Studies on Amino-hexoses. I. A New Method for Preparing Crystalline D-Arabinose

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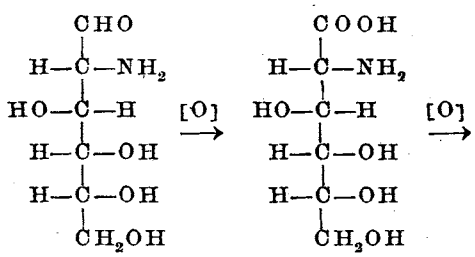
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The preparation of D-arabinose has been dependent on the methods of Ruff,[1] of Wohl-Zemplen[2] or of Weermann[3], all of which being consisted of degrading D-glucose.

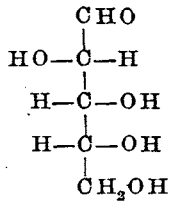
The author took up D-glucosamine as a starting material which is much accessible in this country. The author's aim was that D-glucosamine, a sort of α-amino-aldehyde, would undergo an oxidation analogous to the oxidation of α-amino-acids as exhibited by Dakin[4] and Langheld[5] who obtained aldehydes with one carbon atom less than the starting amino-acids. In fact, Herbst[6] has oxidized glucosamine with chloramine-T (*p*-toluenesodium sulfonchloramide) and has

(1) O. Ruff, *Ber.*, **31**, 1573(1898); R. C. Hockett and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 1632(1934).
(2) G. Zemplén, *Ber.*, **59**, 1254 (1926); "Organic Syntheses" **20**, 14, 74.
(3) R. A. Weermann, *Rec.*, **37**, 16 (1917); *C. A.*, **12**, 1453(1918).
(4) H. D. Dakin, *J. Biol. Chem.*, **1**, 171(1905).
(5) K. Langheld, *Ber.*, **42**, 2360(1909).
(6) R. M. Herbst, *J Biol. Chem.*, **119**, 85(1937).

obtained D-arabinose in the form of benzylphenylhydrazone in 20—40% yields. He concludes that the reaction proceeds as below from the fact that the intermediate product, glucosaminic acid, is also oxidized by chloramine-T giving D-arabinose in about 40% yield

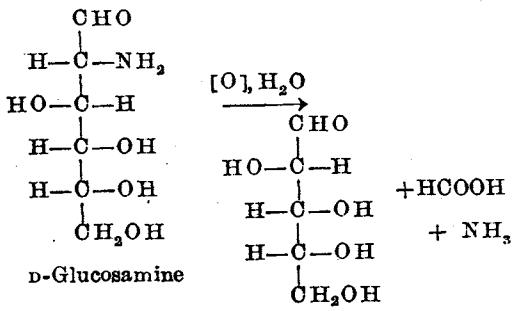


D-Glucosamine D-Glucosaminic acid



D-Arabinose

The author oxidized D-glucosamine with sodium hypochlorite, the amount of which at first being two oxygen-equivalents, assuming that the reaction would proceed as above. Were the mentioned reaction scheme correct, the first carbon atom of glucosaminic acid should be liberated as carbonic acid. But the fact is that D-arabinose is produced in about 30% yield without liberating any carbonic acid. Then, one must conclude that the reaction formulae described above does not represent the fact and a reasonable interpretation of the reaction may be that glucosamine decomposes into arabinose, formic acid and ammonia with one oxygen-equivalent of the oxidant and water as represented below:



D-Glucosamine D-Arabinose

The author's interpretation was confirmed by the experiment in which D-glucosamine was oxidized with one oxygen-equivalent of hypochlorite under ice-water cooling, where D-arabinose-diphenylhydrazone was isolated in a yield above 70% to the theoretical amount. Furthermore, the production of formic acid was confirmed by its reducing action on mercuric chloride and by the analysis of the calcium salt. The application of the method of sugar analysis proposed by Moore and Link[7] revealed also the production of D-arabinose in a yield as high as 75% theoretically. The good yield of the reaction enabled the author to isolate directly crystalline D-arabinose, the yield of the specimen melting at above 150° being round 20% and in the most favourable case 27% yield was secured.

From the results mentioned above it is concluded that glucosamine exhibits a novel type of reaction when oxidized with hypochlorite. It may be worth trying to ascertain that other aminoaldehydes, a type of compound not so familiar to us, have the same character with glucosamine. To this purpose the author took up chondrosamine, a naturally occuring aminoaldehyde, as an example from which it is expected that D-lyxose is formed. The author treated chondrosamine with hypochlorite equivalent to one atom oxygen in the like manner as glucosamine and obtained D-lyxose as its *p*-bromphenydrazone in a yield of 21% to the theory. The formation of formic acid was also confirmed. The low yield of the *p*-bromophenylhydrazone in comparison with the D-arabinose derivative from glucosamine may be due to the incompleteness of the combination of lyxose and *p*-bromophenylhydrazine.

Experimental Part

Preparation of Glucosamine Hydrochloride.— 3 kg. raw dried crab shell is immersed in 20 l. of 2*N*-hydrochloric acid for 3 days with occasional agitation. After washing and drying 1.1 kg. decalcified material is obtained. 500 g. of this material is thrown into 1.5 l. of hydrochloric acid (about 30%) and is heated on a boiling water-bath for 5 hours. To this hydrolysate 1.5 l. water is added and the solution is filtered through Buchner's funnel. The filtrate is concentrated *in vacuo* to about one third of its volume and is placed in a cold room. The colored raw crystals of glucosamine hydrochloride separate and are filtered without washing. The raw crystals are dissolved in as little hot water as possible and are treated with charcoal. The decolorized filtrate is concentrated *in vacuo* until considerable amount of crystals separate which are filtered and washed with a small amount of cold water. The twice

(7) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 293 (1940).

repetition of decolorization and concentration of the mother liquor enables above 80% of the raw crystals to be obtained in practically pure state. The yield is 150 g., $[\alpha]_D$ (final), +70.15°.

Oxidation of Glucosamine with Hypochlorite Equivalent to One Atom Oxygen. (a) **Isolation of D-Arabinose as its Diphenylhydrazone.**—5 g. D-glucosamine hydrochloride is dissolved in 200 cc. water and under ice-water cooling and mechanical stirring the solution of 1 g. sodium hydroxide is added. To this, the solution of sodium hypochlorite is added dropwise at the reaction temperatures below 8°. The oxidation takes place very fast and the reaction is almost completely finished as soon as the oxidant is added. The reaction mixture is neutralized with dilute hydrochloric acid to a weak acid reaction and concentrated *in vacuo* at the temperatures below 40°. The syrup thus obtained is filtered from inorganic salts which have separated during the concentration and the salts are washed with 80% alcohol. A definite portion of the alcoholic solution thus obtained is treated with the alcoholic solution of diphenylhydrazine and the diphenylhydrazone formed of D-arabinose is filtered after over-night standing and washed with 50% alcohol. The crystals melt at 197—198° with decomposition, the yield of which is 73% theoretically to starting D-glucosamine. Recrystallization from alcohol raises the melting point to 198—200° and no melting point depression is observed when mixed with the authentic specimen. Analyses give the following results:

Found: C, 64.88; H, 6.55; N, 8.44.
Calcd. for $C_{17}H_{20}O_4N_2$:
C, 64.54; H, 6.37; N, 8.86.

(b) **Analysis of the Oxidation Mixture by the Method of Moore and Link**[7].—Using 22.6 g. glucosamine hydrochloride the oxidation is carried out as above. The syrupy product separated from most of the inorganic salts is dissolved in 140 cc. of methanol of which 70 cc. is taken for analysis: 26 g. iodine in 370 cc. methanol is combined with the above methanolic solution of sugar and to this mixture 4% methanolic solution of potassium hydroxide is added dropwise under stirring at 40° until the color of iodine is extinguished. After standing overnight at room temperatures the fine crystals which have separated are collected and washed with methanol. Potassium arabonate thus obtained melts at 210—212° with decomposition and weighs 6.6 g. which corresponds to 62% of theoretical amount to the starting aminosugar. It has a specific rotation $[\alpha]_D^{28}$ − 6.3°. The melting point and the specific rotation given in the literature[7] for the L-isomer is 211° and $[\alpha]_D^{25}$ + 4.5° respectively.

1 g. of potassium arabonate is condensed with *o*-phenylene-diamine and 0.9 g. arabo-benzimidazole melting at 235° with decomposition is obtained. After recrystallization from 50% alcohol the specimen melts at 237—237.5° and shows no melting point depression when mixed with authentic specimen. The specific rotation $[\alpha]_D^{28}$ is −48.8° in 5% citric acid solution, while $[\alpha]_D^{25}$ given in the literature[7] for the L-isomer is +49.2°. Analysis gives the following results;

Found: C, 55.14; H, 6.00; N, 11.75.
Calcd. for $C_{11}H_{14}O_4N_2$:
C, 55.45; H, 5.92; N, 11.76.

The calculation from the yield of potassium arabonate shows a 75% yield of D-arabinose when one takes account of the empirical factor given in the literature[7].

The distillate of the acidified oxidation mixture gives calcium formate when treated with calcium carbonate in the usual way. The calcium salt in hydrochloric acid solution reduces mercuric chloride to insoluble mercurous chloride and its calcium content is 30.48% while the theoretical value is 30.80%.

Preparation of Crystalline D-Arabinose.—21.6 g. glucosamine hydrochloride dissolved in 100 cc. of water is placed with 200 g. of cracked ice in a beaker surrounded by ice-water. The mixture is then added with 4 g. of sodium hydroxide dissolved in 20 cc. water. Sodium hypochlorite solution (which is prepared by dissolving 7.1 g. of chlorine in a ice-cold solution of 20 g. sodium hydroxide dissolved in 50 cc. water) is run in immediately from the separating funnel under good mechanical stirring. The reaction proceeds smoothly at −3°. After consumption of the oxidant the reaction mixture is neutralized with 6*N*-hydrochloric acid to *p*H 3, and is as far concentrated as possible *in vacuo* at the bath temperature below 45°. The syrup thus obtained is filtered from the considerable amount of inorganic salts and the latter is washed with as little methanol as possible. The methanolic solution of the sugar is again concentrated *in vacuo* and the small amount of salts which have deposited is filtered off. The brown colored thick syrup thus obtained is added with 40 cc. of glacial acetic acid and put in an ice-box. Crystallization takes place shortly. After letting it stand for 2 days the crystal cake is filtered and washed completely with glacial acetic acid. The almost colorless crystals thus obtained weigh 6.7 g. and melt at 127—129°. The raw crystals are dissolved in 4.3 cc. of hot water and then added with 25 cc. of methanol, treated with charcoal, and after seeding put in an ice-box for 2 days. 4.0 g. of D-arabinose meltiug at 150—151° is obtained which is quite free from chlorine ion.

The recrystallized specimen which melts at 155—156° has the specific rotation −103.0° (final) and the following composition:

Found: C, 39.84; H, 7.16.
Calcd. for $C_5H_{10}O_5$: C, 40.00; H, 6.71.

Oxidation of Chondrosamine to D-Lyxose.—Chondrosamine was prepared from the cartilage of shark or from bovine trachea through

chondroitin-sulfuric acid following the methods of Kondo[8], or Fuerth, Bruno, Boyer and Peschek[9] and Levene and La Forge[10]. Chondrosamine hydrochloride obtained was not quite pure though it was a colorless fine crystal.

4.8 g. chondrosamine hydrochloride is dissolved in 100 cc. water and under ice-water cooling and mechanical stirring, 17 cc. of *N*-sodium hydroxide is added. 18 cc. of sodium hypochlorite which is equivalent to one atom oxygen is dropped within the next 10 minutes. After 30 minutes more the reaction mixture is slightly acidified with dilute hydrochloric acid and evaporated *in vacuo* at the temperature below 40°. The distillate contains formic acid. The syrup is freed from inorganic salts and condensed with *p*-bromophenylhydrazine.

The water solution of the syrup is mixed with the alcoholic solution of *p*-bromophenylhydrazine and a small amount of precipitate which is inorganic salts is filtered off after over-night standing. A thick syrup which is obtained by concentrating the filtrate *in vacuo* becomes crystalline when seeded. After letting at stand over-night the crystals are filtered and washed with water to be freed from a syrupy matter. The orange-yellowish crystals thus obtained melt at 148—151° and weigh 1.1 g.

0.5 g. of raw crystals is recrystallized from 30% alcohol. Slightly yellowish needles thus obtained weigh 0.4 g. and melt at 154—155° and show no melting point depression when mixed with the *p*-bromophenylhydrazone of D-lyxose which is prepared from calcium D-galactonate by the Ruff-degradation. Analyses give the following results:

Found: C, 41.01; H, 4.78; N, 8.43.
Calcd. for $C_{11}H_{15}O_4N_2Br$:
C, 41.39; H, 4.74; N, 8.78.

$[\alpha]_D^{28}$ in pyridine in as follows:

Time after solution	$[\alpha]_D^{28}$	$[\alpha]_D$ in the literature[11]
10 min.		+31.8°
15	+33.9°	
60	+27.9	
24 hrs.	+ 9.97	+ 7.8
48		+ 7.8

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(9) O. Fuerth, T. Bruno, R. Boyer and K. Peschek, *Biochem. Z.*, **294**, 153 (1937).
(10) P. A. Levene and La Forge *J. Biol. Chem.*, **18**, 123 (1914).
(11) P. A. Levene and La Forge, *J. Biol. Chem.*, **18**, 325 (1914).