

aminopropionamide-2-methyl-5-arsonic acid and N-phenyl- β -propionamide-2-methyl-4-arsonic acid have been described for the first time.

3. 3-Amino-4-hydroxy-4'-propionamido-arsenobenzene and its hydrochloride have been prepared.

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NEW CONDITIONS FOR THE FORMATION OF GLUCOSAZONE

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Hydrazine derivatives of recently described compounds in which uronic acids are conjugated with sugars¹ have not yet been studied. We desired to prepare such derivatives of the aldobionic acid isolated from gum arabic^{1b} in order further to characterize it. While a number of hydrazine derivatives of glucuronic acid have been prepared, the lack of agreement in the results of various investigators in this field seems to indicate that the reaction is not a simple one.²

A preliminary investigation of the action of phenylhydrazine on the simpler sugars, glucose, mannose and fructose was therefore made. The chemistry of glucose phenylhydrazone³ is complicated by the existence of at least two isomeric forms. Although we have spent a considerable amount of time attempting to prepare the pure isomers, our results indicated that these are not readily produced by any of the published methods. Since our results were not conclusive, further discussion in this paper will be confined to a description of the formation of glucosazone from glucose and fructose, and from their phenylhydrazones under conditions not ordinarily considered favorable to osazone formation, and of the behavior of mannose phenylhydrazone under similar conditions.

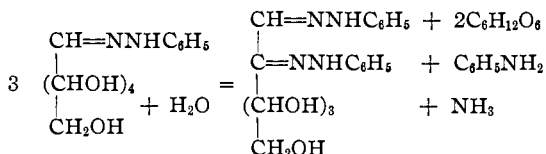
The precipitation of glucosazone from a cold dilute acetic acid solution of 1 equivalent of glucose and 1.5 equivalents of phenylhydrazine was observed by Jacobi.^{3c} This investigator did not examine the reaction further. It was found in the present work that when glucose is treated under

¹ (a) Heidelberger and Goebel, *J. Biol. Chem.*, **74**, 613, 619 (1927); (b) Butler and Cretcher, *THIS JOURNAL*, **51**, 1519 (1929); (c) see also Anderson and Sands, *ibid.*, **48**, 3172 (1926).

² References to the literature of this subject may be found in (a) Van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren," Gebrüder Borntraeger, Berlin, 1920, Chapter VI, and in (b) Tollens, "Kurzes Handbuch der Kohlenhydrate," Barth, Leipzig, 1914, pp. 766-767.

³ (a) Fischer, *Ber.*, **20**, 824 (1887); (b) Skraup, *Monatsh.*, **10**, 406 (1889); (c) Jacobi, *Ann.*, **272**, 172 (1892); (d) Simon and Benard, *Compt. rend.*, **132**, 564 (1901); (e) Behrend, *Ann.*, **353**, 106 (1907); (f) Behrend and Lohr, *ibid.*, **362**, 78 (1908); (g) Behrend and Reinsberg, *ibid.*, **377**, 189 (1911).

similar conditions with an equimolecular proportion of phenylhydrazine, a precipitate of glucosazone is formed after a few hours. On prolonged standing a 62.5% yield of glucosazone, based on the amount of phenylhydrazine used, was obtained. In the absence of acetic acid this reaction did not take place.⁴ No other crystalline product could be isolated from the mother liquors. It was also shown that glucose phenylhydrazone itself, when allowed to stand at room temperature in dilute acetic acid, changes to glucosazone. The yield was 85% based on the equation



The formation of glucosazone under these conditions probably involves an intramolecular oxidation and reduction the mechanism of which will not be discussed in the present paper. That this is also the main reaction in alcoholic solutions of glucose and phenylhydrazine containing acetic acid was shown by the isolation of a 63% yield of glucosazone on attempting to repeat the preparation^{3f} of Behrend's glucose α -phenylhydrazone. It was not possible to isolate any hydrazone from the glucosazone mother liquors.

Attempts to prepare crystalline fructose phenylhydrazone were unsuccessful. The reaction product in the absence of solvent was a gummy mass which could not be crystallized. However, equimolecular proportions of fructose and phenylhydrazine in dilute acetic acid at room temperature gave a 67% yield of glucosazone. The gummy reaction product of fructose and phenylhydrazine also changed to glucosazone when treated in a similar manner.

Mannose phenylhydrazone was found to be stable under these conditions. However, when warmed in dilute acetic acid it gave rise to black resinous material which could not be purified. That mannose was liberated in this reaction was shown by the fact that the mother liquor, after removal of the resinous material by filtration, deposited mannose phenylhydrazone on treatment with phenylhydrazine.

Experimental

Attempted Preparation of Glucose Phenylhydrazone by the Method of Behrend and Lohr.^{3f}—To a solution of 20 g. of glucose in 15 g. of glacial acetic acid and 5 cc. of water was added a solution of 15 g. of phenylhydrazine in 160 cc. of absolute alcohol. On thorough mixing a clear yellow solution was obtained. After standing for three days 10.5 g. of crude yellow crystals was filtered from the solution. On washing with acetone 7.5 g. of bright yellow crystals, melting at 206–207°, was obtained. The

⁴ Cameron, *THIS JOURNAL*, **48**, 2233 (1926), has noted the effect of acetic acid as a catalyst in the reaction of weak bases such as aniline with glucose. His conclusions are confirmed by the experiments herein reported.

mother liquor from these crystals was allowed to stand for two days, and deposited during this time a further quantity of crystalline material which after washing with acetone weighed 2.0 g. One additional gram of crystals was recovered from the mother liquors on concentration *in vacuo* to about 60 cc. This material was recrystallized from 70% alcohol. The purified substance, which melted at 210°, did not depress the melting point of an authentic sample of glucosazone which melted at 210°. The total yield of glucosazone was 10.5 g. or 63.2% of the theoretical based on the amount of phenylhydrazine used.

Preparation of Glucose Phenylhydrazone by the Method of Fischer.^{3a}—The yield from 5 g. of glucose, 5 g. of phenylhydrazine and 2.5 g. of water was 4.5 g. or 60% of the theoretical of hydrazone melting at various temperatures between 140 and 150°. On addition of ether to the concentrated sirupy mother liquor, 1.7 g., or 22.7% of the theoretical of Skraup's phenylhydrazone^{3b} melting at 111–112° was obtained. The total hydrazone yield was thus 6.2 g. or 82.7% of the theoretical.

A modification of this method was tried in the hope of obtaining the glucose α -phenylhydrazone of Behrend. To a suspension of 18 g. of glucose in 6 cc. of water and 25 cc. of absolute alcohol was added a solution of 11 g. of phenylhydrazine in 25 cc. of absolute alcohol. The resulting clear solution was allowed to stand overnight at room temperature and then for several days in the ice box. The crystalline mass was filtered and the crystals were washed with ether. The material was then precipitated several times from alcohol solution by ether. The yield was 17 g. of Skraup's phenylhydrazone melting at 110–112°, or 63% of the theoretical.

Preparation of Mannose Phenylhydrazone.—This compound was prepared from 3.6 g. of mannose and 2.2 g. of phenylhydrazine in the usual way. The yield was 4.8 g. (89% of the theoretical) of pure mannose phenylhydrazone melting at 200–201°.

Attempted Preparation of Fructose Phenylhydrazone.—The method of Landrieu⁵ applied to the production of fructose phenylhydrazone gave unsatisfactory results. The preparation was therefore attempted without solvent. To 1.8 g. of fructose was added 1.8 g. of phenylhydrazine and the mixture was stirred until uniform. It was allowed to stand at room temperature for forty-eight hours. The sticky mass was extracted with ether to remove the excess of phenylhydrazine. On drying about 1.5 g. of a soft gummy mass was obtained. It could not be crystallized.

Preparation of Glucosazone.—Glucosazone was prepared by the usual method. The substance purified by crystallization from 60% alcohol and washing with acetone melted at 210°. The melting point was not changed by recrystallization from 60% alcohol; α_D^{20} -2.625° for $l = 2$ and $c = 2$, ten minutes after solution in a mixture of 1 part of pyridine and 1.5 parts of alcohol; $[\alpha]_D^{20}$ (initial) -65.6° . The rotation became constant after twenty-five hours at -1.44° ; $[\alpha]_D^{20}$ (final) -36.0° .

The Formation of Glucosazone from Equimolecular Proportions of Glucose and Phenylhydrazine.—Thirty-six g. of glucose was dissolved in 200 cc. of water and to the solution was added a mixture of 50 cc. of water, 10 cc. of glacial acetic acid and 21.6 g. of phenylhydrazine. The resulting milky suspension was allowed to stand at room temperature. The following day a large amount of yellow crystalline solid had separated. The amount of solid increased on standing. After six days the material was filtered off and washed with a little water. It melted at 183° with decomposition. The crude product, when purified as described above, melted at 209–210°. Six g. of bright yellow crystals was obtained in this crop. The filtrate from the crude crystals, on standing for several weeks longer, deposited a further quantity of glucosazone which after purification weighed 7 g.

The combined mother liquors were concentrated to about 30 cc. *in vacuo* and diluted

⁵ Landrieu, *Compt. rend.*, **142**, 182 (1906).

with an equal volume of alcohol. Water was then added until no further precipitation occurred and the mixture was filtered. The crude solid material on purification yielded two additional grams of pure osazone. The total yield was thus 15 g. of pure substance or 62.5% of the theoretical based on the equation given in the first part of this paper. The substance did not depress the melting point of pure glucosazone prepared as described above; $[\alpha]_D^{20}$ (initial) -56.3° for $c = 2$ and $l = 2$; $[\alpha]_D^{20}$ (final) -36.5° .

A control experiment in which 1.8 g. of glucose was treated with 1.1 g. of phenylhydrazine in 12.5 cc. of water in the absence of acetic acid was made. No precipitate of glucosazone was formed after standing for two days at room temperature.

Formation of Glucosazone from Glucose Phenylhydrazine.—One cc. of glacial acetic acid was added to a solution of 2 g. of Fischer's glucose phenylhydrazine in 10 cc. of cold water. The solution became cloudy after standing for two hours at room temperature. After standing overnight, a large amount of yellow insoluble material had separated from solution. The mixture was filtered ten days later. The yield was 0.75 g. of recrystallized glucosazone melting at 210° or 85% of the theoretical based on the equation given on page 3162. The substance did not depress the melting point of a sample of pure glucosazone which melted at 210° .

The presence of glucose was demonstrated in the filtrate from the glucosazone precipitate by warming with a little phenylhydrazine and a few drops of acetic acid. The glucosazone which was formed was filtered off and purified in the usual way. It melted at 210° .

A control experiment was run with 2 g. of glucose phenylhydrazine in 10 cc. of water without the addition of acetic acid. There was no glucosazone formation in this experiment.

Two grams of Skraup's phenylhydrazine was treated similarly. The yield of purified osazone melting at 210° was 0.7 g. or 80% of the theoretical.

Formation of Glucosazone from Equimolecular Proportions of Fructose and Phenylhydrazine.—A suspension of 2.2 g. of phenylhydrazine in 5 cc. of water was added to a solution of 3.6 g. of fructose in 15 cc. of water and 1.5 cc. of acetic acid. The mixture became cloudy after several hours and after several days the deposition of glucosazone appeared to have ceased. The mixture was filtered and the glucosazone purified by methods which have been described. The yield was 1.6 g. of pure glucosazone melting at $209-210^\circ$, or 67% of the theoretical.

Formation of Glucosazone from the Crude Reaction Product of Equimolecular Proportions of Fructose and Phenylhydrazine.—One g. of the sticky product was dissolved in 5 cc. of water and filtered from a trace of yellow insoluble material. Several drops of acetic acid were added and the mixture was allowed to stand for two days at room temperature. The yield of pure glucosazone melting at 210° was 0.35 g. or about 80% of the theoretical.

Behavior of Mannose Phenylhydrazine in the Presence of Acetic Acid.—A mixture of 2 g. of mannose phenylhydrazine, 10 cc. of water and 1 cc. of acetic acid did not change after standing for two days at room temperature except for a slight darkening of the liquid. It was then heated for two hours on a water-bath. Some dark resinous product was formed from which nothing could be separated. That the mother liquor contained mannose was shown by the fact that on treatment with a little phenylhydrazine it gave an almost immediate precipitate of mannose phenylhydrazine which after recrystallization melted at $200-201^\circ$. It did not depress the melting point of a sample of pure mannose phenylhydrazine melting at this temperature.

Summary

1. The reaction of glucose or fructose with an equimolecular amount of phenylhydrazine in cold dilute acetic acid gives a high yield of glucosazone,

instead of glucose phenylhydrazone as might be expected. Mannose, on the other hand, yields only the hydrazone under these conditions.

2. Glucose and fructose phenylhydrazones are converted practically quantitatively to glucosazone on standing in dilute acetic acid. Mannose phenylhydrazone is unchanged by this treatment.

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PHOTOCHEMICAL DECOMPOSITION OF LACTIC ACID

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This study of the photochemical decomposition of lactic acid is part of an investigation of the oxidation of lactic acid by oxygen in the presence of ultraviolet radiation. The investigation was undertaken as a result of a consideration of the theories of physiological chemists in regard to the oxidation of lactic acid in the body. It appeared that a study of the oxidation from a purely chemical viewpoint would be of value in connection with these theories.

Before this could be done a more thorough investigation of the decomposition of the acid, in the absence of oxygen, by ultraviolet radiation was necessary. There is a marked conflict between the results of other investigators over the decomposition of lactic acid in solution by the radiations of a quartz mercury arc. The differences found may be explained, in part, by taking into consideration the fact that some observers exposed the irradiated solution to air and some took the necessary precaution to see that no oxygen was present. Another discrepancy arose from the fact that some observers used glass containers. The wave lengths of radiation that decompose lactic acid are absorbed by glass.

Bolin¹ states that there is no decomposition of lactic acid in glass flasks. Euler's² investigation showed that at 70° alcohol and a gas mixture composed of 90% of carbon dioxide and 10% of hydrogen and carbon monoxide resulted. No substance was formed that reduced Fehling's solution. More recently Euler,³ in experiments carried out both at 30 and 70°, confirms these results. He states that no reaction takes place in glass flasks. In all his experiments he finds an induction period of about two hours. This was, doubtless, the length of time necessary to produce enough carbon dioxide to saturate the solution. Euler's results were confirmed by Scharz⁴ with the exception that methane was also found in small

¹ Bolin, *Z. physik. Chem.*, **87**, 497 (1914).

² Euler and Lindberg, *Biochem. Z.*, **39**, 410 (1912).

³ Euler and Ryd, *ibid.*, **51**, 96 (1913).

⁴ Scharz, *Arch. ges. Physiol.*, **170**, 650 (1918).