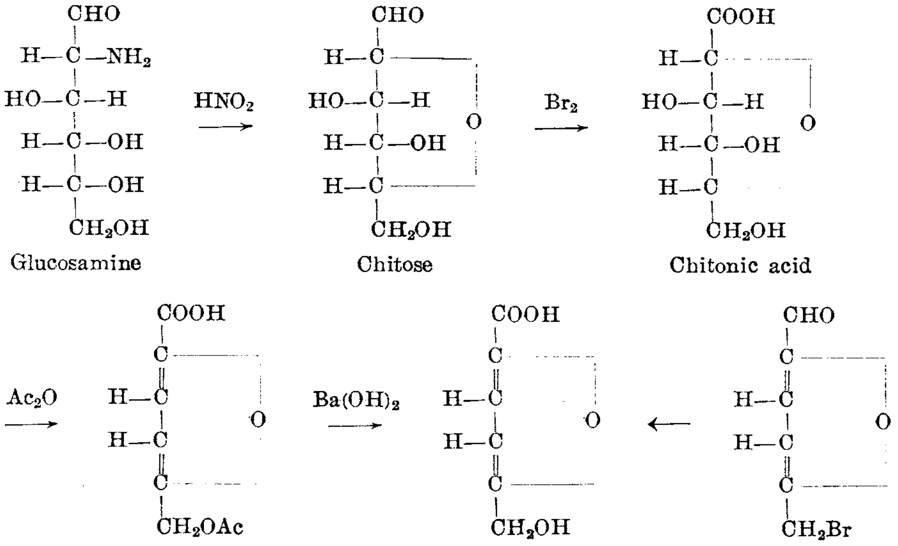
# Studies on Amino-hexoses. II. The Mechanism of the Formation of So-called Chitose from Glucosamine

By Yoshio MATSUSHIMA

(Received September 18, 1950)

The reaction of nitrous acid upon glucosamine (2-amino-2-desoxyglucose) does not proceed, as expected from our "common" knowledge, to the production of glucose or mannose but to the production of so-called chitose. Fischer and Tiemann⁽¹⁾ obtained an acid which they called chitonic acid as its calcium salt by the bromine oxidation of the sugar-like substance which was formed from glucosamine by the action of nitrous acid and named by them chitose. Later, Fischer and Andreae⁽²⁾ treated chitonic acid with acetic anhydride and sodium acetate and obtained hydroxymethyl-pyromucic acid, the constitution of which was confirmed by Haworth, Hirst and Nicholson⁽³⁾ through its preparation from ω-bromo-5-methyl-furfural. Fischer and Andreae⁽²⁾ proposed the 2, 5-anhydro-hexose structure for chitose from the elementary composition of chitonic acid and the reactions mentioned above which can be summerized as follows:

The "abnormal" behavior of glucosamine toward nitrous acid caused several workers to repeat the Fischer's experiment and the complicated results have been reported. Armbrecht⁽⁴⁾ believed that he obtained a hexosazone which was different from glucosazone, but the formation of osazone must be impossible if chitose has the structure mentioned above. Zechmeister and Toth⁽⁵⁾ reported the formation of glucose and they attributed the non-fermentability of the deamination mixture to the co-existence of some inhibitory substance. Schorigin and Makarowa-Semljanskaya⁽⁶⁾ approved of the Fischer's result and obtained the crystalline diphenylhydrazone of chitose. The complexity of the results let the authors of the text-books of sugar chemistry state that "Unsere bisherigen Kenntnisse von der chitose sind recht lueckenhaft. Auch Zweifel an der Einheitlichkeit des Produktes erscheinen berechtigt,"⁽⁷⁾ or "Die Chemie der Chitose bedarf jedoch noch einer eingehenden Bearbeitung."⁽⁸⁾



Hydroxymethylpyromucic acid

---

(1) E. Fischer and F. Tiemann, *Ber.*, **27**, 138 (1894).

(2) E. Fischer and F. Andreae, *Ber.*, **36**, 2587 (1903).

(3) W. N. Haworth, E. L. Hirst and V. S. Nicholson, *J. Chem. Soc.*, **1927**, 1513.

(4) W. Armbrecht, *Biochem. Z.*, **95**, 108 (1919).

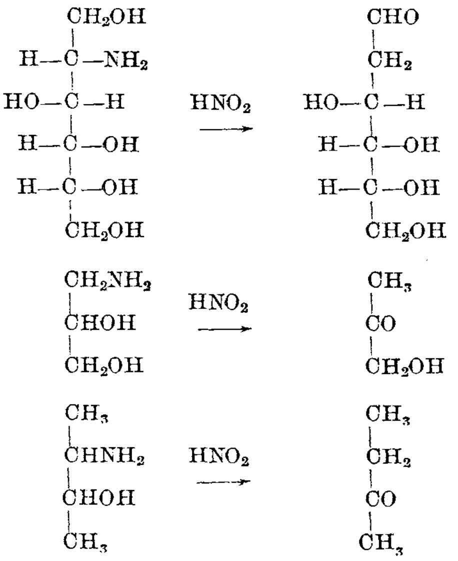
(5) L. Zechmeister and G. Toth, *Ber.*, **66**, 522 (1933).

(6) P. Schorigin and N. N. Makarowa-Semljanskaya *Ber.*, **68**, 965 (1935).

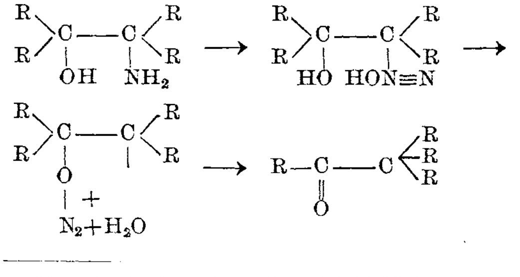
(7) Tollens-Elsner, "Kurzes Handbuch der Kohlenhydrate", 1935, S. 521.

(8) F. Micheel, "Chemie der Zucker und Polysaccharide," 1939, S. 161.

However, the author's deamination experiment on glucosaminol, the hydrogenated product of glucosamine, and on some model substances, together with the search into literatures enabled him to put a reasonable interpretation upon the formation of chitose from glucosamine. The author found that glucosaminol, when treated with nitrous acid, did not produce any trace of sorbitol nor mannitol. The reaction mixture exhibited a reducing action upon Fehling's reagent and crystalline diphenylhydrazone was isolated from it. This was identified with the diphenylhydrazone of 2-desoxy-glucose. The experiments on two model substances proceeded equally on the same line with glucosaminol. Thus, 1-amino-propane-diol-(2, 3) produced acetol and 2-amino-butanol-(3) produced methylethylketone when treated with nitrous acid.



McKenzie(9), (10) first observed the "abnormal" reaction of nitrous acid upon α-hydroxyl-amines and named the reaction "semi-pinacolinic deamination" and explained the reaction by the following scheme.



Several workers(11) have been engaged in the works of this line and the mobility of radicals has been discussed.

On the other hand, the "abnormal" reaction of nitrous acid upon common amines has been observed for a long time. Meyer and Forster(12) reported the production of iso-propyl alcohol beside *n*-propyl alcohol when *n*-propyl amine was deaminated with nitrous acid. Freund and Schoenfeld(13) observed the production of olefinic hydrocarbon $CH_2=C(CH_3)C_6H_{13}$ and tertiary alcohol $(CH_3)_2C(OH)C_6H_{13}$ from the amine $NH_2CH_2CH(CH_3)C_6H_{13}$. According to Levy and Gallais(14) $Ph_2CHCH_2NH_2$ gives stilben $PhCH=CHPh$. Enlargement of the ring is reported by Wallach(15) and Ruzicka(16) when amino-methyl cyclo-paraffin is treated with nitrous acid.

The author's interpretation of these "abnormal" reactions originates in the Arndt-Eistert's(17) synthesis of carboxylic acid homologues:

$$RCOCl \xrightarrow{CH_2NH} RCOCH\overset{-}{N}\equiv\overset{+}{N} \longrightarrow$$

$$RCOCH{<}+N_2 \longrightarrow CO=CHR \xrightarrow{H_2O} HOCOCH_2R$$

The reaction mechanism has an analogy in the Curtius' degradation of acid azide or in the Hofmann's degradation of acid amide.

$$RCO\overset{-}{N}\overset{+}{N}\equiv N \longrightarrow RCON{<} +N_2 \longrightarrow CO=NR$$

$$RCONHX \longrightarrow RCON{<}+HX \longrightarrow CO=NR$$

In these reactions the intermediate bivalent radicals cause the rearrangements of atomic groups. The reactions of amines with nitrous acid can also be interpreted in the light of the same reaction mechanisms which are summerized as follows (the upper schema of p. 146).

McKenzie(9) has already pointed out the similarity between glucosamine and α-hydroxyl-amines in respect of "unusual" behavior toward nitrous acid but he could not interpret explicitly the formation of 2, 5-anhydro-ring in chitose. The author offers the following mechanism for the formation of chitose.

---

(9) A. McKenzie, *J. Chem. Soc.*, **123**, 79 (1923).

(10) A. McKenzie, *J. Chem. Soc.*, **125**, 844 (1924); cf. A. McKenzie and W. S. Dennler, *J. Chem. Soc.*, **125**, 2105 (1924); A. McKenzie and A. K. Mills, *Ber.*, **62**, 284 (1929); A. McKenzie and A. D. Wood, *Ber.*, **71**, 358 (1938).

(11) M. Tiffeneau and J. Lévy, *Compt. rend.*, **183**, 969 (1926); *C. A.*, **21**, 908 (1927); M. Migita, This Bulletin **3**, 308 (1928); S. Kanao and T. Yaguchi, *J. Pharmac. Soc. Japan*, **48**, 252(1928); A. K. Mills, *J. Chem. Soc.*, **1934**, 1565; M. Tiffeneau, J. Levy and E. Dètz, *Bull. Soc Chim.*, (5) **2**, 1871 (1935); *C. A.*, **30**, 1781 (1936).

(12) V. Meyer and F. Forster, *Ber.*, **9**, 535 (1876).

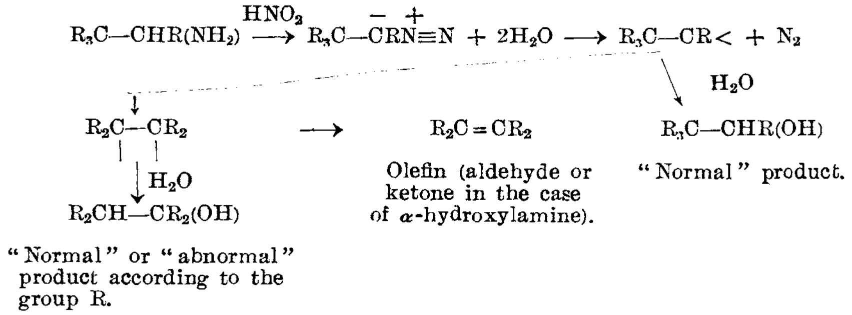
(13) M. Freund and F. Schoenfeld, *Ber.*, **24**, 3350 (1891).

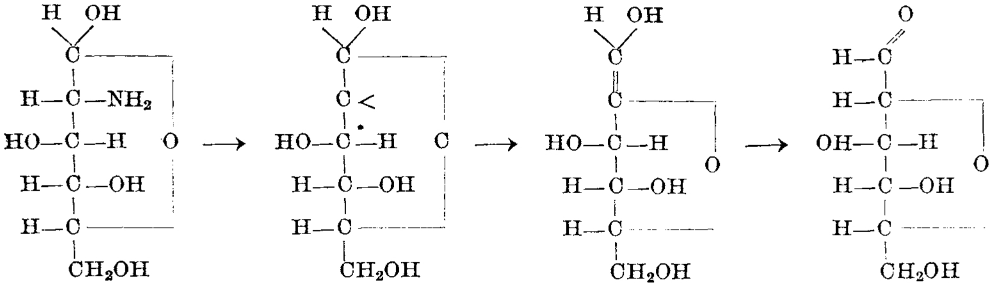
(14) J. Lévy and P. Gallais, *Bull. Soc. Chim.*, **43**, 862 (1928); *C. A.*, **22**, 4504 (1928).

(15) O. Wallach, *Ann.*, **353**, 325 (1907).

(16) L. Ruzicka, *Helv.*, **9**, 399 (1926).

(17) F. Arndt and B. Eistert, *Ber.*, **68**, 200 (1935).

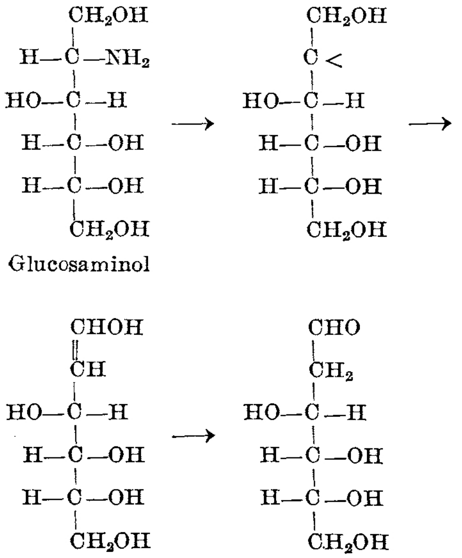




Glucosamine

Chitose

The pyranoid structure of glucosamine has been established by Irvine and Hynd.(18) The intermediate bivalent radical causes the rearrangement of lactol ring, thus forming the furan ring of chitose. Glucosaminol converts into 2-desoxy-glucose following the same reaction mechanism.



2-Desoxy-glucose

The author emphasizes with Berk and Hermans(19) that the replacement of amino-group by hydroxyl is merely one of the probable reaction courses when primary amines of the type $>CHNH_2$ are treated with nitrous acid.

## Experimental

**Preparation of Glucosaminol.**—High pressure hydrogenation of glucosamine in small scale has been reported by Karrer and Meyer.(20) The author hydrogenated a considerable quantity of glucosamine. The solution of 130 g. D-glucosamine hydrochloride in 500cc. of water added with Raney's nickel catalyser prepared from 50 g. of the alloy is heated under shaking at 100° for several hours until the hydrogen pressure becomes constant (initial pressure, 80 atm.). The reaction mixture is filtered from nickel and concentrated *in vacuo*. The resulting thick syrup is added with 400 cc. of absolute alcohol and the seed crystals. Crystalization takes place immediately. The yield of raw crystals of glucosaminol hydrochloride which show a trace of reducing action amounts to 122 g. 40 g. of raw crystals is dissolved in 40 cc. of water and 160 cc. of absolute alcohol is added under vigorous agitation. Colorless fine crystals precipitate immediately which weigh 24.5 g. and melt at 159-160°.

The method of obtaining free base described by Karrer and Meyer(20) which consists in shaking the hydrochloride with alcoholic diethylamine solution is tedious and incomplete. The author proceeded as follows:

---

(18) J. C. Irvine and A. Hynd, *J. Chem. Soc.*, **101**, 1128 (1912).

(19) J. Berk and P. H. Hermans, *Chem. Weekblad*, **22**, 270 (1925); *C. A.*, **19**, 2473 (1925).

(20) P. Karrer and J. Meyer, *Helv.*, **20**, 626 (1937).

10 g. of the hydrochloride is dissolved in 10 cc. of water and 5 g. of diethyl amine is added slowly. After adding 150 cc. of alcohol, the rubbing of the wall accelerates the growth of crystals. The colorless crystals of the free base thus obtained are quite free from chlorine and melt at 131-132°. The yield amounts to 75% of theory.

**Deamination of Glucosaminol.**—6.0 g. of glucosaminol (free base) dissolved in 50 cc. of water is added with 3 g. of sodium nitrite dissolved in 50 cc. of water and then with 3 cc. of acetic acid. Evolution of nitrogen gas ceases after over-night standing at room temperatures. The reaction mixture is concentrated *in vacuo* and is added with 2 g. of diphenylhydrazine dissolved in 5 cc. of alcohol. After an over-night standing the rubbing of the wall accelerates crystalization. The crystals thus obtained weigh 2.6 g. and melt at 175-177°. 0.2 g. of the same substance is recovered from the mother liquor. The substance which melts at 178-179° after being recrystalized twice from 96% alcohol shows no melting point depression when mixed with the diphenylhydrazone of 2-desoxyglucose which is prepared from D-glucal following the method of Bergmann, Schotte and Lechinsky.(21) Analyses give the following results:

Found: C, 65.56; H, 6.74.
Calcd. for $C_{16}H_{22}O_4N_2$: C, 65.44; H, 6.71.

**Preparation of 1-Amino-propane-diol and 2-Amino-butanol-(3).** (a) 1-Amino-propane-diol was prepared following the method of Knorr(22) taking account of the report of Hirata.(23) The substance boiling at 140-141°/9 mm. in redistillation is colorless viscous liquid which has no reducing action toward Fehling's reagent or ammoniacal silver solution.

(b) 2-Amino-butanol-(3) was prepared following the method of Strauss(24) which was somewhat modified.

Diacetyl monoxime is reduced with sodium amalgam under cooling with ice and salt in acid conditions. The reaction mixture is made alkaline and extracted with ether in a automatic apparatus for 5 days. The substance boiling at 53.5-55.5°/7 mm. in redistillation is colorless viscous liquid which has a pyridine-like odor. The yield is 2.3 g. from 5 g. of diacetyl monoxime.

**Deamination of 1-Amino-propane-diol and 2-Amino-butanol-(3).** (a) 10 g. of 1-amino-propane-diol is dissolved in 50 cc. of water and the solution of 8.5 g. sodium nitrite in 10 cc. water is added. 25 cc. of 6 *N*-sulfuric acid is dropped into the mixture under stirring and ice-water cooling. After letting it stand for 4.5 hours at room temperatures hydrogen gas is passed until no trace of nitrous acid remains. Van Slyke amino determination carried out with a portion of the reaction mixture reveals that 2.8 g. of the starting amino-alcohol remains intact. The reaction mixture which exhibits now a strong reducing action upon Fehling's reagent or upon ammoniacal silver solution is neutralized with barium carbonate and the filtrate from barium sulfate is divided into two equal portions. One is treated with phenylhydrazine acetate and the other with semicarbazide. Precipitation takes place immediately in each case and the yield of raw phenylosazone of acetol melting at 137° with decomposition is 2.28 g. and that of semicarbazone melting at 197.5° with decomposition is 1.09 g. The yield of both derivatives of acetol corresponds to about 20% of the starting amino-alcohol consumed. The osazone melts at 146.5-147° with decomposition when once recrystalized from alcohol. The analyses give the following results:

Found: C, 71.75; H, 6.47; N, 22.42.
Calcd. for $C_{15}H_{16}N_4$: C, 71.40; H, 6.39; N, 22.21.

The semicarbazone melts at 197-198° with decomposition when once recrystalized from water. The analyses give the following results:

Found: C, 36.54; H, 7.21; N, 32.56.
Cald. for $C_4H_9O_2N_3$: C, 36.66; H, 6.91; N, 32.04.

(b) 1.06 g. of 2-amino-butanol-(3) is dissolved in 10 cc. of water and the solution of 1.4 g. of sodium nitrite in 15 cc. of water is added. 3 cc. of acetic acid is dropped at room temperatures into the mixture under stirring. After an over-night standing the mixture is neutralized with sodium hydroxide to a *p*H value of 5 and distilled to about 80% of the whole. The distillate upon treatment with dimedone solution gives a precipitate which may, be isobutylaldomedone(25) but can not be identified owing to the small amount of the material. The mixture is again distilled and the distillate is now added with acetic solution of *p*-nitrophenylhydrazine. Precipitation takes place immediately. The yellow crystals thus formed weigh 0.88 g. and melt at 90-100°. After being recrystalized three times the specimen melts at 124-124.5° alone or at 125-126° with the authentic specimen of *p*-nitrophenylhydrazone of methyl ethyl ketone which melts at 127.5-128°. Analyses give the following results:

Found: N, 20.37.
Calcd. for $C_{10}H_{13}C_2N_3$: N, 20.28.

The author expresses his gratitudes for the guidance of Prof. Dr. S. Akabori and for the assistance from Mr. K. Okawa and Mr. T. Ikenaka.

*Science Department, Osaka University, Osaka*

---

(21) M. Bergmann, H. Schotte and W. Lechinsky, *Ber.*, **55**, 165 (1922).

(22) L. Knorr and E. Knorr, *Ber.*, **32**, 750 (1899).

(23) Y. Hirata, K. Inukai and T. Tsujiuchi, *J. Chem. Soc. Japan*, **69**, 59 (1948).

(24) E. Strauss, *Ber.*, **33**, 2827 (1900).

(25) S. Akabori, *J. Chem. Soc. Japan.*, **59**, 1132 (1938).