TABLE III

RESULTS ON UNKNOWN MIXTURES

Mixture	Composition, %	Guaiacol carbonate found, %
1	Guaiacol carbonate, 31.5; sucrose, 0.5; starch, 68.0	29.38, 30.64
2	Guaiacol carbonate, 51.5; sucrose, 9.5; acacia, 1.0; starch, 38.0	49.16, 49.76
3	Guaiacol carbonate, 63.7; tragacanth, 0.3; starch, 36.0	63.5, 63.89

A sample of commercial 5-grain guaiacol carbonate tablets was prepared by pulverizing twenty tablets, the average weight of each being 0.3865 g. or 5.96 grains. Three-tenths gram samples were analyzed according to the method for mixtures, with the check results 75.53 and 75.56%. The average content, therefore, was 4.50 grains instead of the declared 5 grains.

Summary

A new method for the estimation of guaiacol carbonate, depending on the conversion of guaiacol carbonate into a monobromo derivative, and a new compound, monobromo guaiacol carbonate, are described.

Acknowledgement is made to L. E. Warren of the Food, Drug and Insecticide Administration for submitting the problem and furnishing the "unknowns."

DENVER, COLORADO

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MISSOURI]

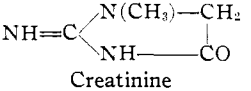
NEW DERIVATIVES OF CREATININE AND DIKETOPIPERAZINE[1,2]

BY L. R. RICHARDSON AND CLAUDE E. WELCH WITH S. CALVERT

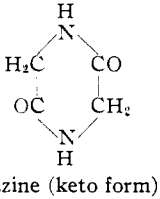
RECEIVED MAY 25, 1929 PUBLISHED OCTOBER 5, 1929

Creatinine, since it is excreted by the animal body, and diketopiperazine, because recent research has indicated that it is an integral part of the protein molecule, are both of great physiological importance. Extensive investigations of the physiological nature of creatinine have been completed but the known chemical reactions of both compounds are comparatively few in number.

A consideration of the formulas of these substances shows that both contain methylene groups



Creatinine



Diketopiperazine (keto form)

[1] An abstract of the theses submitted by L. R. Richardson and Claude E. Welch in partial fulfilment of the requirements for the degree of Master of Arts in Chemistry at the University of Missouri.

[2] Revised paper; original manuscript received August 6, 1928.

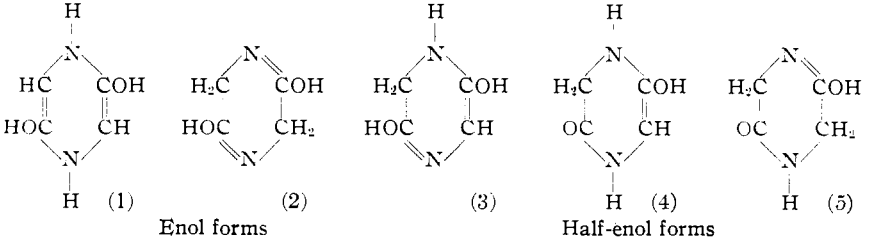
Methylene groups, if sufficiently affected by one or more negative groups such as the carbonyl group, tend to react with aldehydes,[3] substituted amidines and at times with an anilide such as benzylidene aniline. It seemed not unreasonable that similar condensations might be expected with creatinine and diketopiperazine.

Efforts to condense diphenylformamidine with creatinine were successful. We have not yet, however, succeeded in determining the precise formula of the product obtained. This work is being continued.

It was found that aldehydes condense very easily with creatinine upon fusion. Several aromatic aldehydes were condensed in this manner. The derivative that was prepared with benzaldehyde was prepared by Greenwald from creatine[4] and, during the course of these experiments, by Nicolet and Campbell[5] from the dihydrochloride of 5-benzalcreatinine. We have prepared this compound by two different methods: by fusing creatinine with benzaldehyde and with benzylidene aniline. Creatinine was condensed with vanillin and *m*-nitrobenzaldehyde in the same manner.

Diketopiperazine, however, fails to condense with aromatic aldehydes on fusion. It seems that the only suitable method of effecting condensation is an application of the Perkin synthesis first applied to diketopiperazine by Sasaki:[6] several hours' heating of a mixture of the parent compounds in the presence of sodium acetate and acetic anhydride. 3,6-Derivatives of diketopiperazine are prepared in this manner without acetylation of the imino nitrogen. However, as such, the reaction is not general; aliphatic aldehydes do not form simple 3,6-derivatives and, though it is generally applicable to the aromatic series, *o*-hydroxy-substituted aldehydes give poor yields. By this reaction condensations of vanillin, cinnamaldehyde, piperonal, salicylaldehyde, tolualdehyde and *o*-chlorobenzaldehyde with glycine anhydride were effected. Diketopiperazine failed to condense with benzylidene aniline on fusion. Likewise, all attempts to condense diketopiperazine with substituted amidines have been unsuccessful.

Diketopiperazine exists, theoretically, in five enol forms



Enol forms Half-enol forms

[3] Bamberger and Berle, *Ann.*, **273**, 269 ff. (1880); F. Henrich, *Ber.*, **32**, 673 (1899).

[4] Greenwald, THIS JOURNAL, **47**, 1443 (1925).

[5] Nicolet and Campbell, *ibid.*, **50**, 1115 (1928).

[6] Sasaki, *Ber.*, **54**, 163-171 (1921).

The proof of the presence of the hydroxyl groups in enol-diketopiperazine is based mainly upon color tests such as a positive xanthoproteic and a negative picric acid reaction. Definite confirmation of their presence is furnished by the fact that two molecules of α-naphthyl isocyanate condense easily with one of the diketopiperazine.

It is probable that not more than one or two of the various enol forms exists in more than a negligible amount in the equilibrium mixture. Evidence of the structure of the ordinary form of enol-diketopiperazine may be obtained from the chemical conduct of the α-naphthyl isocyanate derivative.

Since two molecules of the isocyanate react with one of diketopiperazine it seems that the more stable form must contain two hydroxyl groups. It is necessary, then, to decide among forms (1), (2) and (3). When the isocyanate derivative is formed it is evident that an equilibrium mixture of the keto and enol forms is no longer present but that a derivative of one or more of these forms will be produced. Since the reactions of these forms with aldehydes will differ because of their structure, an attempt was made to determine the predominant enol form by condensation with *m*-nitrobenzaldehyde.

If the ordinary enol form is (1), the compound should not condense with aldehydes, for no methylene groups are present; (2) should condense with two aldehyde groups while (3) should react with only one.

On condensation the product showed a nitrogen content of 14.73%. The unexpected fact is that this compound is, according to the analysis, virtually identical with that obtained from the condensation of glycine anhydride and *m*-nitrobenzaldehyde. Such a derivative has a nitrogen content of 14.73%. If the enol form had been (2), the condensed product would have contained 11.70% nitrogen, and if it had been (3), 13.21%.

The most probable explanation seems to be that during the course of the reaction the isocyanate derivative first hydrolyzed, forming enol-diketopiperazine, which reverted to the more stable keto form and then condensed with the aldehyde. It is to be expected that (2) would condense with the aldehyde without hydrolysis; on the other hand, before (1) could react with an aldehyde, it would necessarily have to hydrolyze. The analysis showed that nearly complete hydrolysis had taken place. This indicates that (1) is the ordinary form of diketopiperazine.

To confirm this statement another condensation was made with tolualdehyde. The condensation product formed from tolualdehyde and glycine anhydride has a nitrogen content of 8.81%. If the conclusion drawn from the preceding analysis is correct, the product resulting from the reaction of the α-naphthyl isocyanate derivative of diketopiperazine with tolualdehyde should agree quite closely with this figure. The compound formed contained 8.92% of nitrogen. The decomposition point of this product also

checked closely with that of 3,6-*bis*-tolual-2,5-diketopiperazine. Hence the probability of the carbon–carbon double bond is supported by this reaction.

Other reports on methylene condensations which have been in progress in this Laboratory will appear at an early date.

Experimental Part

1. Creatinine

5-Benzalcreatinine.—The method employed in the preparation of this compound is typical of the derivatives of creatinine which were made. Five grams of creatinine and an excess of benzaldehyde were thoroughly mixed and then heated at 150° in a flask attached to an air reflux. The creatinine dissolved in about forty-five minutes, giving a reddish-yellow solution which formed a yellow solid on cooling. Flaky, golden-yellow crystals were obtained by recrystallization from boiling alcohol. The compound was difficultly soluble in boiling alcohol and water, and slightly soluble in ether, benzene and chloroform; m. p. 244°, uncorrected.

Anal. Calcd. for $C_{11}H_{11}ON_3$: N, 20.89. Found: N, 20.76, 20.64.

Fusion of benzylidene aniline and creatinine (equivalent quantities) for an hour at 150° produced the same compound. Aniline was liberated, as was shown by the bleaching powder test.

5-*m*-Nitrobenzalcreatinine.—Five g. of creatinine and an equivalent quantity of *m*-nitrobenzaldehyde were fused for three hours at 150°, and then for two hours more at 180°. The product was purified by extracting first with alcohol and then with water. It is very slightly soluble in each. A 70% yield of the yellow powder was obtained; it decomposes at 288°, uncorrected.

Anal. Calcd. for $C_{11}H_{10}O_3N_4$: N, 22.76; C, 53.75; H, 4.1. Found: N, 22.63; C, 53.85; H, 4.19.

5-(*m*-Methoxy-*p*-hydroxybenzal)-creatinine.—Half an hour's fusion of equivalent quantities of vanillin and creatinine gave a 75% yield of the condensation product. It was purified by extracting first with alcohol and then with water. It was very slightly soluble in each of these solvents and slightly soluble in ether, chloroform and benzene; it melts with decomposition at 267°, uncorrected.

Anal. Calcd. for $C_{12}H_{13}O_3N_3$: N, 17.0; C, 58.3; H, 5.30. Found: N, 16.78; C, 58.04; 58.06; H, 5.43, 5.55.

II. Diketopiperazine

Preparation of Glycyl-glycine Anhydride.—Diketopiperazine was prepared according to the method of Emil Fischer[7] from glycine ethyl ester hydrochloride.

3,6-Bis-(*m*-methoxy-*p*-acetoxybenzal)-2,5-diketopiperazine.—The method employed in the preparation of this derivative is typical of the 3,6-derivatives which were prepared. One and four-tenths grams of glycine anhydride, 4.5 g. of vanillin, 4 g. of sodium acetate and 8 g. of acetic anhydride were thoroughly mixed and heated at 120–130° for eight hours. During this period the liquid solidified into a brown mass of crystals. After cooling the product was washed several times with hot water and then extracted for a short time with alcohol. The bright yellow crystals were recrystallized from boiling acetic acid and, for analysis, dried for three hours at 130°.

A list of the six aldehyde derivatives is given in Table I. They were prepared in a similar manner. All of this series of compounds are soluble

[7] Fischer, *Ber.*, **39**, 2930 (1906).

in boiling acetic acid, but they are insoluble in water, alcohol, ether, benzene and chloroform.

TABLE I

DATA ON ALDEHYDE DERIVATIVES

3,6-Bis-( )-2,5-diketopiperazine	Formula	Diketopiperazine condensed with	Nitrogen, % Calcd.	Found		Brown at °C.	Dec., °C.
m-Methoxy-*p*-acetoxy	$C_{24}H_{22}O_8N_2$	Vanillin	6.00	5.88	5.85	290	310
Cinnamal	$C_{22}H_{18}O_2N_2$	Cinnamaldehyde	8.18	8.00		335	350
Piperonylidene	$C_{20}H_{14}O_6N_2$	Piperonal	7.41	7.29		290	320
m-Acetoxybenzal	$C_{22}H_{18}O_6N_2$	Salicylaldehyde	6.90	6.77	6.90	M. p. 255	
Tolual	$C_{20}H_{18}O_2N_2$	Tolualdehyde	8.81	8.69	8.80	305	320
o-Chlorobenzal	$C_{18}H_{12}O_2N_2Cl_2$	*o*-Chlorobenzaldehyde	7.80	7.82		330	340

α-Naphthyl Isocyanate Derivative of Diketopiperazine.—One gram of thoroughly dried glycine anhydride was finely powdered and covered with 8 g. (excess) of α-naphthyl isocyanate. The mixture was heated in the sulfuric acid bath to 175°. At this temperature it rapidly turned into a white solid. After fifteen minutes it was cooled. The compound was insoluble in ligroin, ether, water, benzene and chloroform; it was very slightly soluble in boiling acetic acid. To remove the excess isocyanate the mass was extracted with ligroin for three hours; two hours' extraction with water removed any unchanged glycine anhydride. The fine white crystals were dried for three hours at 130°. They melt with decomposition at 232°.

Anal. Calcd. for $C_{26}H_{20}O_4N_4$: N, 12.39. Found: N, 12.15, 12.18.

Condensation of the Isocyanate Derivative with *m*-Nitrobenzaldehyde.—Three grams of the isocyanate derivative, 10 g. of *m*-nitrobenzaldehyde, 8 g. of sodium acetate and 20 g. of acetic anhydride were heated at 125°. After eight hours the product was cooled, digested with warm water and boiling alcohol, recrystallized from boiling acetic acid and dried for three hours at 130°.

Anal. Calcd. for $C_{18}H_{12}O_6N_4$: N, 14.73. Found: N, 14.38.

Condensation of the Isocyanate Derivative with *m*-Tolualdehyde.—Five grams of the isocyanate derivative, 2 g. of tolualdehyde, 2 g. of sodium acetate and 4 g. of acetic anhydride formed the condensation product on heating for several hours at 125°. It was digested with water and alcohol and recrystallized from boiling acetic acid; yellow crystals, decomposing at 320°.

Anal. Calcd. for $C_{20}H_{18}O_2N_2$: N, 8.81. Found: N, 8.92.

Summary

1. A method of preparation of condensation products of creatinine with aldehydes and benzylidene aniline has been described.

2. Six new derivatives of diketopiperazine with aldehydes have been prepared.

3. The presence of hydroxyl groups in the enol form of diketopiperazine has been confirmed by the formation of the α-naphthyl isocyanate derivative.

4. The α-naphthyl isocyanate derivative condenses with aldehydes only after hydrolysis. It, therefore, appears that the ordinary enol form of diketopiperazine has double bonds between carbon atoms.

COLUMBIA, MISSOURI

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

PARA-CYMENE STUDIES. XII. 2-PARA-CYMYL-4-SEMICARBAZIDE AND CERTAIN DERIVATIVES

BY ALVIN S. WHEELER AND J. G. PARK[1]

RECEIVED MAY 27, 1929 PUBLISHED OCTOBER 5, 1929

This paper presents a continuation of studies of semicarbazides as ketone reagents.[2] In view of the fact that cymene is a fair priced product, its derivatives are more available than formerly. Ketone reagents such as hydroxylamine, phenylhydrazine and semicarbazide differ in their properties from strongly basic to neutral. This series is extended in the acid direction by using phenylsemicarbazide with its negative phenyl group and finally the substituted phenylsemicarbazides containing bromine or the nitro group. It is desirable to have such a variety for detecting and characterizing ketones since no one is applicable to all cases.

2-*p*-Cymyl-4-semicarbazide, not known hitherto, was prepared by known methods. 2-Amino-*p*-cymene was converted into *p*-cymylurea, a compound first mentioned by Kremers and Demonbreun,[3] who made only a very brief note about it and gave no analysis. We believe their product was impure since our product melts six degrees higher. Three methods of preparation were tested. The first was Davis and Blanchard's method[4] of preparing phenylurea. An aqueous solution of aminocymene hydrochloride and urea was heated. This was unsatisfactory on account of the simultaneous production of di-*p*-cymylurea, a compound not hitherto described. The second method was that of Kelbe and Warth,[5] who prepared *m*-cymylurea. The hydrochloride of aminocymene was heated with potassium cyanate. No dicymylurea was produced but the yield was poor. The method adopted was to heat an acetic acid solution of aminocymene with potassium cyanate. In a similar way Walther and Wlodkowski[6] made *o*-tolylurea. The yield was better and the product readily purified.

[1] This paper is an abstract of a thesis submitted by J. G. Park in partial fulfilment of the requirements for the degree of Master of Arts at the University of North Carolina in June, 1929.

[2] Wheeler and Bost, THIS JOURNAL, **46**, 2813 (1924); Wheeler and Walker, *ibid.*, **47**, 2792 (1925).

[3] Kremers and Demonbreun, *J. Am. Pharm. Assocn.*, **12**, 591 (1923).

[4] Davis and Blanchard, THIS JOURNAL, **45**, 1816 (1923).

[5] Kelbe and Warth, *Ann.*, **221**, 171 (1883).

[6] Walther and Wlodkowski, *J. prakt. Chem.*, **59**, 266 (1899).