CLXVIII. A NEW GUANINE HYDROLYTIC PRODUCT [4-(OR 5-)GUANIDINOGLYOXALINE], AND ITS HYDROLYTIC PRODUCT [4-(OR 5-)CARBAMIDOGLYOXALINE].

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WITH A NOTE ON THE DISSOCIATION, AND ULTRAVIOLET ABSORPTION SPECTRA.

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(Received 23 May 1936.)

THE formulation of the new base obtained from guanine by hydrolysis as 4-(or 5-)guanidinoglyoxaline [Hunter, 1936, 1, 2] is based on the evidence which will now be submitted.

Prima facie its formation as a simple hydrolytic product of guanine through the removal as CO_2 of C in position 6, with simultaneous reduction of the adjacent N and C atoms, seems probable.

That the base retains the glyoxaline ring of the original guanine is indicated by its readiness to couple in sodium carbonate solution with sodium diazo-benzene-*p*-sulphonate. The formation of a blue coupled product is unique among the known glyoxalines but a side-chain of the nature of guanidine might well lead to such a colour.

With the Sakaguchi test the base yields an intense green-blue colour, in contrast to the red typical of monosubstituted guanidines. After oxidation with bromine or potassium permanganate in an acid medium, the solution fails to give a Sakaguchi test but responds positively to Tiegs's test; which is evidence for the appearance of guanidine and the absence of a substituted guanidine. After oxidation with permanganate, about 75 % of the guanidine theoretically derivable from the base has been isolated as picrate, and after oxidation with bromine the guanidine released according to colorimetric estimation with Tiegs's reagent is over 90 % of the theoretical amount.

After oxidation, the diazo-test on the solution is negative, which suggests disruption of the glyoxaline nucleus. Along with guanidine there may readily be detected in solution oxalic acid,¹ ammonia, formic acid and a small proportion of

¹ The appearance of oxalic acid in a solution resulting from permanganate oxidation needs comment. The explanation appears to be that as the oxalic acid is produced the formation of an exceedingly insoluble guanidine oxalate removes it from the sphere of the reaction. This compound was first observed following the addition of slight excess of bromine to a solution of $\text{C}_4\text{H}_7\text{N}_5 \cdot 2\text{HCl}$, but it may also be observed in either a cold or hot acid solution of the base treated with permanganate. Almost 50 % of the weight of the original dihydrochloride may be recovered as guanidine

glycine. The main results of the oxidation appear to be representable as follows:

In an oxidation experiment with standardized bromine water 83% of the oxygen required by the above expression was actually used, and in a similar determination with permanganate in hot solution, about 93%. The deficiency is readily explicable by the appearance of glycine originating in the manner suggested below.

Further evidence in support of the formulation adopted is concerned with certain observations on the alkaline hydrolysis of the base. These comprise the isolation of an intermediate product, apparently the corresponding carbamido-glyoxaline, and secondly the identification of glycine as an end-product of the hydrolysis of both the guanidino- and carbamido-compounds. The apparent significance of glycine as a matter of evidence will first be considered.

Purines, without substituents in the 7, 8 or 9 positions, on drastic hydrolysis with HCl, yield glycine, along with ammonia, CO₂ and sometimes CO and formic acid. Thus Schmidt [1883] showed that xanthine when heated for 6 hours at 180–200° with conc. HCl left glycine, and Krüger [1892] showed its presence in similar hydrolysates from adenine and from hypoxanthine. Its production from guanine under the less drastic conditions reported in the previous paper is thus not unexpected. When the purine carries a methyl substituent in position 7 glycine is replaced in the hydrolysate by sarcosine, as shown in the case of theobromine by Schmidt & Pressler [1883], and in the case of caffeine by Rosengarten & Strecker [1871]. The latter, however, hydrolyzed the caffeine with hot saturated barium hydroxide until ammonia was no longer evolved.

Fargher [1920] isolated glycine in an attempt to prepare 4-(or 5)-aminoglyoxaline by reduction of the corresponding nitro derivative. Fargher supposed the aminoglyoxaline to be unstable and transformed into 4-(or 5)-glyoxalone, which readily disintegrates at the 1 : 2 and 3 : 4 positions with the production

oxalate by the addition of slight excess of bromine and keeping the solution at room temperature for a few hours, for the separation of the small doubly-pointed needle-like crystals. They burn without melting. Boiling water or dilute acid has no apparent effect on the material. A little of the substance suspended in water readily dissolves on addition of a small amount of NaOH. Curiously enough the solid is insoluble in cold 2.5 N NaOH. If the slightly alkaline solution is acidified, the material quickly recrystallizes. If however the alkaline solution is kept for some time at room temperature, or boiled and quickly cooled, and made acid, then no separation occurs. Guanidine is then readily precipitated from the solution by picric acid, oxalic acid by Ca, and in presence of excess H₂SO₄ the solution avidly absorbs permanganate. Determination by this means indicates about 60% oxalic acid in the substance in question. Guanidine is present to the extent of about 40% of the substance according to measurement by the Tiegs method. Nessler's test for ammonia is negative, and indeed the total N of the substance is accounted for by the guanidine content. Micro-analysis of the substance (Dr Stantial) gave: C, 26.82, 26.56; H, 4.18, 4.10; N, 29.82, 29.64%. C₆H₁₂O₇N₆ requires C, 25.70; H, 4.32; N, 30.01%. The values are not very good for C₆H₁₂O₇N₆, representing a complex of equal proportions of guanidine oxalate (CH₅N₃.C₂H₂O₄) and oxalyl guanidine (CH₄N₃.C₂H₂O₃). There is good evidence that the substance contains only guanidine and oxalic acid, but attempts to prepare it from these constituents have so far failed. No substance of a like composition appears to have been described, and the product obviously calls for further study.

of glycine. From 4-nitro-5-methylglyoxaline Fargher similarly obtained α -alanine.

Glyoxaline, and glyoxalines with alkyl substituents or a saturated side-chain, e.g. histidine, are exceedingly resistant to oxidizing or hydrolytic agents. They do not absorb permanganate and may be heated for long periods with strong acid or base without appreciable change.

The stability of the glyoxaline ring is apparently lost in such a substance as 4-(or 5-)aminoglyoxaline, in the substances to be described in this paper, and presumably as it exists in the purine nucleus. The writer has observed that a small amount of urocanic acid treated in cold acid solution with permanganate readily yields a positive ninhydrin test, most likely attributable to glycine; which suggests that the presence of an unsaturated aliphatic side-chain leads to lability of the glyoxaline nucleus.

It thus appears at the outset that the likelihood of obtaining a stable glyoxaline from a structure such as 4-(or 5-)guanidinoglyoxaline is slight. But, on the other hand, from the foregoing discussion, the formation of glycine by hydrolysis with acid or base or by oxidation with permanganate of the substance in question would seem to be strong evidence for the presence in it of the glyoxaline nucleus.

From $\text{Ba}(\text{OH})_2$ hydrolysates of the base, glycine has been isolated and identified as its copper salt and as glycine sulphate. Experiments have not so far been specially designed to obtain a maximum yield of glycine (most probably exhaustive hydrolysis with $\text{Ba}(\text{OH})_2$ or HCl) but about 30% of the theoretical yield has been obtained as copper glycine. Under the conditions no more was to be expected, so that little doubt can be entertained about its quantitative significance.

The mechanism of the hydrolysis of the base has not been fully elucidated. On the analogy of arginine it was expected that baryta hydrolysis would yield urea and 4-(or 5-)aminoglyoxaline. The picrate obtained from the hydrolysate had a much lower m.p. than guanidinoglyoxaline dipicrate, but it was soon discovered to be a mixture of this with the picrate of a different substance. The guanidinoglyoxaline dipicrate was less soluble in hot water than the unknown picrate and a mixture, estimated from this property roughly as two parts of the unknown picrate to one part of the known, was readily obtained, m.p. 185–190°. The further separation was extremely tedious as the two picrates had like solubilities at ordinary temperature, and further fractional crystallization appeared impossible. The mixture, with as large a proportion of the unknown picrate as was readily attainable, was freed of picric acid in the presence of HCl by ether and evaporated to a small volume. On the addition of a suitable amount of absolute ethyl alcohol, rhombic or square-shaped crystals appeared. These were readily distinguishable under the microscope from the needles or spars formed by guanidinoglyoxaline dihydrochloride, which was always present in the filtrate from such crystals. The filtrate was re-treated with picric acid and the procedure repeated. About 0.5 g. of a hydrochloride was thus obtained from about 2.5 g. of guanidinoglyoxaline sulphate. After hydrolysis about 25% of the material is recoverable unchanged, and it appears that part of the compound here sought decomposes further to glycine and ammonia. With the application of less tentative methods a better yield may be attainable.

I have again to thank Dr Stantial, Toronto, for the C and H values in the following analysis of the substance thus prepared. (Found: C, 29.36, 29.72; H, 4.34, 4.23; N, 34.61, 34.42; Cl, 21.80, 21.76%. $\text{C}_4\text{H}_6\text{ON}_4 \cdot \text{HCl}$ requires C, 29.54; H, 4.34; N, 34.46; Cl, 21.80%.)

With the diazo-reagents the substance yields an exquisite red colour which shows only a tinge of blue in presence of slight excess of NaOH. After oxidation with permanganate there is no trace of guanidine according to Tiegs's test. A positive ninhydrin test, almost certainly attributable to glycine, is obtained on hydrolysis of the substance with Ba(OH)₂. The relationship of the substance to the guanidinoglyoxaline would thus appear to be correctly represented by

and on the arginine analogy, the citrulline analogue has been produced.

The changes intermediate between the carbamidoglyoxaline and glycine are still obscure, but may yield to further study. The Ba(OH)₂ hydrolysates from both the guanidino- and carbamido-compounds are characterized by a number of colour tests suggesting the presence of 4-(or 5-)aminoglyoxaline [Fargher & Pyman, 1919], or perhaps of 4-(or 5-)glyoxalone. Of these a strongly marked sodium nitroprusside test would appear to suggest the presence of a linkage of the type HC=C.OH. It appears unlikely that aminoglyoxaline would yield a positive nitroprusside test. Furthermore, the hydrolysate in alkaline solution acquires a blue colour on exposure to air. If a little of the acidified hydrolysate is treated with a drop of NaNO₂ solution, and the mixture added to a NaOH solution of β-naphthol a brown colour is produced. Fargher and Pyman [1919] attribute a similar reaction to diazotization of an NH₂ group on the benzenoid 4- or 5-C atom of the glyoxaline nucleus. The last test which may be mentioned is the production of a grass-green colour with ninhydrin. The solution shows an absorption band at about 6460 Å., and its colour is quite unlike that obtained in the usual amino-acid test. Whether all of the tests here mentioned are attributable to one or more substances can only be decided by further investigation.

A number of colour tests for the two new bases will be described in the experimental section. These are both highly reactive substances, and often respond in such an extraordinary manner that certain of such tests seem to merit detailed consideration. Some of the tests, not already mentioned, have a bearing on the constitution of the substances.

EXPERIMENTAL.

Preparation of salts and free base.

4-(or 5-)Guanidinoglyoxaline dihydrochloride. The preparation and analysis of this salt are reported in the preceding paper. Recrystallization is best carried out by dissolving in a minimum amount of water, if necessary boiling with charcoal and filtering, evaporation *in vacuo* to copious crystal formation, release of vacuum and heating the tube over a flame to dissolve the crystals, followed by the gradual addition of absolute alcohol with shaking. Two to three volumes of absolute alcohol suffice for the separation of 80–90% of the material present. The crystals are fine needles or spars according to the rate at which they are formed. After washing with absolute alcohol and drying at 110°, they have the composition C₄H₇N₅·2HCl. m.p. 288° (decomp.) with quick heating; darkening begins about 270°. The substance is readily soluble in water.

4-(or 5-)Guanidinoglyoxaline sulphate. This is readily prepared from the dihydrochloride by the addition of the theoretical amount of 5 *N* H₂SO₄ and drying in a vacuum desiccator in presence of NaOH; e.g. 2.0 g. C₄H₇N₅·2HCl were treated as described, and the residue was dissolved in 5–6 ml. water. This was concentrated *in vacuo* to 2–3 ml. and 2–3 vol. absolute alcohol were added. When the tube was cooled and scratched with a glass rod crystallization quickly set in. (There is always a tendency for the sulphate, even in pure form, to separate first as an oil on addition of excess alcohol, but scratching the tube invariably initiates crystal formation.) The tube was left in the refrigerator overnight, the crystals collected, washed with absolute alcohol and dried in the air for some hours; yield 2.282 g. Dried at 110° to constant weight, 1.972 g. Loss of 0.310 g. H₂O or 13.6%. (Calculated for C₄H₇N₅·H₂SO₄·2H₂O, 13.9% H₂O.) The crystals are square or rhomboid, M.P. 219°.

4-(or 5-)Guanidinoglyoxaline dipicrate. When picric acid is added even to dilute aqueous solutions of either of the above salts an insoluble precipitate is quickly formed. When the picrate is dissolved in a minimum of hot water and allowed to cool slowly long heavy spars with uneven edges and golden colour appear. There are frequently also fused masses of short stout prisms with well-defined crystal faces; M.P. 216–217°. Found by the nitron method of Busch & Blume [1908] 77.0 and 77.2% picric acid. (Calculated for C₄H₇N₅·2C₆H₃O₇N₃, 78.5% picric acid.)

Guanidinoglyoxaline. 0.3 g. guanidinoglyoxaline sulphate was dissolved in a little water, and titrated in an atmosphere of N with 0.5 *N* Ba(OH)₂ until the SO₄ was removed. The solution was centrifuged in the stoppered tube and the supernatant fluid transferred to a small basin, which was quickly placed in a desiccator and left to dry *in vacuo* in presence of solid NaOH and H₂SO₄. At this stage a drop of the aqueous solution on a tile was slightly alkaline to Porrier's blue, indicating *pH* near 11.0. After drying for a day the material in the basin showed no sign of crystallization, but remained as a thick gum, readily thinned by warming.

Guanidinoglyoxaline carbonate. The free base described above was dissolved in a little warm absolute alcohol and CO₂ led through the solution. The solution remained alkaline to litmus and showed no sign of crystal formation. An excess of dry ether was finally added; a clear oil separated. After the stoppered tube had been kept for a few hours definite crystal formation took place at points on the sides, but the main body of oil was only semi-solid after keeping 2 days in the refrigerator. Some was removed to a watch glass, but it remained sticky and apparently hygroscopic. On placing the watch glass in an oven at about 110°, the substance quickly melted and did not resolidify on cooling. On the addition of a drop of conc. HCl there was vigorous effervescence with loss of CO₂. The addition of a little alcohol to the watch glass was soon followed by the formation of the typical spars of the dihydrochloride over its whole surface. A little of the carbonate tested on a tile with thymol blue had a *pH* near 8.5.

Oxidation with bromine.

20.1 mg. C₄H₇N₅·2HCl titrated with 0.112 *N* Br required 3.0 ml. for slight excess in the cold. After keeping for a short time the excess Br disappeared and guanidine oxalate separated. When the solution was made slightly alkaline with NaOH the precipitate dissolved. It was brought to the boil, cooled and made up to 10 ml. With Tiegs's reagents and a colour standard of pure guanidine hydrochloride the solution was found to contain 5.43 mg. guanidine from the possible 5.96 mg., or a yield of 91%.

3.0 ml. 0.112 *N* Br yield 2.69 mg. O. 20.1 mg. $C_4H_7N_5 \cdot 2HCl$ using 2 atoms of O require 3.25 mg.; the O utilized is 82.8% of theoretical amount.

By nesslerization of a similar solution it was found that about 80% of the non-guanidine-N was present as ammonia in the solution. At least part of the remainder was present as glycine as shown by the ninhydrin test.

The solution prepared as above and acidified with acetic acid precipitates calcium oxalate on the addition of $CaCl_2$ solution. The precipitate dissolves in excess H_2SO_4 , and the resulting solution absorbs permanganate.

When the solution prepared as above, is acidified with H_2SO_4 , and distilled *in vacuo* into dilute NaOH solution, the distillate readily reduces permanganate in alkaline solution in the cold, indicating the presence of formic acid.

Oxidation with $KMnO_4$.

54.5 mg. $C_4H_7N_5 \cdot H_2SO_4$ were treated with 5 drops 5 *N* H_2SO_4 and 10 ml. 0.1 *N* $KMnO_4$. When the solution was heated to boiling, a copious brown precipitate, mixed with a white precipitate of guanidine oxalate, persisted. The solution was made alkaline with 2.5 *N* NaOH and treated with excess H_2O_2 . MnO_2 was filtered off, the solution made slightly acid to litmus and treated with excess picric acid; wt. of picrate 53.2 mg. (theory 70.4 mg. guanidine picrate); yield 75.7%.

In a similar manner, but adding $KMnO_4$ until the purple colour persisted, 54.1 mg. $C_4H_7N_5 \cdot H_2SO_4$ gave 52.7 mg. guanidine picrate (theory 69.9 mg.); yield 75.4%.

The picrates thus obtained were mixed and recrystallised without change of m.p. (324–327°, according to rate of heating). 9.8 mg. of the picrate gave 18.0 mg. nitron picrate, or 7.62 mg. picric acid, 77.75%. 8.8 mg. gave 16.9 nitron picrate, or 7.15 mg. picric acid, 81.3%. (Calculated for guanidine picrate 79.5% picric acid.)

50.9 mg. $C_4H_7N_5 \cdot H_2SO_4$ titrated in hot solution in presence of excess H_2SO_4 showed a persistent brown end-point after 8.5 ml. 0.1 *N* $KMnO_4$ had been added. This is the equivalent of an uptake of 6.8 mg. O. 50.9 mg. of the salt require on the basis of 2 atoms 7.3 mg. O. Oxygen used is thus 93.2% of theoretical amount.

Oxalic acid, ammonia, formic acid and glycine can be shown to be present in the solution by the means adopted above for the material oxidized with bromine.

Hydrolysis with $Ba(OH)_2$.

Preparation of 4-(or 5-)carbamidoglyoxaline hydrochloride. 0.97 g. $C_4H_7N_5 \cdot H_2SO_4$ was placed in a small flask with a reflux condenser, on top of which was fixed a Folin bulb containing standard acid. A tube was fused through the side of the flask, leading close to the bottom, so that nitrogen could be bubbled through the mixture during hydrolysis. There were placed in the flask 3.5 g. $Ba(OH)_2$ and 30 ml. water, giving a medium approximately *N* for hydrolysis. A slow current of N was admitted and the flask boiled with a micro-burner for 4.5 hours. It was allowed to cool while the stream of N flushed the remaining NH_3 into the standard acid. The flask was detached from the condenser and 6.0 ml. 5 *N* H_2SO_4 added, at which point the reaction was slightly acid to Congo red. The $BaSO_4$ was removed, and the filtrate evaporated to about 10 ml. *in vacuo* in a boiling-tube. About 1 g. of picric acid, dissolved in a minimum quantity of boiling water was then added to the solution. After cooling the picrate was collected, washed with water and absolute alcohol and dried; yield 0.99 g.; m.p. 176°. (The filtrate

was kept.) It was transferred to a 50 ml. centrifuge-tube, 20 ml. water were added, and the mixture was boiled. It was centrifuged hot and the supernatant liquid poured into another tube. The residue was re-extracted with 3 ml. water in the same fashion and the supernatant fluid added to the first. From the combined extracts 0.626 g., M.P. 184° , was recovered. The residue was dissolved in a minimum amount of boiling water (23 ml.), then allowed to crystallize; yield 0.252 g.; M.P. 217° , the same as the dipicrate of guanidinoglyoxaline.

At this stage the 0.626 g. picrate was combined with similar material giving in all 1.98 g. It was put in a separating funnel with about 50 ml. water and 2 ml. conc. HCl and the picric acid removed with ether. The resulting solution was concentrated *in vacuo* to about 20 ml., boiled with a little charcoal and the filtrate concentrated to about 3 ml. About 2 vol. absolute alcohol were added and crystals soon separated. After keeping for 1–2 hours in the refrigerator the mixture was filtered, washed with absolute alcohol and dried; yield 0.539 g.; M.P. 204° . The filtrate was kept for re-treatment with picric acid.

The 0.539 g. was boiled in a little water with charcoal, the filtrate evaporated to about 4 ml. and 2 vol. of absolute alcohol were added. There was a slow appearance of large flat plates, appearing sometimes in clusters on edge as spars; yield 172 mg.; M.P. 210° , with yellow melt. By adding an equal vol. of alcohol to the filtrate a further crop of 50 mg. of identical material was obtained. By evaporation of the filtrate and again adding alcohol a further amount of 146 mg. of material of M.P. 206° was obtained. This crop was contaminated with guanidinoglyoxaline dihydrochloride, and with its filtrate it was re-treated with that above, with picric acid. By repeating the whole procedure a further crop of 59 mg. of pure carbamidoglyoxaline hydrochloride was obtained. The three crops with M.P. 210° were combined and recrystallized without change in M.P. or behaviour towards the diazo-reagents. When dried their composition was represented by $C_4H_6ON_4 \cdot HCl$.

Carbamidoglyoxaline sulphate. 31 mg. of the hydrochloride were treated in a small basin with 0.19 ml. $N H_2SO_4$ and dried in a vacuum desiccator. The crystal mass was dissolved in about 0.5 ml. water and filtered through a micro-funnel into a small tube. The volume was reduced *in vacuo* to about 0.1 ml. when signs of crystallization appeared. A few drops of absolute alcohol were added and the tube placed in the refrigerator. The filtered, air-dried crystals weighed 29.624 mg. After heating to constant wt. at 110° they lost 2.651 mg. or 8.95% H_2O . (Calculated for $(C_4H_6ON_4)_2 \cdot H_2SO_4 \cdot 2H_2O$, 9.33% H_2O .) M.P. of dry sulphate is 197° with slow decomposition and foaming. (Found: S, 9.05%. Calculated for $(C_4H_6ON_4)_2 \cdot H_2SO_4$, S, 9.15%.)

Carbamidoglyoxaline. 22.73 mg. of the sulphate were dissolved in about 2 ml. water and 0.26 ml. 0.5 $N Ba(OH)_2$ was added, so that the solution was neutral to litmus and free from Ba and SO_4 ions. It was filtered and evaporated to a small drop, and a small amount of a mixture of equal parts absolute alcohol and ether added. A slight pinkish colour appeared in the solution. The crystals, wt. 4 mg., formed broad spars; M.P. 194 – 195° with golden melt. They were just perceptibly alkaline to sensitive litmus, and to bromothymol blue, so that carbamidoglyoxaline is apparently only feebly basic. (Found: N, 40.87% on somewhat impure material. $C_4H_6ON_4$ requires N 44.41%.)

Carbamidoglyoxaline picrate. To about 20 mg. of the hydrochloride an excess of picric acid was added. The precipitate was washed with absolute alcohol, wt. 46 mg.; M.P. 210° . The material was recrystallized from 2 ml. water; beautiful iridescent needles and spars formed on slow cooling, of a much more homogeneous appearance than is obtained with guanidinoglyoxaline dipicrate;

M.P. 210° . 11.624 mg. air-dried material, on heating to constant wt. at 110° , lost 0.554 mg. or 4.77 %. (Calculated for $C_4H_6ON_4 \cdot C_6H_3O_7N_3 \cdot H_2O$, 4.83 % H_2O .) By nitron method, found 61.9 and 62.2 % picric acid. (Calculated for $C_4H_6ON_4 \cdot C_6H_3O_7N_3$, 64.5 % picric acid.)

Note on the $Ba(OH)_2$ hydrolysis.

By titration of the contents of the Folin bulb it was found that 100 mg. N had been lost as NH_3 during the 4.5 hours' hydrolysis of 0.97 g. $C_4H_7N_5 \cdot H_2SO_4 = 0.5437$ g. $C_4H_7N_5$. (Total N 304 mg.) Tests for urea on such hydrolysates have invariably been negative. It has been recorded that approximately 1 g. of a mixed picrate was obtained from 0.97 g. of the original salt. A rough computation would indicate that this is composed of about 0.6 g. guanidinoglyoxaline dipicrate and 0.4 g. carbamidoglyoxaline picrate. This means that 0.130 g. of the original base remained unchanged, leaving 0.4137 g. taking part in the hydrolysis. Of the latter 0.142 g. is accounted for by formation of the carbamido compound, thus leaving approximately 0.27 g. to be accounted for. Under the conditions of the hydrolysis it may thus be concluded that about 25 % of the original guanidinoglyoxaline remains unchanged, 25 % is converted into carbamidoglyoxaline, and 50 % of the material is present as product(s) not yet recognized. Guanidine may be present in such hydrolysates but only in traces, and the bulk of the NH_3 obtained by titration probably arises from this side-chain. There is also little glycine present in the freshly prepared hydrolysate.

Isolation of glycine from $Ba(OH)_2$ hydrolysates.

Filtrates from the picrates, as obtained under the preparation of carbamidoglyoxaline hydrochloride, were collected from a number of batches representing about 3.0 g. $C_4H_7N_5 \cdot H_2SO_4$, with a view to the study of the substance giving a positive nitroprusside test. After keeping for 2-3 weeks in the refrigerator it was observed that this test had become faint, but that the ninhydrin test had become very marked. The combined picric acid filtrates were accordingly evaporated to about 10 ml. and the excess picric acid, along with a little more of the mixed picrates, was filtered off. Picric acid was removed from the solution with ether and the aqueous residue treated with Ag and $Ba(OH)_2$. Only the filtrate from the latter was kept. It was freed from Ag by H_2S , concentrated, made neutral to litmus and boiled with $CuCO_3$; wt. of Cu salt 200 mg. (= 140 mg. glycine). It was dissolved in 2 ml. $N H_2SO_4$ and saturated with H_2S . On evaporating to about 1 ml. and adding excess absolute alcohol, there were obtained 136 mg. of typical glycine sulphate, M.P. 225° .

According to the computation above only about half of the starting material is available for glycine formation in such hydrolysates; in this case about 1.5 g. $C_4H_7N_5 \cdot H_2SO_4$, or 840 mg. $C_4H_7N_5$, which could theoretically yield 504 mg. glycine. The actual yield of 140 mg. is thus 28 % of theory. It may be observed that several of the batches in the combined picric acid filtrates were subjected to milder hydrolysis than that detailed, and an appreciable amount of material, which may have produced glycine on further hydrolysis, was discarded in the Ag- $Ba(OH)_2$ precipitate.

Oxidation of 4-(or 5-)carbamidoglyoxaline.

10.4 mg. $C_4H_6ON_4 \cdot HCl$ were treated with acid permanganate as described for the guanidino-compound. The resulting solution gave a negative diazo-test, a negative Tiegs test, but a strongly positive ninhydrin test. Urea could not be detected in the solution.

Ba(OH)₂ hydrolysis of 4-(or 5-)carbamidoglyoxaline.

7.5 mg. $C_4H_6ON_4 \cdot HCl$ were heated with 2 ml. N $Ba(OH)_2$ in a small side-arm tube for $4\frac{1}{4}$ hours. 0.95 mg. N was evolved as NH_3 . The solution was neutralized with 2 ml. N H_2SO_4 and the $BaSO_4$ separated; the filtrate gave a positive nitroprusside test, a slowly developing purple colour with $NaOH$ and, after diazotization, a brown colour with β -naphthol.

On treating a little of the solution with an equal vol. of 1 % ninhydrin and a drop of pyridine a greenish yellow colour appeared immediately, and with very slight heat a grass green colour quickly developed. On prolonging the heating, the unstable green disappeared and was replaced by the blue colour typical of glycine. From these tests it is apparent that the carbamido-compound is an intermediate in the formation of the unknown substance(s) discussed above as arising from 4-(or 5-)guanidinoglyoxaline. It may well also be an intermediate in the formation of glycine.

General properties and colour tests of the two bases.

Both bases and their salts above mentioned, with the exception of the picrates, are readily soluble in water. Both bases, but especially guanidinoglyoxaline, are soluble in absolute alcohol. Their salts are insoluble in alcohol and in most organic solvents. The salts of guanidinoglyoxaline are colourless, those of the carbamido-compound tend to have a faint pink tinge. The picrates of course are yellow and not markedly different in colour from guanidine picrate. Both bases are precipitable by phosphotungstic acid, salts of mercury and by silver in presence of $Ba(OH)_2$.

Diazo-test. When a drop of a dilute solution of the guanidino-compound is added to a mixture of 1.25 ml. 1.1 % Na_2CO_3 and 0.5 ml. Koessler and Hanke reagent, a dirty yellowish green may be momentarily observed followed immediately by a bright methylene blue-like colour. If more than about 0.1 mg. of the substance has been added the blue colour persists for some time, but if the colour is faint at the outset it quickly fades. Under the latter circumstances it can be stabilized for some time by the addition, quickly after coupling, of a few drops of 2.5 N $NaOH$. If the blue-coloured alkaline solution is made acid, it changes to a garnet-like red.

When a drop of a dilute solution of the carbamido-compound is similarly added to the mixed diazo-reagent, a momentary yellow colour is first perceptible followed by the immediate formation of a brilliant red colour. In dilute solution this fades but again may be stabilized by the addition of a few drops of dilute $NaOH$, with little tendency towards blue. When large excess of strong alkali is added, however, the colour is changed to a deep pure blue. In acid solution the colour is again red.

With either substance the test is very sensitive. The addition of as little as 0.1 γ of either of the hydrochlorides to the above amount of reagent is sufficient to give a perceptible colour.

Sakaguchi test. (According to the modification of Weber [1930, see also Poller, 1926].) When about 0.05 mg. guanidinoglyoxaline dihydrochloride in 0.5 ml. water is treated with 1 drop of 2.5 N $NaOH$ and 1 drop of 0.02 % α -naphthol solution, the tube cooled in ice, and 1 drop of sodium hypobromite solution (1 g. Br in 50 ml. 5 % $NaOH$) added, followed quickly by a few drops of 40 % urea solution, a bright green colour, rapidly changing to blue and slowly fading to purple and brown, appears.

An equal quantity of the carbamido-compound similarly tested gives a pink colour which fades rapidly.

Both tests are abnormal. The carbamido-compound, as stated above, contains no guanidine yet gives a test characteristic of monosubstituted guanidines according to Poller. Weber mentions that tyrosine, histidine and tryptophan interfere with the test. I have found that tyrosine gives no colour, histidine some colour, and tryptophan a marked red colour with the test. With urocanic acid a brilliant pinkish red colour, which quickly fades, is produced. So, apparently, the Sakaguchi test is not entirely specific for substituted guanidines. With the guanidino-compound the normal red is replaced by a blue, perhaps by an influence similar to that at work in the diazo-test.

Tiegs's test. When about 0.05 mg. of the guanidino-compound in 0.5 ml. water, to which has been added a drop of 2.5 *N* NaOH, is mixed with 0.3 ml. Tiegs's reagent, a clear deep green colour, gradually changing to blue, is produced. When a somewhat greater amount of the carbamido-compound is similarly tested a dirty greenish brown colour appears.

Both tests are again abnormal. The behaviour of the guanidino-compound in this test is analogous to its behaviour in the diazo- and Sakaguchi tests. The behaviour of the carbamido-compound is here quite anomalous.

Behaviour with Cu. A neutral solution of either substance when heated with CuCO_3 yields a blue solution, and a black precipitate is formed. A very dilute solution of the guanidino-compound added to Benedict's solution leads to the formation of a greyish purple precipitate. If shaken with a little freshly prepared $\text{Cu}(\text{OH})_2$, with subsequent addition of a solution of Na_2CO_3 , a similar salt is formed in the cold. On heating, the salt blackens. The Cu salt is soluble in excess of strong alkali and does not appear in Fehling's solution, but when the latter is heated a deeper blue or violet colour appears. The carbamido-compound yields no precipitate with cold Benedict's solution, but a black precipitate appears on heating.

Urea tests. (a) *Ehrlich's.* A greenish yellow colour is obtained when a 1% solution of carbamidoglyoxaline hydrochloride is treated with a little *p*-dimethylaminobenzaldehyde. On heating, a clear pink colour is produced—an anomalous behaviour. The guanidino-compound shows a negative test.

(b) *Schiff's.* The carbamido-compound responds like allantoin to this test, the colour being more blue than that given by urea. The guanidino-compound shows a negative test.

Neither substance is precipitable by xanthydrol or affected by urease.

Ninhydrin test. Both substances yield an apparently identical buff-red colour when a little of their salts in solution is heated with an equal volume of 1% ninhydrin in presence of a drop of pyridine.

Folin's uric acid test. When 1 drop of the Folin & Marenzi [1929] reagent is added to a little solution of either base, and made alkaline with Na_2CO_3 , or a slight excess of NaOH, a blue colour is produced. A rough estimate indicated that 1 part of guanidinoglyoxaline dihydrochloride gave the same amount of colour as 2.83 parts of carbamidoglyoxaline hydrochloride or 2 parts of uric acid. The *biuret test* is positive for the carbamido-compound and negative for the guanidino-compound.

With the *murexide test* the guanidino-compound leaves a lemon-yellow residue made slightly orange with alkali, whilst the carbamido-compound leaves a blackish residue which goes quite black with either NaOH or NH_4OH .

The guanidino-compound gives a negative *xanthoproteic test*, the carbamido-compound gives a deep reddish colour on addition of ammonium hydroxide.

The *Jaffé test* is positive for both substances.

The following tests are negative for both substances: *Millon*, *Adamkiewicz*, *Mörner*, *Molisch*, *Obermeyer* and *nitroprusside*.

When 10 *N* NaOH is brought in contact with a dry guanidinoglyoxaline salt a bright pinkish red colour appears and then rapidly fades.

A solution containing 10·13 % of guanidinoglyoxaline dihydrochloride in a 2 dm. tube when examined in a Bellingham and Stanley polarimeter with the green Hg line showed no optical activity.

I should like to acknowledge the great help given me throughout this work by my colleague, Dr M. M. Cantor. The Carnegie Research Grant Committee of the University has assisted financially.

NOTE ON THE DISSOCIATION AND ULTRAVIOLET ABSORPTION SPECTRA.

BY WILLIAM DOUGLAS MCFARLANE.

Guanidine is a base of the strength of potassium hydroxide. It will be seen (Fig. 1) that guanidinoglyoxaline is also a relatively strong base. The influence of

Fig. 1. Titration curves of (A) guanidine hydrochloride and (B) guanidinoglyoxaline dihydrochloride.

salt concentration on the dissociation of these highly ionized bases may, for present purposes, be considered as negligible so that the ionic strength may be

determined directly from the titration curves. The dissociation constant of guanidinyglyoxaline is at approximately pKH 10.5 while, according to Hall & Sprinkler [1932], that of phenylguanidine is at pKH 10.77. The introduction of an unsaturated negative substituent, such as a phenyl or glyoxaline group reduces, therefore, the basicity of the parent compound. Davis & Elderfield [1932] have shown that on the other hand a saturated positive substituent such as piperidine has no such effect.

Ring compounds of the unsaturated pyrimidine series exhibit, according to Heyroth & Looffbourov [1934], a marked degree of selective absorption. The compounds examined in this study (Fig. 2) showed end absorption typical of

Fig. 2. Absorption curves of 0.1 *M* guanidine hydrochloride; 0.02 *M* glyoxaline; 0.000287 *M* carbamidoglyoxaline monohydrochloride and 0.000174 *M* guanidinoglyoxaline dihydrochloride in various solvents: A, 0.1 *N* NaOH; B, phosphate buffer; C, 0.1 *N* HCl.

glyoxaline itself. It is well known that the absorption curves of most organic acids and bases change in some regular way with the pH of the solution, owing to the difference in the absorption exhibited by the anions, cations or undissociated molecules. In this regard the compounds examined are no exception. Previous measurements on glyoxaline and guanidine have been made by others without taking this into consideration. The shift in ultra-violet absorption by guanidinoglyoxaline at varying pH is typical of a highly ionized base whilst the behaviours of glyoxaline and carbamidoglyoxaline are characteristic of weakly ionized compounds.

The following figures indicate the relative absorption intensities of these compounds in 0.1 *N* NaOH.

	Molecular extinction coefficient at wave- length 2300 Å.
Guanidinoglyoxaline	5680
Carbamidoglyoxaline	5600
Glyoxaline	300
Guanidine	90

EXPERIMENTAL.

1. *Titration curves.*

The data represented in Fig. 1 were obtained as follows. Solutions of the free base, approximately 0.033 *M*, were prepared by dissolving 119.4 mg. guanidine hydrochloride, or 246.1 mg. guanidinoglyoxaline dihydrochloride in distilled water; adding NaOH to neutralize the HCl exactly and diluting to 50 ml. with distilled water. A hydrogen half-cell of the Hildebrand type and a saturated calomel half-cell were used for the pH measurements made during the titration of the two solutions with 0.2 *N* HCl.

These measurements have been repeated using the procedure of Davis and Elderfield [1932] in which the solution of the free base is prepared by adding the required amount of Ba(OH)₂ to a solution of the sulphate. The results obtained by the two methods were identical, within the limits of accuracy of the technique.

2. *Absorption curves.*

To obtain solvents which were completely transparent down to the limit 2160 Å. of the photographic plates employed, it was found necessary to recrystallize the primary and secondary phosphates used in the preparation of the phosphate mixtures, and to make the NaOH solution from NaOH prepared from sodium. The pH of the buffered solutions was determined by the quinhydrone electrode.

The absorption spectra were determined with a Bellingham and Stanley quartz spectrograph fitted with a rotating sector photometer. The wave-length measurements were made with a plate-measuring instrument of the same manufacture. The sector readings gave directly the value of the logarithm of the transmission *T*. From this the molecular extinction coefficient (ϵ) was obtained by Beer's law

$$I = I_0 10^{-\epsilon cl},$$

where *c* is the concentration in g. mol. per litre and *l* is the thickness (in this case 1.0 cm.) of the absorbing solution.

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