5. Cinnamic esters of the cresol trypan-reds and of the iodocresol trypan reds of definite composition were prepared.

In conclusion the author wishes to express his gratitude to Paul A. Lewis, who suggested the present investigation.

PHILADELPHIA, PA.

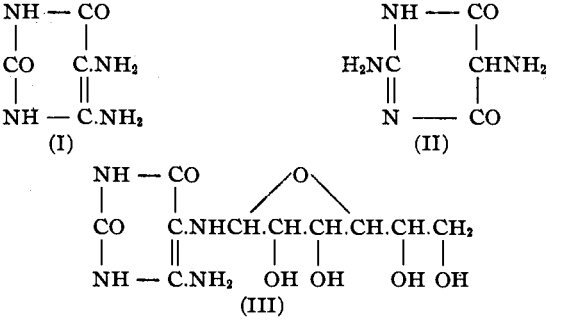
[CONTRIBUTIONS FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY.]

RESEARCHES ON PYRIMIDINES. LXIX. ON A COLOR TEST FOR 5-AMINOPYRIMIDINES.

BY TREAT B. JOHNSON AND CARL O. JOHNS.

Received February 25, 1914.

In the last paper from this laboratory on pyrimidines entitled, "The Structure of Ritthausen's Divicine,"[1] the writers emphasized the significance of the interesting observation made by Ritthausen,[2] that this base interacts with phosphomolybdic acid, in aqueous solution, giving an intense blue color. We contributed the interesting data that 4,5-diaminouracil (I) and 2,5-diamino- 4,6-dioxypyrimidine (II) likewise interact with this reagent, giving apparently the same blue color as *divicine*. Phosphotungstic acid also gave a characteristic blue color with the 2,5-diamino-4,6-dioxypyrimidine (II), but, on the other hand, the isomeric 4,5-diaminouracil interacted with formation of an amorphous green precipitate. This behavior is in accordance with that of divicine towards phosphotungstic acid. In fact, all the known experimental evidence seems to indicate that Ritthausen was actually dealing with this pyrimidine and, consequently, that his *vicine*, which he isolated from vetch seeds, is a glucoside of this interesting base as represented by formula (III).[3] Whether these assumptions are correct will be determined by an examination of the natural products. This work is now in progress.



Attention was also called, in our paper, to the fact that both of the diaminopyrimidines contain an amino group in position 5 of the pyrimidine

[1] Johnson and Johns, THIS JOURNAL, 36, 545 (1914).

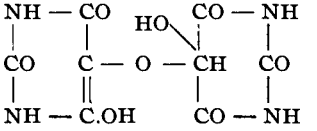
[2] *J. prakt. Chem.*, 59, 482.

[3] The sugar might also be linked to the 4-amino group of the pyrimidine base.

ring, and we stated therein that we should present, in a later paper, new evidence in support of the assumption that it is probably this particular amino group which actually participates in this characteristic color reaction. This work has been continued, and the new data, which we have already obtained, are now recorded in this paper.

Folin and Dennis[1] have recommended as an uric acid reagent a phosphotungstic acid solution, which is prepared by treatment of sodium tungstate in aqueous solution with a definite amount of phosphoric acid. They found that this reagent not only interacts with uric acid in the presence of sodium carbonate, with formation of a blue color, but also gives characteristic blue solutions with monohydric phenols containing an amino group in the benzene ring, and also with di- and polyhydric phenols. As a specific phenol reagent, these investigators recommend a phosphotungstic acid solution which contains 10% of sodium tungstate, 2% of phosphomolybdic acid and 10% of phosphoric acid. This mixture not only gives a blue color with uric acid, but also with all phenols which Folin and Dennis examined, with the exception of *o*-, *m*- and *p*-nitrophenols.[2] In fact, they state that the solution can be used as a substitute for Millon's reagent. Both reagents are applied by mixing them with an aqueous solution of the compound to be tested, and then adding the required amount of alkali to produce the color. Folin and Dennis state that the color is produced in alkaline solutions only, and is not obtained if the alkali is added before the phosphotungstic acid reagent. They recommend sodium carbonate as the alkali to be used and expressly state that potassium carbonate and ammonium hydroxide can not be used, because they give precipitates with the reagents.

These solutions of Folin and Dennis were later used by Funk and Macallum[3] in an investigation of the chemical nature of substances from alcoholic extracts of various foodstuffs. They found, for example, that this solvent dissolved nitrogenous substances, which reacted, in many cases, with the uric acid and phenol reagents giving blue colors. In order to acquire a knowledge of the nature of compounds capable of reacting in this characteristic manner they examined the behavior of the two reagents towards various substances of biochemical interest. Several pyrimidine and purine compounds were included among those tested. Alloxantine (IV), however,



(IV)

[1] *J. Biol. Chem.*, **12**, 239 (1912).

[2] The behavior of this reagent towards new types of phenols will be discussed in a future paper (T. B. J.).

[3] *Biochem. J.*, **7**, 356 (1913).

was the only pyrimidine compound, of those examined, which interacted with formation of a blue color. Thymine, uracil, uridine and cytidine gave no color. The purines xanthine, hypoxanthine, guanine, 3-methyl- and 7-methyluric acids gave blue solutions with the phenol reagent while no color was obtained with the uric acid reagent. Adenine, paraxanthine, theophylline, guanosine and adenosine failed to react with either reagent.

This work of Funk and Macallum's was recently extended by an investigation of Lewis and Nicolet,[1] who examined the behavior of the two reagents towards about seventy different organic substances. These included a variety of hydantoin, pyrimidine and purine compounds. No generalizations, however, can be established from their data. The most striking results obtained by them were the observations, that certain hydantoins react positively, and that the only two sulfur-free pyrimidines, which interacted with formation of a blue color, were 2,4,5-triamino-6-oxypyrimidine and 2,5-diamino-4,6-dioxypyrimidine (II). Of the twelve sulfur-free purines examined not one reacted positively.

Our investigation has so far been confined exclusively to the study of pyrimidine compounds. We have used both the uric acid and the phenol reagents of Folin and Denis, and also a third reagent or 2% phosphomolybdic acid solution. We have not applied the tests, however, as previously recommended and consequently have obtained some interesting and unexpected results. In fact, a systematic application of the three reagents has revealed some very interesting structural relationships. These are apparent by inspection of Table I.

Every compound represented in the table has been tested under the same conditions, *viz.*: in neutral,[2] acid and alkaline solutions. We have used acetic acid and ammonium hydroxide as the acid and alkali reagents, respectively. Twenty-nine different pyrimidines have been tested and also three related acyclic compounds, namely, cyanacetylurea, $NH_2.CO.NH.CO.CH_2.CN$, benzoylaminothioureaacrylic acid, $H_2NCSNHCOCH(NHCOC_6H_5)COOH$ and benzoylaminopseudo-ethylthioureaacrylic acid, $C_2H_5SC(NH_2) = NCH(NHCOC_6H_5)COOH$.

Methods Employed for the Application of the Tests.—Five to ten milligrams of the pyrimidine to be tested are dissolved in about 2–3 cc. of water, heat being applied if necessary. For a neutral test this solution was then cooled to the temperature of ordinary tap water and the respective reagent added directly. In applying the tests in acetic acid solution, the aqueous solution of the pyrimidine was first acidified with the reagent and the pyrimidine reagent then added. For testing in alkaline solution ammonium hydroxide was always used. The aqueous solution of the pyrimidine was made distinctly alkaline with this base and the pyrimidine reagent

[1] *J. Biol. Chem.*, 16, 369 (1913).

[2] By neutral is meant without use of either acetic acid or ammonia.

TABLE I.

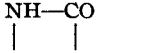
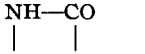
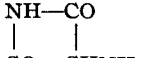
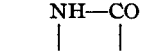
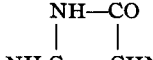
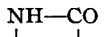
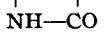
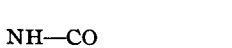
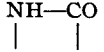
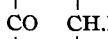
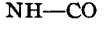
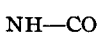
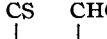
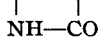
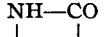
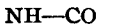
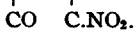
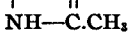
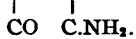
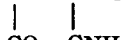
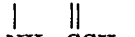
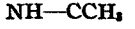
	Pyrimidines.	Phosphotungstic and phosphomolybdic acids. (Phenol reagent.)	Phosphomolybdic acid. (2% solution.)	Phosphotungstic acid. (Uric acid reagent.)	Solution.
		—	—	—	Acid (1)
1.	(barbituric acid).....	—	—	—	Alkaline (2)
		—	—	—	Neutral (3)
		—	—	—	1
2.	.....	+ +	+ +	—	2
		—	—	—	3
		—	—	—	1
3.	(uramil).....	+ + + +	+ + + +	+ + + +	2
		—	—	—	3
		—	—	—	1
4.	.....	+ +	+ +	—	2
		—	—	—	3
		+ + + +	+ + + +	—	1
5.	.....	+ + + +	+ + + +	+ + + +	2
		+ + + +	+ + + +	—	3

TABLE I (*Continued*).

	Pyrimidines.	Phosphotungstic and phosphomolybdic acids. (Phenol reagent.)	Phosphomolybdic acid. (2% solution.)	Phosphotungstic acid. (Uric acid reagent.)	Solution.
		+ + + +	+ + +	—	1
6.		+ + + +	—	+ + + +	2
		+ + + +	+ + +	—	3
		green	green	—	1
7.		+ + + +	—	+ + + +	2
		+ + + +	+ + + +	+ + +	3
		—	—	—	1
8.		+ +	—	—	2
		—	—	—	3
		—	—	—	1
9.		+ +	—	—	2
		—	—	—	3
		+ + + +	+ + + +	—	1
10.		+ + + +	+ +	+ + + +	2
		+ + + +	+ + + +	+ + +	3

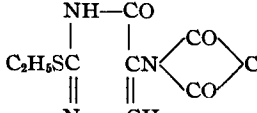
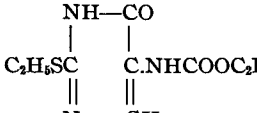
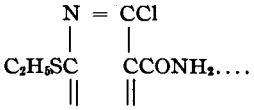
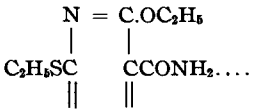
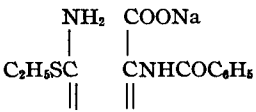
	NH_2 CN	—	—	—	1
11.	CO CH_2	+ + + +	+	—	2
	NH—CO	—	—	—	3
	N = CNH_2	—	—	—	1
12.	CO CH (cytosine)	—	—	—	2
	NH—CH	—	—	—	3
	NH—CO	—	—	—	1
13.	NH_2.C CH (isocytosine)	+ +	—	—	2
	N——CH	—	—	—	3
	NH—CO	—	—	—	1
14.	CO CH	—	—	—	2
	NH—CNH_2	—	—	—	3
	NH—CO	—	—	—	1
15.	CO CNH_2	+ + + +	+ + + +	+ + + +	2
	NH—CH	—	—	—	3
	NH—CO	+ + + +	+ + + +	—	1
16.	CO C.NH_2 (divicine?)	+ + + +	+	+ + + +	2
	NH—C.NH_2	+ + + +	+ + + +	—	3

TABLE I (*Continued*).

	Pyrimidines.	Phosphotungstic and phosphomolybdic acids. (Phenol reagent.)	Phosphomolybdic acid. (2% solution.)	Phosphotungstic acid. (Uric acid reagent.)	Solution.
		—	—	—	1
17.		—	—	—	2
		—	—	—	3
		—	—	—	1
18.		+ + + +	+ + + +	+ + + +	2
		—	—	—	3
		—	—	—	1
19.		+ + + +	+ + + +	+ + + +	2
		—	—	—	3
		—	—	—	1
20.		+ + + +	+ + + +	+ + + +	2
		—	—	—	3
		—	—	—	1
21.		+ + + +	+ + + +	+ + + +	2
		—	—	—	3

		—	—	—	1
22.		—	—	—	2
		—	—	—	3
		—	—	—	1
23.		—	—	—	2
		—	—	—	3
		—	—	—	1
24.		+ +	+ +	—	2
		—	—	—	3
		—	—	—	1
25.		+ +	+ +	—	2
		—	—	—	3
		—	—	—	1
26.		+ +	+ +	—	2
		—	—	—	3
		+ + + +	+ (strong)	—	1
27.		deep green	deep green	+ (strong)	2
		+ + + +	+ + + +	—	3

TABLE I (*Concluded*).

	Pyrimidines.	Phosphotungstic and phosphomolybdic acids. (Phenol reagent.)	Phosphomolybdic acid. (2% solution.)	Phosphotungstic acid. (Uric acid reagent.)	Solution.
		—	—	—	1
28.		+ +	+ +	—	2
		—	—	—	3
		—	—	—	1
29.		+ + +	+ + +	+ +	2
		—	—	—	3
		—	—	—	1
30.		—	+ +	—	2
		—	—	—	3
		—	—	—	1
31.		—	+ +	—	2
		—	—	—	3
		—	—	—	1
32.		+ +	+ +	—	2
		—	—	—	3

	NH_2	—	—	—	1
33.	CO (biuret).....	—	—	—	2
	$NH—CONH_2$	—	—	—	3
	$\left[\begin{matrix} & NH_2 \\ & \vert \\ HN : & C \\ & \vert \\ & NH_2 \end{matrix}\right]$	—	—	—	1
34.	$.H_2CO_3$ (guanidine carbonate).....	—	—	—	2
	$_2$	—	—	—	3

Explanations for Table I.

Intense or deep blue color = + + + + .
Light blue color = + + + .
Very pale blue = + +.
Greenish blue = + .
No formation of a blue color = —.

Literature References for Table I.

(1, 2, 4) Michael, *J. pr. Ch.*, [2] **35**, 456; *Am. Chem. J.*, **9**, 221. (15, 17) Behrend, *Ann.*, **240**, 3; Behrend and Grünwald, *Ann.*, **309**, 256. (5, 16, 23, 14) Traube, *Ber.*, **26**, 2536; **33**, 1382; *Ann.*, **331**, 71. (12, 13, 18, 25, 26, 27, 28, 29, 32) Johnson, *Am. Chem. J.*, **34**, 199; Wheeler and Johnson, *Am. Chem. J.*, **29**, 498; **29**, 501; Johnson and Clapp, *Ibid.*, **32**, 142; Johnson, Johns and Heyl, *Ibid.*, **36**, 166; Johnson and Heyl, *Ibid.*, **38**, 668; Johnson and Johns, *J. Biol. Chem.*, **1**, 314; Johnson and Shepard, THIS JOURNAL, **35**, 1003. (30, 31) Wheeler and Johns, *Am. Chem. J.*, **38**, 597; **40**, 241. (19, 20, 21, 22, 24) Johns, *Am. Chem. J*, **41**, 61; *J. Biol. Chem.*, **1**, 449; **11**, 397; **9**, 164; **16**, 140. (11, 33, 34) Kahlbaum's samples.

added last. Where a blue color was formed it generally developed immediately, before the formation of any precipitate. The colors produced in some cases were extremely intense and very permanent. As stated above 4,5-diaminouracil gives no color with the uric acid reagent in acid solution. When the test is applied according to the above conditions, in the presence of ammonia, an intense blue color is produced.

An examination of Table I will reveal the interesting fact that the 5-amino group is apparently the functionating group when phosphotungstic acid (uric acid reagent) produces a blue color in an ammoniacal solution. In every case examined by us the presence of an amino or imino group in the 5-position of the ring was necessary in order to obtain a blue color by use of the uric acid reagent. If both hydrogens were replaced by other groups or the 5-amino group placed in any other position in the ring or substituted in some side chain this reagent always failed to give a color in alkaline solution. It is interesting to note at this time that alloxantine also gives a blue color with the uric acid reagent in an ammoniacal solution. Whether other 5-hydroxypyrimidines behave in a similar manner will be determined by further work. 5-Hydroxypyrimidines possess many properties in common with the aromatic phenols.

NEW HAVEN, CONN.

NEW PROCESSES FOR THE PREPARATION OF BUTADIENE-1,3 HYDROCARBONS. II. PYROGENETIC DECOMPOSITION OF HYDROXY-COMPOUNDS.

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Part I. Catalytic Dehydration of Glycols.

In the first paper on these processes, it was shown that methyl-2-butylene-oxide-1,2 could be easily and smoothly dehydrated to isoprene. As oxides are anhydrides of the glycols, it occurred to me that the latter substances also might be made to give hydrocarbons of the butadiene-1,3 series. In the literature on the subject of dihydroxyl compounds, we read that α-glycols are easily transformed to aldehydes or ketones, after partial dehydration. This transformation is effected most readily by heating the glycols with dehydrating agents or dilute acids. The reactions[1] are explained on the assumption that the dehydration of the glycols results in the formation of either the corresponding oxide, or the homologue of vinyl alcohol. It is known that oxides are metamerized to aldehydes or ketones by the action of acid substances or high temperatures; while alcohols of the *vinyl* series, being incapable of existence in the free state, are metamerized to the carbonyl substances at the moment of their formation. Among the α-glycols, those containing two tertiary hydroxyl groups, the

[1] Lehrbuch der org. Chemie, Meyer-Jacobson, 2nd ed., Vol. I, Part I, pp. 648–649.