduced at the double bond by digestion with hydriodic acid.



Experimental Part.

2-Thio-5-carbethoxy-6-aminopyrimidine,  .— This pyrimidine was apparently the only compound formed by condensation of thiourea with ethyl α-cyano-β-ethoxyacrylate.[2] Ten grams of the acrylate (1 mol.) and 6.0 grams of thiourea (1.3 mols.) were dissolved in 50 cc.

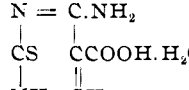
[1] Johnson and Speh, *Am. Chem. J.*, **38**, 602.

[2] *Loc. cit*

of absolute alcohol, containing a molecular proportion of sodium, and the solution digested on the steam bath. The alcohol assumed a red color and the pyrimidine began to separate within a few minutes after heating. After heating about 6 hours to complete the reaction, the alcohol was then cooled and the undissolved pyrimidine separated by filtration. By concentration of the filtrate more of the compound was obtained. This pyrimidine is very difficultly soluble in water and insoluble in cold alcohol. It is somewhat soluble in boiling, glacial acetic acid and crystallizes from this solvent or hot water in needles. It darkens at about 250°, when heated in a capillary tube, and then decomposes from 260–265° according to the rate of heating. The pyrimidine is soluble in cold alkali and is precipitated unaltered by addition of mineral acids. It is a weak base and its salts easily dissociate in aqueous solution. The hydrochloride separates from a ho: solution of 25 per cent. hydrochloric acid in colorless prisms, which decompose at 209–211° with violent effervescence. When dissolved in water the salt is decomposed and the pyrimidine separates in needles melting at 260–265°. The yield of purified pyrimidine was 8.4 grams. Analysis (Kjeldahl):

Calculated for $C_7H_9O_2N_3S$: N, 21.10. Found: 20.77, 21.1.

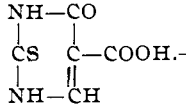
2-Thio-5-carboxyl-6-aminopyrimidine (2-Thiocytosine-5-carboxylic Acid),



$C_5H_5O_2N_3S.H_2O$.—A quantitative yield of this acid was obtained by saponification of its above ethyl ester with potassium hydroxide. It was precipitated from its alkaline solution, by addition of acetic or hydrochloric acids, in the form of colorless prisms, which decomposed from 253–263° with strong effervescence. A mixture of the acid and its ester decomposed below 240°. This pyrimidine is soluble in hydrochloric acid, insoluble in acetic acid and difficultly soluble in boiling water. It deposits from hot water in needles containing one molecule of water of crystallization. The aqueous solution also gives an acid reaction when tested with litmus.

Calculated for $C_5H_5O_2N_3S.H_2O$: H_2O, 9.52; N, 22.22.
Found: H_2O, 9.32; N, 22.3, 21.8.

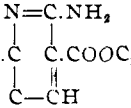
Action of 20 Per cent. Sulfuric Acid on 2-Thio-5-carboxyl-6-aminopyrimidine.

2-Thio-5-carboxyl-6-oxypyrimidine,  —One gram of the hydrous amino acid was dissolved in 40 cc. of 20 per cent. sulfuric acid and the solution boiled for 20 hours. After cooling, the above pyrimidine separated as a heavy, yellow powder. It was purified for

analysis by recrystallization from hot water and separated, on cooling, in granular crystals which melted with decomposition at 246–247°. It gave a strong test for sulfur and the yield of purified material was 0.42 gram. Analysis (Kjeldahl):

Calculated for $C_5H_4O_3N_2S$: N, 16.39.
Found: N, 16.35, 16.47.

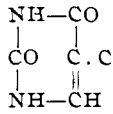
5-Carbethoxy-6-aminopyrimidine-2-thioglycolic Acid,



$HOOC.CH_2S.C(N{=}C.NH_2)C(C.COOC_2H_5){=}CH$.—This pyrimidine was obtained in an attempt to desulfurize 2-thio-5-carbethoxy-6-aminopyrimidine by digestion with chloroacetic acid in aqueous solution. It was difficultly soluble in hot water and separated as a light brown powder, which decomposed at 174–177° with effervescence. Analysis (Kjeldahl):

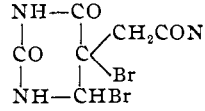
Calculated for $C_7H_7O_4N_3S$: N, 18.34.
Calculated for $C_9H_{11}O_4N_3S$: N, 16.35.
Found: N, 16.9.

2,6-Dioxypyrimidine-5-acetamide,



$NH{-}CO)(CO)(C.CH_2CONH_2){=}NH{-}CH$. — This pyrimidine, which has been described in a previous paper,[1] was prepared for the following experiments by heating the corresponding ethyl 2,6-dioxypyrimidine-5-acetate with aqueous ammonia. An attempt to prepare the amide by heating the ammonium salt of 2,6-dioxypyrimidine-5-acetic acid was unsuccessful.

2,6-Dioxy-4,5-dibromohexahydropyrimidine-5-acetamide,



$NH{-}CO)(CO)(C(CH_2CONH_2)(Br)){-}NH{-}CHBr$. — Eight grams of potassium hydroxide (8 mols.) were dissolved in 50 cc. of cold water and the solution divided into two portions of 37.5 cc. and 12.5 cc., respectively. In the first portion (37.5 cc.) were dissolved 5.6 grams of bromine (2 mols.), and 3.0 grams of finely pulverized 2,6-dioxypyrimidine-5-acetamide were then slowly added while keeping the temperature of the solution below 20°. After complete solution of the amide the second portion of potassium hydroxide (12.5 cc.) was added and the solution finally heated at 85° for half an hour. There was only a slight evolution of ammonia, under these conditions, and a dark red-colored solution was obtained. After cooling and filtering from a small amount of amorphous precipitate the solution was acidified with glacial acetic acid, filtered, and finally evaporated to

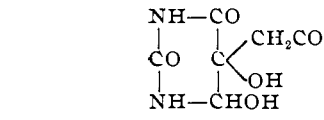
[1] Johnson, *Loc. cit.*

dryness. We obtained a dark colored residue, which was again dissolved in water and the solution decolorized with bone-coal. On evaporation to dryness a yellow crystallin substance, mixed with sodium acetate, was obtained. By extraction of this material, however, with absolute alcohol, the dibromohexahydropyrimidine was obtained as a light yellow powder, which was very soluble in water. It was identified by means of its picrate which crystallized from water in beautiful yellow needles. This compound decomposed when heated above 280° and gave a strong test for bromine. Analysis (Kjeldahl):

Calculated for $C_6H_7O_3N_3Br_2.C_6H_3O_7N_3$: N, 15.10.
Found: 15.4.

When the material above, insoluble in alcohol, was digested with alkali ammonia was evolved and a clear solution obtained. After cooling and acidifying with hydrochloric acid a crystallin substance separated which was identified as 2,6-dioxypyrimidine-5-acetic acid.

2,6-Dioxy-4,5-dihydroxyhexahydropyrimidine-5-acetamide,



. — In this experiment 2.75 grams of 2,6-dioxypyrimidine-5-acetamide were treated with bromine (5.13 grams) as in the previous experiment, except that 10 molecular proportions of potassium hydroxide were used in the reaction instead of eight. After allowing the solution to stand a few minutes and then warming to 85° it was cooled and acidified with hydrochloric acid. The solution was then evaporated to dryness when an orange-colored powder, mixed with potassium chloride, was obtained, which was digested with alcohol. About 1.2 grams of a yellow substance were dissolved by this solvent. It was purified by crystallization from hot water, in which it was quite soluble, and separated on cooling in aggregates of minute lemon-yellow prisms, resembling in crystallin appearance that of uracil. They decomposed at about 270–280°, giving a tar, and then effervesced when heated to about 285–286°. The pyrimidine was soluble in alkali, and ammonia was evolved when the alkaline solution was heated. It did not give a test for halogens. The low analysis for nitrogen was probably due to the presence of a small amount of 2,6-dioxypyrimidine-5-acetic acid as impurity. Analysis (Kjeldahl):

Calculated for $C_5H_7O_2N_3$: N, 29.78.
Calculated for $C_6H_9O_5N_3$: N, 20.6.
Found: N, 19.8.

Reduction of 2,6-Dioxy-4,5-dihydroxyhexahydropyrimidine-5-acetamide to 2,6-Dioxypyrimidine-5-acetic Acid.—About 0.8 gram of the above hexahydropyrimidine was dissolved in 15 cc. of colorless hydriodic acid and

the solution boiled for 2 hours. Iodine was evolved immediately and deposited on the surface of the condensing tube. After cooling and diluting with water, the iodine was reduced by addition of sulphur dioxide solution, when we obtained a clear pale yellow solution. This was finally concentrated, decolorized with charcoal, and cooled. Beautiful, colorless prisms deposited, which did not melt below 300°. The compound was identified as 2,6-dioxypyrimidine-5-acetic acid. The yield was nearly quantitative. A mixture of this and thymine melted below 260°. The compound contained no halogen, was soluble in alkali, and was precipitated from its alkaline solution by addition of acids. When the above mother liquor (HI solution) was digested with an excess of sodium hydroxide, ammonia was evolved, showing the presence of ammonium iodide in the solution. Analysis (Kjeldahl):

Calculated for $C_6H_6O_4N_2$:	N, 16.47.
Found:	N, 16.44.

2,6-Dioxypyrimidine is not reduced at the double bond in the 4,5-positions by the action of hydriodic acid. One-half a gram of the acid was recovered unaltered after digestion for 3 hours. There was no coloration of the acid, due to the formation of any free iodine. This stability of the unsaturated ring, in presence of hydriodic acid, is remarkable.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

METHYL PHENYLIMINOMALONATE AND ITS REACTIONS.

BY RICHARD SYDNEY CURTISS AND F. GRACE C. SPENCER.

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We have for some time sought a method of making the above-named compound, $C_6H_5N=C(CO_2R)_2$, but without avail. Considered as a dicarboxyl addition product of phenyl isocyanide, $C_6H_5N=C$, or as an analog of phenyl isocyanate, $C_6H_5N=C=O$, containing the negative carbomethoxyl groups in place of oxygen, one might predict its chemical properties and behavior with some degree of accuracy. As Nef[1] and others have so well shown in the case of isocyanides and isocyanates, they possess a great reactivity on the N=C double bond—adding on at this point many compounds having easily dissociable hydrogen atoms. In such cases of addition with isocyanides, RN=C, in which the carbon is bivalent, it is the higher valences of the carbon atom which show the greatest reactivity. They first become saturated, yielding compounds of the isocyanate type, $RN=C=O$ and $RN=C\langle^{H}_{X}$; further addition takes place on the N=C double bond; for instance with ammonia; the phenyl

[1] *Ann.*, **270**, 268–335; **280**, 291–342; **287**, 265–359; and other papers.