CLVIII. STUDIES ON THE INTERACTION OF AMINO-COMPOUNDS AND CARBOHYDRATES.

III. THE ACTION OF NITROUS ACID ON CERTAIN NITROGENOUS SUGAR DERIVATIVES AND RELATED COMPOUNDS.

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IN continuation of our investigations on the behaviour of glucose ureide [Hynd, 1926] it was necessary to study the action of nitrous acid on the compound, even though Schoorl [1903] has reported the formation of glucose from the ureide as the result of the interaction of nitrous anhydride.

From the results of preliminary experiments carried out by one of us (A. H.) over a year ago, it seemed that, under conditions similar to those of Van Slyke's technique for the determination of NH_2 -groups in amino-acids, glucose ureide failed to react with nitrous acid, but on the addition of a mineral acid fully half of the total nitrogen was liberated rapidly. As this result was in agreement with the findings of Werner [1917] regarding the behaviour of urea towards nitrous acid, but contrary to those of other workers, a closer study of the reaction seemed desirable. Consequently an investigation of the behaviour of urea and related compounds towards nitrous acid under various conditions was planned and experiments were in progress when Plimmer [1925] published the results of experiments covering this same field, though undertaken for a somewhat different purpose.

Reference to the experimental part will show that our results for urea are in close agreement with those reported by Plimmer, and as a similar agreement was obtained in the case of urethane, guanidine carbonate, creatine, and creatinine, our results for these compounds need not be given in detail. Accordingly, it is only necessary to report fully the nitrogenous sugar derivatives investigated, though reference will be made to the other results in the course of the discussion.

EXPERIMENTAL.

All the experiments were carried out in Van Slyke's micro-apparatus, the usual method being followed. In those cases where mineral acid was required, 40 % sulphuric acid was used and was introduced by means of the side burette. The reaction chamber was shaken continuously in experiments of short dura-

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tion, but during the first and last half-hours only, when the reaction was continued for a long period.

The nitrogen evolved was measured, and by making use of Van Slyke's table [1912] was converted into milligrams; all the results are expressed as mg. nitrogen per 100 cc. of solution. The concentrations of the solutions employed were checked either by carrying out total nitrogen determinations by Kjeldahl's method or by the rotations in the case of the optically active compounds.

Compound	Medium	Time	Temp. ° C.	Amino-N found mg.	% of total N
Urea	20 % acetic acid	$\frac{1}{2}$ hr.	19	12.7	5.53
	"	1 "	16	47.6	20.6
	"	$1\frac{1}{2}$ hrs.	16	81.2	35.1
	"	$2\frac{1}{2}$ "	16	177.4	66.8
	"	22 "	16	232.0	100.6
	" + 1 % H_2SO_4	5 mins.	20	52.7	22.8
	" + 3 % "	5 "	20	213.2	92.3
	" + 6 % "	5 "	21	183.4	79.5
	"	5 "	20	180.8	78.3
	"	5 "	20	174.9	75.8
	" + 10 % "	10 "	19	172.7	74.5
	"	15 "	19	176.6	76.5
	"	30 "	18	230.7	99.9
	"	30 "	19	234.1	101.4
Urethane	20 % acetic acid	22 hrs.	15	11.4	6.28
Guanidine carbonate	" + 8 % H_2SO_4	22 "	14	188.3	103.9
	20 % acetic acid	2 "	16	0	0
	" + 12 % H_2SO_4	23 "	14	135.8	102.3
Guanidine nitrite	" + 6 % "	21 "	15	136.3	102.7
	20 % acetic acid	24 "	14	0	0
	" + 6 % H_2SO_4	21 "	15	83.2	93.1
Creatine	20 % acetic acid + 6 % H_2SO_4	21 "	14	52.8	67.9
Creatinine	20 % acetic acid	1 "	17	153.2	36.1
	"	2 "	18	159.6	37.6
	"	18 "	14	166.4	39.2
Creatine methyl ester hydrochloride	" + 3 % H_2SO_4	23 "	16	150.9	35.5
	20 % acetic acid	2 "	18	79.8	45.5
	" + 3 % H_2SO_4	17 "	15	114.2	65.
"Creatine methyl ester"	20 % acetic acid	2 "	16	55.1	41.3
	20 % acetic acid	10 mins.	16	41.22	100.3
	" + 6 % H_2SO_4	10 "	15	41.36	100.7
Glucosimine	20 % acetic acid	$\frac{1}{2}$ hr.	19	158.7	81.35
	"	1 "	19	176.0	87.1
	"	$1\frac{1}{2}$ hrs.	19	181.2	92.9
	"	2 "	19	196.8	101.2
	" + 6 % H_2SO_4	10 mins.	19	168.9	86.6
	"	$\frac{1}{2}$ hr.	19	194.0	99.5
Glucosamine	20 % acetic acid	1 "	19	18.0	25.0
Glucosamine hydrochloride	"	18 hrs.	19	74.3	103.4
	20 % acetic acid	$\frac{1}{2}$ hr.	19	137.36	95
	"	$\frac{1}{2}$ "	19	144.0	100.3
	" + 5 % H_2SO_4	$\frac{1}{2}$ "	19	137.3	95.6
	"	2 hrs.	19	147.6	102.8
Glucose ureide	20 % acetic acid	20 mins.	19	4.58	1.8
	"	20 "	19	0	0
	"	24 hrs.	19	118.3	46.36
	" + 5 % H_2SO_4	5 mins.	19	153.8	60.2
	"	$\frac{1}{2}$ hr.	19	167.0	66.5
	"	$2\frac{1}{2}$ hrs.	19	169.6	67.6
	"	24 "	19	165.5	65.9
	" + 10 % H_2SO_4	15 mins.	19	151.26	60.2
	"	$\frac{1}{2}$ hr.	19	163.1	63.8
	"	1 "	19	167.0	66.5
	"	$2\frac{1}{2}$ hrs.	50	218.5	87.0

Compound	Medium	Time	Temp. ° C.	Amino-N found mg.	% of total N
Glucose ureide	"	3 "	50	232.3	92.5
	"	4 "	50	252.5	100.5
Glucose ureide-urea	20 % acetic acid	20 mins.	19	43.0	7.16
	"	$\frac{1}{2}$ hr.	19	64.1	10.68
	"	1 hr.	19	110.3	18.3
	"	2 hrs.	19	162.1	26.98
	"	20 "	19	506.7	84.2
	+ 5 % H ₂ SO ₄	$\frac{1}{2}$ hr.	19	422.2	86.4
	"	2 hrs.	19	427.8	87.5
	"	24 "	19	430.0	87.9
	"	2 "	50	427.8	87.5
	"	5 $\frac{1}{2}$ "	50	478.5	97.9
	"	7 "	50	490.1	100.3
Lactose ureide	20 % acetic acid	$\frac{1}{2}$ hr.	19	0	0
	"	18 hrs.	19	52.98	47.3
	+ 5 % H ₂ SO ₄	20 mins.	19	65.36	58.7
	"	$\frac{1}{2}$ hr.	19	72.06	63.9
	"	2 hrs.	19	72.98	64.7
	"	19 "	19	78.94	70.05
	"	4 "	50	113.9	101.03
	"	4 "	50	116.5	103.3
"Glucosamine + urea product"	20 % acetic acid	24 "	18	0	0
	+ 5 % H ₂ SO ₄	24 "	18	0	0
	"	10 "	50	0	0

DISCUSSION OF RESULTS.

With the one exception of the product obtained in an attempt to condense urea with glucosamine, all the compounds examined react with nitrous acid under suitable conditions. But the compounds are divided, though perhaps not sharply, into three groups according as the reaction proceeds (1) rapidly, or (2) slowly in the presence of a weak organic acid such as acetic acid, or (3) requires the presence of a strong mineral acid, such as hydrochloric acid or sulphuric acid, to bring about a significant evolution of nitrogen gas. Typical examples in the first group are glycine and glucosimine; in the second group, urea, glucose ureide, glucosamine and creatinine; and in the third group, urethane, guanidine and creatine.

To account for these differences it is evident that the nitrogen atom or atoms involved must be linked in various ways, which it should be possible to indicate by means of structural formulae. Although guanidine, on this basis of structure, may be regarded as the parent compound, it will be advisable to deal first with urea, as its structure has been more fully investigated [Werner, 1923].

Urea. Werner [1917] claims that urea is not attacked by pure nitrous acid alone or even when a second very weak acid is present. Consequently the practically complete, though slow, decomposition of urea by nitrous acid in the presence of acetic acid here recorded may seem at first sight contrary to this statement, but it is to be noted that the experimental conditions were widely different. In the first place, the concentrations of both acetic acid and nitrous acid in our experiments were high, and secondly, conditions for the partial decomposition of the nitrous acid, according to the equation

are favourable during Van Slyke's process, especially when the reaction is allowed to proceed for a long period such as 24 hours at 20°. However, as Werner's experiments lasted from 1 to 3 days at 13°, neither the temperature nor the duration of the reaction appears to be an important factor, though both Dunn and Schmidt [1922] and also Wilson [1923] have observed the rate of deamination of certain amino-compounds to be affected by temperature. It seems reasonable to suppose that in our experiments the hydrogen ion concentration of the medium became sufficiently high slowly to convert urea from its inactive cyclic structure into its reactive open form. Further support is given to this view by the fact that the addition of a strong acid rapidly brought about complete deamination. Accordingly, urea in a neutral medium exists as a ring compound, represented by I or III, either of which becomes converted into the *iso*-carbamide structure II on increase of hydrogen ion concentration. The free amino-group is then

attacked by nitrous acid, nitrogen being liberated, and the residual cyanic acid, as explained by Werner, yields a second atom of nitrogen.

Structure I is that proposed by Werner, but we are inclined to favour III, for, as will be clear from what follows, its adoption brings urea, guanidine and creatine better into line.

Guanidine. We find that guanidine, in presence of acetic acid, reacts with nitrous acid only very slightly if at all, while in presence of the necessary amount of mineral acid practically the whole of the nitrogen is evolved after 24 hours. These results agree with the experimental findings of Plimmer [1925], but are contrary to the observations of Krall [1915], who states that nitrous acid reacts slowly and liberates nitrogen corresponding with no more than one of the three nitrogen atoms, even when excess of a strong acid is present. Consequently, this worker suggested that guanidine in solution as the free base is a mixture, in approximately equal proportions, of the isomerides IV and V, formulated below, strong acid increasing the proportion of the latter, whereas alkali hydroxides increase the proportion of the former. This view is also supported by the recent study of the hydrolysis of guanidine carried out by Bell [1926]. Plimmer, on the other hand, considers guanidine to have formula IV, changing to VI in the presence of mineral acid, which he holds explains the liberation of two-thirds of its nitrogen in the presence of hydrochloric acid.

Taking cognisance of the fact that all attempts to prepare a dihydro-

chloride from guanidine have been unsuccessful, we take the view that guanidine is best represented by the structure:

which, as indicated, would dissociate as a strong base, and form stable salts even with weak acids. Such salts would be hydrolytically dissociated and accordingly their solutions would have an alkaline reaction. This is in agreement with the well-known fact that guanidine carbonate $(\text{CH}_5\text{N}_3)_2 \cdot \text{H}_2\text{CO}_3$ is comparable with sodium carbonate and forms strongly alkaline solutions. Further, we found that a solution of guanidine nitrite can be obtained by mixing equivalent solutions of guanidine carbonate and barium nitrite and then removing the precipitated barium carbonate by filtration. No evolution of nitrogen was observed from a $M/20$ guanidine nitrite solution thus prepared which had p_{H} 9 approximately. This stability is consistent with the isolation by Thiele [1893] of nitrosoguanidine, to which he assigned the formula

Accordingly, owing to the stability and strongly basic nature of the guanidine ion, no reaction with nitrous acid takes place in the presence of acetic acid, that is, decomposition does not occur so long as the hydrogen ion concentration remains low. On increasing the hydrogen ion concentration, as by the addition of hydrochloric acid, evolution of nitrogen ensues. During this process, there is probably conversion to the structure

the free amino-group then reacting normally with nitrous acid, nitrogen being liberated and the rest of the molecule appearing as structure III above, which in the presence of mineral acid would pass into II. Consequently, urea is to be regarded as the first intermediate product in the decomposition of guanidine by nitrous acid. The subsequent stages being those already indicated for urea, the whole of the nitrogen is liberated, a result in accordance with the behaviour of arginine under similar conditions (Plimmer). It is to be noted, however, that it would be impossible to detect the intermediate formation of urea, as, under the conditions necessary for the first stage of the reaction which is a slow one, the urea would be decomposed as fast as it was formed.

The conversion of form IV into V is not regarded by us as a simple tautomeric change produced by the wandering of a hydrogen atom from the NH in the ring to the NH outside the ring. The initial step seems to involve rupture of the bond between the two ring nitrogen atoms. Redistribution of the hydrogen

atoms then occurs with the formation of a ring structure, in which the nitrogen atom outside the original ring structure now takes part, while that one of the original ring-nitrogen atoms, which is now outside, carries the additive salt molecule. This gives a satisfactory explanation of the existence of mono-acid salts of guanidine. However, probably owing to the presence of the double bond, the ring structure in V is not so stable as that in IV and can be ruptured by even a weak acid, so that in solution a salt of guanidine with a strong acid may exist as represented in form VI.

Creatine. From the fact that both Volhard and Horbaczewski used sarcosine as the starting material for their syntheses of creatine, it is clear that the methyl and the carboxy-methyl groups must be linked to the same nitrogen atom. Further, as the compound reacts towards nitrous acid in the same way as guanidine, it appears to us to have structure VII, in solutions of low hydrogen ion concentration, which, as explained in the case of guanidine, tautomerises to the form VIII when the hydrogen ion concentration is sufficiently high. In solution conversion to IX would occur, and consequently on reaction with nitrous acid one nitrogen atom would be evolved with production of an intermediate X similar in structure to the acid amides (Plimmer), so that a second nitrogen atom would be liberated.

Creatinine. Further action of hydrochloric acid on compound VIII brings about rupture of the bond between the nitrogen atoms, and dehydration occurs, probably without the intermediate formation of IX, so that creatinine results with structure XI. Consequently we find, as previously reported by Plimmer [1925] and also by Wilson [1923], that creatinine reacts with nitrous acid in the presence of acetic acid to lose one of the three nitrogen atoms. The same result is obtained in presence of hydrochloric acid unless excessive amounts are used. Plimmer explains the effect of mineral acid in decreasing the amount of nitrogen evolved as due to a change to the alternative structure XII. However, as a similar retarding influence was noticed by us in the case of urea when excessive amounts of sulphuric acid were used, the correct explanation is not clear. In this connection it may be noted that no difference

was produced in the result of the decomposition of glycine by nitrous acid in the presence of acetic acid by adding excess of sulphuric acid.

Creatine methyl ester hydrochloride. This compound has been claimed by Kapfhammer [1925] to be a derivative of creatinine, but, as 65.1 % of the total nitrogen is evolved on reaction with nitrous acid in the presence of sulphuric acid, the compound was correctly described by Dox and Yoder [1922] as the hydrochloride of creatine methyl ester. However, as 41.3 % of the total nitrogen is liberated from the compound when its solution is shaken with a slight excess of silver carbonate and then decomposed by nitrous acid in the presence of acetic acid, removal of the salt molecule evidently involves destruction of the ester with elimination of methyl alcohol and the resulting formation of creatinine. This would seem to give a satisfactory explanation of Kapfhammer's observations.

Glucosimine and glucosamine. The former compound, like glycine, was found to yield the same results with acetic and sulphuric acids. In both cases, rapid decomposition of glucosimine with nitrous acid occurs, as noted by Levene [1916], and also by Schmuck [1923].

This behaviour forms a marked contrast to that of glucosamine, which required 18 hours for complete decomposition in the presence of acetic acid. This supports the view that glucosimine should be formulated with a free amino-group, as in XIII, while glucosamine, as it reacts in a manner similar to urea, must contain in its molecule, as previously suggested by Irvine and Hynd [1913], the modified betaine ring structure, as in XIV, which in the presence of mineral acid changes to XV; accordingly the compound yields the total amount of nitrogen in about half an hour.

Ureides. (1) *Glucose ureide.* The formula XVI originally assigned to this compound by Schoorl [1903] requires modification in two respects. Alteration of the glucose portion to an oxidic ring structure [cf. Helferich and Kosche, 1926], probably amylene oxide, as in XVII, gives a better explanation of the results obtained on acetylation [Schoorl, 1903]. With regard to the urea portion, no free amino-group is present, as with nitrous acid in the presence of acetic acid only slow evolution of nitrogen amounting to about 50 % of the total nitrogen was obtained. Consequently a ring structure like that in urea is

indicated, as the alternative form XVIII, on the analogy of the behaviour of urethane and the acid amides, would not be expected to yield any nitrogen in the presence of acetic acid.

With regard to ring structures three possibilities exist, which, if we write G for the glucose residue, may be represented as follows:

In the presence of acetic acid these would exist as XXII, XXIII, and XXIV respectively:

XXIII then passing into XXII. With the data at present available it is impossible to decide which of the above ring structures should be adopted for the compound, but from the results of another series of experiments which are all but complete, it is hoped that it will be possible to make a definite choice.

The fact that 50 % of the total nitrogen is readily obtained by reaction with nitrous acid either in the presence of acetic acid or sulphuric acid, while even in the presence of mineral acid the reaction requires to be continued for several hours at 50° before 100 % of nitrogen is liberated, suggested that it might be possible to isolate an intermediate product and thereby furnish experimental evidence in support of Werner's views on the structure of urea and its derivatives. Various experiments were carried out with this end in view, using either silver nitrite and hydrochloric acid or barium nitrite and sulphuric acid to generate the necessary nitrous acid, but all ended in failure. The only crystalline product isolated was a small proportion of unchanged glucose ureide (mixed m.p. 203°). The main organic product obtained was an extremely hygroscopic syrup, which could not be obtained crystalline. It reacted like glucose.

(2) *Glucose ureide-urea*. The behaviour of this compound towards nitrous acid supported the view that the substance is a simple additive compound. No further comment is required.

(3) *Lactose ureide*. This product obtained according to the method described for glucose ureide [Hynd, 1926] has the same melting-point and

specific rotation as given by Schoorl, and reacts with nitrous acid in a similar manner to glucose ureide.

(4) Product obtained from glucosamine-urea. The preparation and properties of this compound, which so far has not been fully examined, will be described later. It may be mentioned, however, that it does not react with nitrous acid under any conditions—a rather unexpected result in view of the character of the starting materials used in its preparation.

CONCLUSIONS.

1. The so-called amino-compounds can be subdivided into three groups according to their behaviour towards nitrous acid.

2. Glycine and glucosimine are typical examples of the group in which nitrogen is liberated readily on reaction with nitrous acid in the presence of a weak acid.

3. Urea, glucose ureide and glucosamine are representative of a group in which reaction with nitrous acid proceeds slowly in the presence of a weak acid but rapidly in the presence of a strong acid.

4. Urethane, guanidine and creatine are typical of those amino-compounds which on treatment with nitrous acid require the presence of a strong acid before any appreciable evolution of nitrogen takes place.

5. Structural formulae are assigned to each of the compounds investigated. These are in most cases slight modifications of those generally accepted, and account for the difference in behaviour towards nitrous acid observed. They are also, at the same time, consistent with the other properties of the respective compounds.

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