stance were present. The soap was filtered off, the filtrate evaporated and the residue purified by a number of recrystallizations from absolute ether and from aqueous alcohol. There was thus obtained a few tenths of a gram of a substance which separated from alcohol as a voluminous precipitate resembling precipitated aluminum hydroxide. It gave a strong qualitative test for nitrogen and a rather weak one for bromine, but its ether solution did not yield a precipitate with alcoholic platinic chloride or with hydrogen chloride. Neither did its alcoholic solution give a precipitate with alcoholic silver nitrate. A Dumas nitrogen determination on a small sample gave 2.64% nitrogen. Another small sample (m. 88–90°, uncorr.) prepared in a similar manner, gave 2.69% nitrogen. The amino diglyceride would give 2.25% nitrogen. It is possible that the products obtained were mixtures, though no evidence for that fact could be obtained by use of the microscope or from the change of character on recrystallization. The analyses agree with the theoretical values for stearylbromoglyceryl-dipropyl amine, but the formation of this does not seem likely.
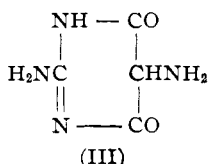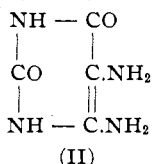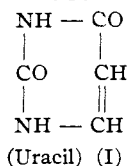
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RESEARCHES ON PYRIMIDINES. LXVIII. THE STRUCTURE OF RITTHAUSEN'S DIVICINE.

BY TREAT B. JOHNSON AND CARL O. JOHNS.

In a recent publication[1] from this laboratory were recorded some new speculations regarding the origin of purines in plants. Attention was called to the most interesting fact that the nitrogenous substance *divicine*, which Ritthausen[2] obtained from *vicine* of vetch seeds by hydrolysis with sulfuric acid, may be a pyrimidine compound. In fact, the formula $C_4H_7O_2N_4$, which was assigned to the base by Ritthausen, differs from that of a diamino-derivative of uracil (II), or its isomer 2,5-diamino-4,6-dioxypyrimidine (III) by only one hydrogen atom. The low per-

centage of hydrogen, and the significant carbon and nitrogen ratio of 1 : 1, exclude the possibility of the base being an acyclic compound. Since pyrimidines of this type have never been observed to occur in nature, it

[1] Johnson, THIS JOURNAL, **36**, 337.
[2] For literature references see paper by Johnson, *Loc. cit.*

was, therefore, of the greatest importance, from a biochemical standpoint, to compare critically the properties of these two pyrimidine bases with those of Ritthausen's divicine.[1] This has now been done, and the description of the interesting relationships, which the work has revealed, forms the subject of this paper.

We wish to call attention first to the analytical results obtained by Ritthausen, which are recorded in this investigator's last paper on divicine.[2] These, together with the theoretical values for carbon, hydrogen and nitrogen in divicine and 4,5-diaminouracil are recorded in Table I, below.

TABLE I.

Calculated for $C_4H_7O_2N_4$.		Calculated for $C_4H_6O_2N_4$.	
C...............	33.5	C...............	33.8
H...............	4.8	H...............	4.22
N...............	39.16	N...............	39.4

VALUES OBTAINED BY RITTHAUSEN.

Percentage of C.	Percentage of H.	Percentage of N.
32.91	4.88	38.82
33.32	4.59	38.77
33.88	4.89	38.95
33.83	4.47	38.92
33.56	4.84	38.23
34.04	4.62	38.88
33.41	4.59	...
33.69	4.74	...
34.4	4.34	...
33.89	4.50	...
33.80	..	...

While the analytical data are of interest, on the other hand, it is apparent from inspection that one cannot safely conclude, from the results, whether divicine is to be represented by formula $C_4H_7O_2N_4$ or $C_4H_6O_2N_4$.[3] The high percentage of nitrogen and the carbon and nitrogen ratios of 1 : 1 afford evidence of a pyrimidine compound. The substance is evidently not ethylene diurea[4] (glycouril, IV) and its stability, in the presence of
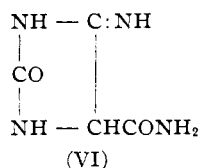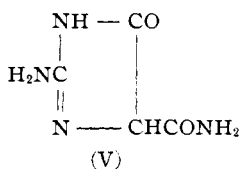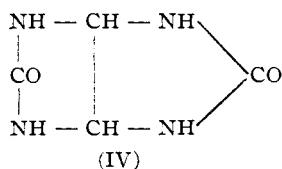
[1] It is of especial interest to note here that Kutscher (Z. physiol. Chem., 38, 176 (1903)) believed he obtained an oxydiaminopyrimidine from yeast nucleic acid, by decomposing this acid at a high temperature with sulfuric acid. This observation has previously received attention from this laboratory and Johnson and Johns (Amer. Chem. J., 34, 554) actually found that 2,5-diamino-6-oxypyrimidine agreed in its chemical behavior, so far as they were able to judge from comparative tests, with Kutscher's unknown base.
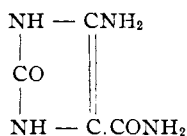
[2] J. prakt. Chem., 59, 482 (1899).

[3] It is important to call attention here to the fact, that this formula of Ritthausen's $C_4H_7O_2N_4$ is impossible unless it be doubled, because it does not conform to the principle that the number of atoms of odd valence in a carbon compound must be an even number.

[4] Ber., 19, 2477.

alkali, excludes the possibility of its being a hydantoin derivative containing an acid amide grouping as represented by formulas V, and VI.

$$\begin{array}{cccc}
NH - CH - NH & & & NH - CO \\
| & | & \diagdown & | & | \\
CO & | & \diagup CO & H_2NC & | \\
| & | & & \| & | \\
NH - CH - NH & & & N --- CHCONH_2 \\
(IV) & & & (V)
\end{array}$$

$$\begin{array}{cccc}
NH - C:NH & & & NH - CNH_2 \\
| & | & & | & \| \\
CO & | & or & CO & \| \\
| & | & & | & \| \\
NH - CHCONH_2 & & & NH - C.CONH_2 \\
(VI) & & &
\end{array}$$

Ritthausen has also summarized, in his last paper on divicine,[1] further results of his work on this base and gives a series of precipitation and color reactions which are characteristic of divicine. These tests are recorded verbatim in Table II, below. Some of these tests are unique and undoubtedly specific for certain types of compounds.

We have now applied all these tests of Ritthausen's with both the 2,5-diamino- and the 4,5-diaminopyrimidines (III) and (II), respectively, and have made the interesting observation that 4,5-diaminouracil (II) responds positively to every one. In fact, the agreement in chemical behavior is so perfect, that one would actually be justified in concluding that this pyrimidine and divicine are identical. On the other hand, as will be observed by inspection of Table II, the isomeric 2,5-diamino-4,6-dioxy-pyrimidine (II), failed to respond to all of Ritthausen's tests. This base is also far less soluble in water than divicine. Furthermore, it is so susceptible to oxidation that it is practically impossible to isolate it in a pure condition. It is of interest to note, in this connection, that both of these pyrimidines were isolated by Traube[2] in the form of their stable sulfates. He writes as follows regarding the 2,5-diaminopyrimidine (III): "Der Amidokörper bildet einen gelblichen, pulverigen, in Wasser kaum löslichen Niederschlag, der sich in feuchtem Zustande an der Luft rasch dunkelroth färbt."

The three most characteristic tests of the ten applied by Ritthausen with divicine (Table II) are 1, 3 and 4. The reduction of silver salts to metallic silver is a characteristic behavior, which is in accord with previous observations, and is a very strong argument, from a chemical standpoint, that Ritthausen was dealing with an orthodiamine $H_2NC = CNH_2$. The observation that both of our pyrimidine bases give the same

[1] *Loc. cit.*
[2] *Ber.*, **26**, 2556; **33**, 1382.

TABLE II.

Ritthausen's Description of Divicine,[1]	Behavior of 2,6 - Dioxy - 4,5 - diaminopyrimidine,	Behavior of 4,6 - Dioxy - 2,5 - diaminopyrimidine,

$C_4H_7O_2N_4(?)$.

| 2,6 - Dioxy - 4,5 - diaminopyrimidine	4,6 - Dioxy - 2,5 - diaminopyrimidine
NH — CO | | CO CNH$_2$ or C$_4$H$_6$O$_2$N$_4$. | || NH — CNH$_2$	NH — CO | | NH$_2$.C CHNH$_2$ or C$_4$H$_6$O$_2$N$_4$. || | N —— CO

Ritthausen's Description of Divicine,[1]

1. Ag—Lösung—wird sofort zu Ag reducirt.

2. HgCl$_2$—giebt augenblicklich HgCl.

3. Phosphor-Molybdänsäure—wird sofort tiefblau.

4. Phosphor-Wolframsäure — sofort schmutzig graublauer Niederschlag bei Zusatz verdünnter Schwefelsäure.

5. Pikrinsäure—reichlicher Niederschlag, gelblich flockig.

6. Kalium-Wismuthjodid—nach längerer Zeit Niederschlag in der Kälte, erwärmt—sofortige rothbraune Fällung.

7. Kalium - Quecksilberjodid — nach längerer Zeit schmutziggrauer Niederschlag.

8. Platinchlorid—wird entfärbt, ohne Niederschlag zu erzeugen.

9. Beim Kochen mit Kalilauge oder Natriumcarbonat wird die Lösung fortdauernd dunkler, endlich rothbraun.

10. Durch Salpetersäure wird die Lösung getrübt und es scheidet sich bald ein krystallinischer Niederschlag ab.

[1] J. prakt. Chem., **59**, 484.
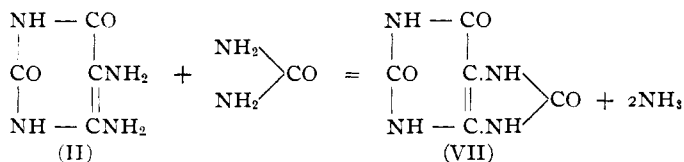
Behavior of 2,6 - Dioxy - 4,5 - diaminopyrimidine,
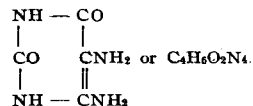
1. Immediate reduction with deposition of silver on adding silver nitrate to an aqueous solution of the pyrimidine.

2. HgCl$_2$ produces a precipitate, which changes to a white powder on heating.

3. Phosphomolybdic acid produced at once a deep blue color.

4. The addition of phosphotungstic acid produced a grayish yellow precipitate.

5. Picric acid gave a yellow precipitate on standing.

6. Potassium-bismuthiodide reacted giving a heavy yellow precipitate.

7. No apparent reaction. On standing a yellowish precipitate finally deposited.

8. Chloroplatinic acid gave no precipitate.

9. On boiling with an aqueous solution of potassium hydroxide an orange color was produced without evolution of ammonia.

10. When nitric acid was added to a strong, aqueous solution of the pyrimidine a turbidity resulted and finally a crystalline precipitate deposited.

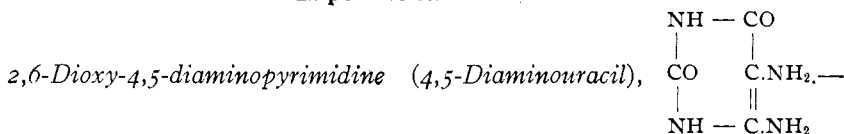**Behavior of 4,6 - Dioxy - 2,5 - diaminopyrimidine,**
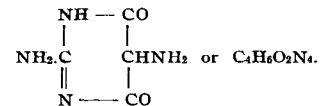
1. Silver nitrate reduced to metallic silver when added to an aqueous solution of the pyrimidine.

2. Mercuric chloride gave a flaky precipitate which collected together on heating.

3. Phosphomolybdic acid produced at once a deep blue color.

4. Phosphotungstic acid gave a characteristic blue color, but no precipitate.

5. Picric acid gave no precipitate.

6. No precipitate on addition of potassium-bismuth iodide solution.

7. No apparent reaction. On standing a yellowish precipitate finally deposited.

8. Chloroplatinic acid gave no precipitate.

9. On boiling with an aqueous solution of potassium hydroxide a violet color is first produced, but finally changes to red on continued heating.

10. No precipitate was obtained on adding nitric acid to an aqueous solution of the pyrimidine.

characteristic blue solutions as Ritthausen's divicine, when combined in aqueous solution with phosphomolybdic acid, is especially significant, because both of the pyrimidines (II), and (III) contain an amino group in the 5-position of the pyrimidine ring. This observation and those of our former co-workers Lewis and Nicolet[1] have aroused a new interest in the practical utility of these acids as color reagents[2] for certain types of organic compounds. That the 5-amino group functionates in these pyrimidine reactions is supported by further evidence which will be discussed in a future paper from this laboratory. It appears probable, therefore, from a consideration of the experimental evidence, that Ritthausen's *divicine* is identical with 4,5-diaminouracil (II). There remained to be developed, however, a reliable test by which such a constitution could be easily established. We are now able to state that this can easily be accomplished by heating divicine with urea. We find that 4,5-diaminouracil (II) is converted quantitatively into uric acid (VII) when heated with urea at 170–180°. The interaction of divicine with urea with formation of this purine (VII) would establish beyond doubt the structure of the base.

$$
\begin{array}{ccc}
\text{NH — CO} & & \text{NH — CO} \\
| \qquad | & \text{NH}_2\!\!\diagdown & | \qquad | \\
\text{CO} \quad \text{CNH}_2 \;+ & \qquad\text{CO} \;=\; & \text{CO} \quad \text{C.NH}\!\!\diagdown \\
| \qquad || & \text{NH}_2\!\!\diagup & | \qquad || \qquad\text{CO} \;+\; 2\text{NH}_3 \\
\text{NH — CNH}_2 & & \text{NH — C.NH}\!\!\diagup \\
\text{(II)} & & \text{(VII)}
\end{array}
$$

Experimental Part.

2,6-Dioxy-4,5-diaminopyrimidine (4,5-Diaminouracil),
$$
\begin{array}{cc}
\text{NH — CO} & \\
| \qquad | & \\
\text{CO} \quad \text{C.NH}_2.— \\
| \qquad || & \\
\text{NH — C.NH}_2 &
\end{array}
$$

This pyrimidine was prepared from its sulfate, which was synthesized according to the directions of Traube.[3] The salt was finely pulverized and then mixed with just enough water to make a thin paste. The sulfuric acid was then exactly neutralized by triturating the paste with the required amount of normal sodium hydroxide solution. The free pyrimidine was obtained as a light gray powder. This was separated by filtration, washed with water to remove sodium sulfate and finally with alcohol and ether. The pyrimidine was then dried at 75° and used for our experiments without further purification. An attempt to purify the base by crystallization from water was not successful because the pyrimidine

[1] *J. Biol. Chem.*, 16, 369.

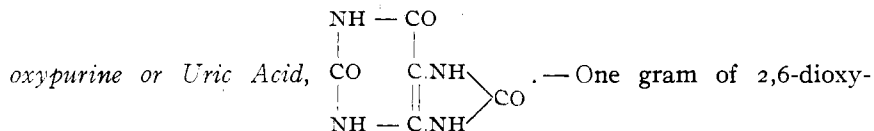[2] Folin and Macallum, *J. Biol. Chem.*, 11, 265; Folin and Denis, *Ibid.*, 12, 239; 14, 95.

[3] *Loc. cit.*

undergoes partial decomposition when warmed with water and separates in an amorphous condition. When sulfuric acid is added to an aqueous solution of the base the corresponding sulfate separates immediately.

$$\begin{array}{ccc}
\text{NH} & — & \text{CO} \\
| & & | \\
\text{H}_2\text{NC} & & \text{CHNH}_2 \\
\| & & | \\
\text{N} & —— & \text{CO}
\end{array}$$
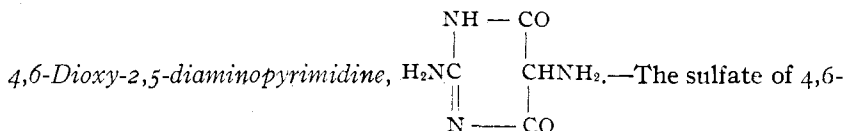
4,6-Dioxy-2,5-diaminopyrimidine, —The sulfate of 4,6-dioxy-2,5-diaminopyrimidine was prepared according to Traube's[1] directions by dissolving the ammonium salt of 2-amino-4,6-dioxy-5-isonitrosopyrimidine in dilute sulfuric acid and then saturating the solution (while warm) with hydrogen sulfide. This treatment was continued until the reduction was complete. The crude sulfate was then separated by filtration and washed with water and alcohol. The free sulfur which was present was then removed by washing with carbon disulfide and the salt finally washed with ether and dried at 75°. The free pyrimidine base was then liberated according to the same procedure as applied in the case of the 4,5-diaminopyrimidine. The pyrimidine was washed in turn with water, alcohol and ether and then dried at 75°. This base is much more unstable than the isomeric 4,5-diaminopyrimidine and if exposed to the air, while moist, it rapidly undergoes oxidation becoming purple. Its aqueous solution rapidly becomes colored, finally assuming a brilliant potassium permanganate color.

The Conversion of 2,6-Dioxy-4,5-diaminopyrimidine into 2,6,8-Trioxypurine or Uric Acid,

$$\begin{array}{ccc}
\text{NH} — \text{CO} & & \\
| \quad | & & \\
\text{CO} \quad \text{C.NH} & \diagdown & \\
| \quad \| & & \text{CO} \\
\text{NH} — \text{C.NH} & \diagup &
\end{array}$$

.—One gram of 2,6-dioxy-4,5-diaminopyrimidine and 1 gram of urea were pulverized together and the dry mixture then heated in an oil bath at 170–180° for one hour. The brown residue which was obtained was then dissolved in hot, dilute sodium hydroxide solution and the solution filtered. On acidifying with dilute hydrochloric acid there was an immediate precipitation of uric acid. This was washed with water and alcohol and dried at 120°. The weight was 1 gram. The formation of the purine is so smooth that extremely small quantities of the pyrimidine can be identified in this manner.

Calculated for $C_5H_4O_3N_4$: N, 33.33%; found: N, 33.19%.

NEW HAVEN, CONN.

[1] *Loc. cit.*