from cleavage of the diglycosylamine, thus forming L-arabinosylamine and L-arabinose; and the final slow increase indicates hydrolysis of L-arabinosylamine to L-arabinose.

The substance that separated in needle-like crystals has not yet been isolated in the pure state.

Amadori rearrangement of D-*glucopyranosylamine in acetic acid.* β-D-Glucopyranosylamine (0.5 g.) was dissolved in 10 ml. of glacial acetic acid and the solution was kept at 20°. The optical rotation, $[\alpha]_D^{20}$, changed from a positive value to −18° in 1 hr. and −65° in 18 hr.; the solution had then become amber-colored. The acetic acid was removed by the repeated addition of toluene and evaporation in a rotary vacuum still. The residue, containing a small amount of acetic acid, was dissolved in 10 ml. of water, and the solution, after standing for several hours for hydrolysis to take place, was again concentrated; this treatment caused decomposition of any remaining D-glucosylamine. The residue was dissolved in 10 ml. of water and passed through a column containing 20 ml. of cation exchange resin (Amberlite IR120-H. Rohm & Haas Co., Philadelphia, Pa.); the resin was then washed with water and the wash liquor discarded. The basic materials held on the resin were eluted with 20 ml. of *N* hydrochloric acid. The eluate and wash liquor were combined, concentrated and then adjusted to a volume of 10 ml. The specific rotation, $[\alpha]_D^{20}$, was −58° on the basis of the original D-glucosylamine. Production of the stable basic substance having a levorotation indicates the presence of 1-amino-1-deoxy-D-fructose. The material is being investigated further.

Amadori rearrangement by a modification of the Hodge and Rist method. D-Glucosylamine (0.2 g.) was dissolved in 5 ml. of dimethylsulfoxide[45] and 5 ml. of diethyl malonate was added. The mixture was heated for 90 min. at 80° and kept at room temperature overnight. The brown solution was diluted with water and allowed to stand for several hours to effect hydrolysis of the remaining D-glycosylamines. The solution was then extracted several times with chloroform, and the aqueous portion was filtered through decolorizing carbon, concentrated in a rotary still, and adjusted to a volume of 10 ml. The optical rotation, $[\alpha]_D^{20}$, was −43.5°, based on the weight of the original D-glucosylamine. The levorotatory product, presumably containing 1-amino-1-deoxy-D-fructose, is being studied further.

WASHINGTON 25, D. C.

(45) Dimethyl sulfoxide has been found to be an exceptionally useful solvent for glycosylamines.

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC ELECTROCHEMISTRY, DEPARTMENT OF CHEMICAL ENGINEERING, TOKYO INSTITUTE OF TECHNOLOGY]

Guanidination of D-Glucosamine*

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Since 1942, quantitative studies on the preparation of several naturally occurring guanidine compounds have been carried out and reported by K. Sugino, K. Odo, and their collaborators.

Both the *S*-methylisothiourea method and improved cyanamide condensation methods were applied for this purpose. The latter involves the following two methods which were newly established by the same authors as the result of researches made on the mechanism of the guanidination of amines with cyanamide. (1) The reaction of amine salt with cyanamide in the fused state.[1,2] (2) The reaction of an amine salt with cyanamide in aqueous solution in the presence of a small amount of free amine.[3]

As of this writing, quantitative studies on the preparation of diguanidines [ethylene-,[2] tetramethylene-(arcain),[2] hexamethylene-,[4] decamethylene-[4]], aminoalkyleneguanidine (agmatine),[5] guanidino amino acid or diguanidino acid [arginine and diguanidino valerianic acid,[6] homoarginine and diguanidino caproic acid[7]), and creatine[8] were completed successfully using one of these methods selectively. The detailed description of each special procedure suitable to each compound has been given in the preceding papers.

In the present work, the synthesis of 2-deoxy-2-guanidino-D-glucose from D-glucosamine has been studied. In regard to this problem, only one paper which turned out to be erroneous has been reported.[9]

Among the three methods of guanidine preparation described above, the cyanamide condensation in the fused state appeared to be out of the question in view of the thermal stability of D-glucosamine. Therefore, the *S*-methylisothiourea method and the cyanamide condensation in aqueous solution were both tried. The condensations of D-glucosamine with S-methylisothiourea were unsuccessful. Instead of a guanidino compound, a resinous product was obtained due to the effect of alkali on D-glucos-

* Cyanamide Derivatives, XLVII.

(1) K. Sugino, *J. Chem. Soc. Japan*, **60**, 421 (1939).

(2) K. Sugino, K. Shirai, and K. Aoyagi, *Bull. Chem. Soc. Japan*, **17**, 126 (1942).

(3) K. Odo, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **71**, 394 (1950).

(4) K. Odo and K. Sugino, *J. Chem. Soc. Japan*, **63**, 336 (1942).

(5) K. Odo, *J. Chem. Soc. Japan*, **67**, 132 (1946).

(6) K. Odo, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **74**, 1, 774 (1953).

(7) K. Odo and E. Ichikawa, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **76**, 228 (1955).

(8) K. Odo and E. Ichikawa, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **77**, 1413 (1956).

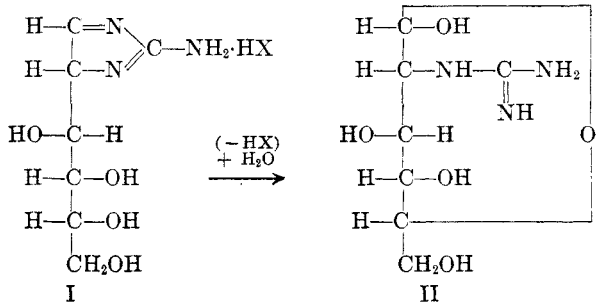
(9) J. Miller, *J. Chem. Soc.*, 2722 (1949).

TABLE I

Run No.	D-Glucosamine HCl, G.	Cyanamide, G.	NaOH Solution N	NaOH Solution Cc.	Water, Cc.	pH	Temp., °C.	Time, Hr.	Yield of I Picrate G.	Yield of I Picrate %	Cyanamide Unconverted, %
1	5.40	2.30	5.6	4.46	15	...	30	72	0	0	0
2	4.30	0.90	...	...	16	...	30	792	0.30	3.5	86
3	21.50	4.50	...	...	60	...	80	5	5.50	12.7	44
4	21.50	4.50	1.0	6.00	54	5.8	80	5	7.90	18.3	54
5	5.40	2.47	5.6	0.75	15	8.3	60	5	2.95	27.3	Trace
6	5.40	1.71	5.6	0.55	20	7.0	60	7	4.10	38.0	0

amine. The reaction of cyanamide with D-glucosamine, therefore, was the only method remaining for this purpose. Fortunately, after many trials, it was found that this condensation occurred most effectively when an aqueous solution of D-glucosamine hydrochloride was treated with cyanamide at *p*H 7 at 60° until the cyanamide disappeared. The condensation product was isolated first as the picrate, which was then converted to the hydrochloride and the nitrate. The analytical values of these salts coincided with the formula $C_7H_{13}O_4N_3 \cdot HX$. The aqueous solution of these salts showed the following characteristic properties. (1) It gave a very weak Sakaguchi's test[10] for guanidine compound. However, after treating with an equivalent amount of alkali, the solution turned to give the distinct positive test indicating the presence of guanidine group. (2) It reduced Fehling's solution when it was heated with the latter as usual.

These facts indicate that these salts may have structure I in the crystalline state but change to formula II when their aqueous solution is treated with alkali to form a free base.



I II

From these results, the compound was characterized as 2-amino-D-*arabino*-tetrahydroxybutyl-4-isoimidazole (I), anhydro-2-guanidino-D-glucose.

In order to ascertain the optimum conditions for this reaction, a set of experiments was made in which the amount of water, alkali, temperature, and time were varied. The results are shown in Table I.

In run 1, when free D-glucosamine was used, condensation did not proceed at all. This is unusual in the reactions of amines with cyanamide and may be due to the instability of D-glucosamine in alkaline medium.

(10) S. Sakaguchi, *J. Biochem. Tokyo*, **5**, 25, 133 (1925).

In runs 2–3, when an aqueous solution of D-glucosamine hydrochloride was used, condensation occurred to some extent even below 60°. This is also an exceptional phenomenon for this kind of reaction and may be explained by the existence of free amino groups[3] due to hydrolysis of the hydrochloride.

In runs 4–6, when the solution of D-glucosamine hydrochloride was neutralized with alkali so as to keep the *p*H at 6–8, condensation proceeded at an increased rate. At *p*H 7, a maximum yield of 38% was obtained.

It was noticed that increased alkalinity or high temperature have a tendency to cause resinification.

In 1949, J. Miller[9] carried out the same reaction, in which D-glucosamine hydrochloride was treated with cyanamide in 60% aqueous-ethanolic solution by boiling it for several hours. After the reaction, a picrate was obtained by adding an ethanolic solution of picric acid to the resulting solution. This picrate melted at 189–190° and was identified as *N'*-D-glucosylbiguanide monopicrate, based solely on the results of elemental analysis. In order to confirm this result and to clarify the difference between Miller's and our results, the authors carried out the same experiment, following precisely the description in his paper. A picrate which had almost the same melting point as that obtained by Miller was obtained. However, it was found that the picrate thus obtained was not *N'*-D-glucosylbiguanide monopicrate, but *O*-ethylisourea picrate by comparing it with an authentic sample. It may be formed by the following reaction.

$$NH_2CN + C_2H_5OH \xrightarrow{HX} NH_2COC_2H_5 \cdot HX \quad (NH)$$

It is well known that this reaction proceeds well in the presence of acid at elevated temperature. *O*-ethylisourea picrate has almost the same elemental composition (C, 34.1; H, 3.50; N, 22.1) as *N'*-D-glucosylbiguanide picrate (C, 34.2; H, 4.1; N, 22.8). It is supposed, therefore, that Miller's result was erroneous and that he did not obtain a definite condensation product of D-glucosamine with cyanamide.

Two other picrates were also obtained in this experiment, one of which melted at 180° and was the picrate of I though the yield was very poor. Neither was reported by Miller.

EXPERIMENTAL

Preparation of 2-amino-D-arabino-tetrahydroxybutyl-4-isoimidazole (I) *picrate.* A 5.40-g. sample of D-glucosamine hydrochloride (Anal. Calcd. for $C_6H_{14}O_5NCl$: N, 6.49, Found: N, 6.50) and 1.71 g. of cyanamide (purity 95%) were dissolved in 20 ml. of water and the *p*H was adjusted to 7 by adding 0.55 cc. of 5.6*N* NaOH. The solution was stirred and heated with reflux at about 60° for 7 hr., until the cyanamide disappeared. After the reaction, a methanolic solution of picric acid (11.5 g./150 cc.) was added to the reaction mixture.[11] After leaving it for two days, the precipitate was filtered and recrystallized from water as long needles, m.p. 180°. A further crop of the same picrate was collected from each filtrate. The total yield was 4.10 g. The compound was slightly soluble in cold water, soluble in hot water, slightly soluble in methanol.

Anal. Calcd. for $C_{13}H_{16}O_{11}N_6$: C, 36.1; H, 3.73; N, 19.4. Found: C, 36.6; H, 3.93; N, 19.1.

Preparation of the hydrochloride, the nitrate, and the sulfate of I. Crude sirup of the hydrochloride was prepared by treating 4.40 g. of the picrate dissolved in 100 cc. water with 30 cc. of 10% HCl and working up as usual. A small amount of ethanol was added to the resulting sirup and the mixture was allowed to stand in an ice box to obtain crude crystals which melted at 175°. The yield was 2.20 g. This was again dissolved in a small amount of water, decolorized, and concentrated, and a small amount of ethanol was added to it for crystallization. The product was finally purified by washing with ethanol to give 2.0 g. of pure hydrochloride in the form of white needles, m.p. 178° $[\alpha]_D^{12.5}$ (C, 2.392, water), −26.49. The compound was very soluble in water, very slightly soluble in methanol and ethanol, insoluble in ether.

Anal. Calcd. for $C_7H_{14}O_4N_3Cl$: C, 35.1; H, 5.89; N, 17.5; Cl, 14.8. Found: C, 35.3; H, 6.19; N, 17.4; Cl, 14.5.

Crude sirup of the nitrate was prepared by treating 4.50 g. of the picrate dissolved in 100 cc. water with 25 cc. of 10% nitric acid. In the resulting sirup, a small amount of ethanol was added to form a clear solution. After adding enough ether to cause a white turbidity, this solution was allowed to stand in a desiccator to obtain crude crystals of nitrate. Recrystallization from methanol gave 2.31 g. of pure nitrate melting at 137° $[\alpha]_D^{17.0}$ (C, 9.283, water) −29.23, very soluble in water, slightly soluble in methanol and ethanol, insoluble in ether.

The sulfate was prepared by treating 9.5 g. of the picrate dissolved in 140 cc. water with 70 cc. 10% sulfuric acid, but the attempt to crystallize it proved unsuccessful.

Sakaguchi's test (and Nessler's test) for I *and* II. A solution of 0.24 g. of I hydrochloride in 10 cc. *N* NaOH was allowed to stand for 3 hr. at room temperature. A few drops of the solution were placed in a test tube and diluted to 2 cc. with water. To it, a few drops of 0.1% α-naphthol solution in 70% ethanol and 5% aqueous sodium hypochlorite solution were added. A distinctly red color appeared which indicated a strongly positive Sakaguchi's test.

The same test was then carried out for a solution of I hydrochloride itself. A light pink color appeared, indicating a very weak Sakaguchi's test.

Neither solution gave the Nessler's test for ammonia. The former gave a black mercury precipitate, indicating the occurrence of reduction. The latter gave a white precipitate which turned gradually to black.

Reaction of D-glucosamine hydrochloride with cyanamide in aqueous ethanolic solution at high temperature (J. Miller's experiment). A 21.5-g. sample of D-glucosamine hydrochloride and 8.8 g. of cyanamide were dissolved in 250 ml. of 60% ethanol and the solution was refluxed for 5 hr. The resulting solution, after decolorizing, was concentrated to 100 ml. at reduced pressure and a solution of 46 g. of picric acid in 200 ml. of methanol was added to it, and heated once to form a clear solution and then allowed to cool in order to separate crude picrate. This picrate was collected by filtration and extracted with 200 ml. of hot water to obtain the picrate solution and the crystal residue. The latter was subjected to fractional crystallization in 200 ml. of water to yield 2.50 g. of picrate A of m.p. 186°. Concentration of the filtrate and recrystallization of the residue afforded 1.16 g. of picrate B, m.p. 192°. The picrate solution was also concentrated to give 0.66 g. of another picrate C, m.p. 180° which was recrystallized from water. The methanolic filtrate was evaporated to dryness at reduced pressure and the residue, after removing picric acid with ether, was extracted with 100 cc. methanol. The final residue was unreacted D-glucosamine hydrochloride and weighed 6.60 g. Evaporation of the methanolic solution and recrystallization of the residue gave 0.05 g. picrate C, m.p. 180°.

Anal. Picrate A. Calcd. for $C_9H_{11}O_8N_5$: C, 34.1; H, 3.50; N, 22.1. Found: C, 34.2; H, 3.52; N, 22.7. Picrate B. Calcd. for $C_8H_9O_8N_5$: C, 31.7; H, 2.99; N, 23.1. Found: C, 31.6; H, 2.88; N, 23.7.

Picrate C was, evidently, the picrate of I since it had an undepressed melting point on admixture with the authentic sample obtained by the former experiment.

These picrates were then converted to sulfates and hydrochlorides, respectively. *Hydrochloride* from picrate A, m.p. 121°. *Sulfate* from picrate A, m.p. 166°.

Anal. Calcd. for $C_6H_{18}O_6N_4S$: C, 26.3; H, 6.61; N, 20.4; S, 11.7. Found: C, 26.1; H, 6.44; N, 20.7; S, 11.8.

Hydrochloride from picrate B, m.p. 127°. *Sulfate* from picrate B, m.p. 168°.

Anal. Calcd. for $C_4H_{14}O_6N_4S$: C, 19.5; H, 5.73; N, 22.8; S, 13.0. Found: C, 20.3; H, 5.96; N, 23.8; S, 13.1.

Hydrochloride from picrate C, m.p. 178°.

The hydrochlorides and the sulfates derived from picrate A and B were identified as those of *O*-ethylisourea and *O*-methylisourea based on the melting point and the elemental analysis. They were also confirmed by reacting them with methylamine hydrochloride to give methylguanidine picrate. M.p. 198–200°.

The hydrochloride from picrate C was identical with that obtained by the former experiment.

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(11) It was noticed that *O*-methylisourea picrate was obtained together with the picrate of I when unreacted cyanamide remained in the solution as in runs 2, 3, 4, and 5 in Table 1. In these cases, it was necessary to separate the two picrates.