and the third to the truxinic acids. This third polymer is formed by the action of sunlight on methyl benzalpyruvate in solution, a reaction that usually gives an isomeric product.

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THE ACTION OF GUANIDINE UPON GLUCOSE IN THE PRESENCE AND ABSENCE OF OXYGEN

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Nef[1] divided the effects of alkali upon sugars into two groups, (1) the more profound reactions involving the destruction or oxidation of the sugars and (2) the less profound effects involving merely transformations, such as the isomerization phenomena described by Lobry de Bruyn and Alberda van Ekenstein.[2] Woodyatt[3] has pointed out the close parallelism between these two groups of effects and the effects exerted on sugars in the normal and diabetic organism, respectively. The diabetic accomplishes transformations of Type 2 very effectively, but fails to a greater or less extent in accomplishing effects of Type 1. When the diabetic is supplied with insulin his lost capacity for transformations of Type 1 can be restored. From this it might appear that insulin acts upon glucose *in vivo* as strong alkalies act upon it *in vitro*. There is, however, no reason for thinking that insulin is a strong alkali like potassium hydroxide.

Recent experiments on the behavior of ammonium hydroxide, in comparison with potassium and sodium hydroxides, on the oxidation of butyric acid with hydrogen peroxide,[4] give a basis for the belief that catalyses attributed to the hydroxyl-ion concentration of the alkali used are also influenced under suitable conditions by other factors such as the nature of the positive ion. It was in pursuit of the idea that somewhat similar relations might hold for the alkali effects of Type 1 that some preliminary fundamental experiments upon the action of guanidine were made.

The experiments here described were carried out before insulin became known and are offered in the present form because they may have considerable interest in other ways than those suggested above. The results in general were indecisive because the effects of guanidine on glucose proved to be much like those of potassium hydroxide.

[1] Nef, *Ann.*, **403**, 204 (1914). See also *Ann.*, **357**, 214 (1907); **376**, 1 (1910).

[2] de Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 158, 203 (1895); **15**, 92 (1896); **16**, 257, 262 (1897).

[3] Woodyatt, *J. Biol. Chem.*, **20**, 129 (1914).

[4] Witzemann, *ibid.*, **49**, 123 (1921).

After the experiments were under way, the interesting study of Morrell and Bellars[5] on the guanidine hexosates was found. Their work relates mainly to transformations of Type 2, while the present experiments have been directed mainly to determine the extent and scope of reactions of Type 1 as brought about by guanidine.

Experimental Part

Optical and Reducing Properties of Guanidine-Glucose Solutions.—To 4.76 g. of free guanidine[6] in 160 cc. of water was added 2 g. of pure glucose. Half of this solution was placed in the incubator at 37°. The other half was kept at room temperature. The solution as prepared contained 1.62 g. of glucose as derived from its optical rotation and 1.95 g. of glucose as determined by the Benedict-Osterburg reduction method.[7]

Three days later the solution kept at room temperature showed a rotation of —0.08° in a 10cm. tube; 0.80 g. of sugar by the Benedict-Osterburg method, that is, 20.0% of the reducing power had disappeared, indicating that 20% of the sugar used had been destroyed. The solution kept in the incubator for three days showed a rotation of 0.00° in the same tube and 0.20 g. of sugar by the Benedict-Osterburg method, that is, an 80% loss in sugar content.

Both solutions became yellow on standing. That at 37° reached its maximum color after about 24 hours and was distinctly yellow. The one kept at room temperature required three or four days to become definitely yellow and reached a maximum in about a week.

Portions of these solutions were analyzed before and after hydrolysis with an excess of hydrochloric acid but no evidence of the formation of non-reducing polysaccharides was obtained.

Parallel experiments were carried out with a similar glucose solution containing 4.52 g. of potassium hydroxide and 2 g. of glucose in 160 cc. The results were very similar. The portion kept at room temperature for three days showed a polariscopic reading of —0.01° in the same tube and 0.712 g. of sugar by the Benedict-Osterburg method, that is, about 29% loss in sugar content as measured by reducing power.

The darkening of these solutions at 37° was about 5 to 6 times greater than with the same molecular concentration of guanidine at 37°. On the other hand, the yellowing with potassium hydroxide at room temperature was very much less than with guanidine. In a week the potassium hydroxide showed a scarcely perceptible effect, while with guanidine almost the maximum effect was developed in this time. The relations observed are qualitatively represented in the diagram and show that there is a distinct difference in the action of these two bases upon glucose as indicated by the qualitative differences in the formation of sugar tar.[8]

The experiments described above were repeated at concentrations of alkali corresponding to 0.1 *N* but the second series did not develop any additional facts. The

[5] Morrell and Bellars, *J. Chem. Soc.*, **91**, 1010 (1907).

[6] This was prepared according to the method used by Morrell and Bellars.

[7] Benedict and Osterburg, *J. Biol. Chem.*, **34**, 195 (1918).

[8] The specificity suggested in this diagram is especially interesting when considered in its biological relations. From the standpoint of living organisms the sugar catalyst that has the lowest temperature coefficient would be best suited for the conditions of life in general. The results represented in the diagram show that guanidine apparently fulfils this requirement much better than potassium hydroxide. Guanidine would be available for both warm- and cold-blooded animals, while a catalyst such as potassium hydroxide would perhaps be out of the question in one of them.

action of ammonium hydroxide and guanidine carbonate upon glucose was also observed at the same time.

With a solution containing 1.43 g. of guanidine carbonate and 1.00 g. of glucose in 160 cc. of water the rotation changed from +0.31° to + 0.24° (in a 10cm. tube) and the reducing sugar by the Benedict-Osterburg method from 1.03 g. to 0.936 g. when kept at room temperature for five days. At 37° the values after five days were for the rotation +0.02° in the same tube and for the reducing sugar content 0.666 g.

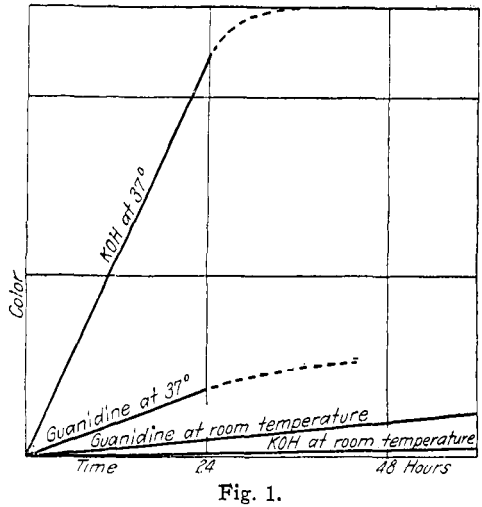

Fig. 1.

With 1 g. of glucose in 160 cc. of 0.1 *N* ammonium hydroxide the rotation remained unchanged at room temperature for five days while the sugar content dropped from 1.02 g. to 0.876 g. as determined by the Benedict-Osterburg method. A similar solution at 37° showed a rotation of +0.21° (+0.25° at the beginning) and a reducing sugar content of 0.718 g. after five days. No yellowing occurred with either reagent.

The results show that even guanidine carbonate acts upon glucose giving rise to both mutarotation and sugar destruction.

The behavior of potassium hydroxide in the experiments described above is well known. Morrell and Bellars studied in detail the mutarotation phenomena caused by guanidine. The loss in reducing power of the solutions was measured by them in terms of the loss in alkalinity on the well-established assumption that salts of polyhydroxy acids are formed by rearrangements of the various isomeric sugars present in an alkaline solution of hexose. The difference in the coloring of glucose solutions with potassium hydroxide in comparison with free guanidine was the only definite indication of marked differences in the behavior of these two bases and could be established only by parallel observations.

The action of these bases will have to be studied with care and in detail by some method like that used by Nef[1] before anything definite can be said about the relative quantitative importance of the various types of chemical processes that occur in these guanidine and guanidine carbonate solutions.

Guanidine as an Oxidizing Catalyst

1. *Using Hydrogen Peroxide.*—It was a matter of considerable interest and importance to explore the capacity of guanidine to catalyze oxidation in order to determine to what extent its effects resemble those of sodium and potassium hydroxides under similar conditions.

The free base from 1.65 g. of guanidine carbonate in 25 cc. water was liberated with barium hydroxide solution, leaving a small amount of the carbonate in excess; 0.1 g. of glucose was added and the solution made up to 75 cc.; 25 cc. of this solution was mixed with 25 cc. of 2.1% hydrogen peroxide. After four days at room temperature the solution was found

to be free from peroxide. The solution was then oxidized completely with potassium permanganate by the method previously described,[9] and the permanganate consumed calculated to its glucose equivalent, namely, 0.281 g. of glucose in this case (0.33 g. used); 25 cc. of the original solution was analyzed in the same way and showed a permanganate consumption equivalent to 0.335 g. of glucose (0.33 g. used). Although peroxide was used in large excess only a small amount of oxidation took place. The evolution of oxygen during the oxidation was plainly visible. The guanidine + glucose solution was yellow in color while the yellow color was absent from the portion to which peroxide had been added. In this experiment the behavior of guanidine resembles that of sodium or potassium hydroxide.

The above experiments were repeated several times and about the same results were obtained. Sugar determinations by the Benedict-Osterburg method showed that at room temperature these solutions lose from 50 to 85% of their reducing power in four or five days. When kept at 37° they contained no reducing substance at all after five days. This result taken in conjunction with the results on the oxidation shows that guanidine, like potassium hydroxide, is a better catalyst for the destructive rearrangements of glucose than for oxidation.

2. *Using Air.*—Sixty cc. of solution containing 3.72 g. of guanidine and 1.0 g. of glucose in 125 cc. was subjected to aëration at room temperature by placing it in a gas wash-bottle and drawing an active stream of carbon dioxide-free air through it with a suction pump; 60 cc. was set aside for the control analysis.

A solution of 3.50 g. of potassium hydroxide and 1.0 g. of glucose in 125 cc. of water was treated in the same way and placed in the same suction train. Air was circulated for 48 hours in the course of 12 days. The total permanganate consumed by all four solutions was then determined. The permanganate consumed by the experiment using guanidine plus air was 13% less than in the blank; with the experiment using potassium hydroxide plus air it was 6% less than in the blank. Apparently guanidine catalyzes oxidation by air a little more than potassium hydroxide. Both guanidine solutions became yellow, while the potassium hydroxide solutions remained colorless.

3. *Using Air Plus Manganese Hydroxide.*—The preceding experiments were repeated with the addition of a solution containing 0.05 g. of manganese chloride to each experiment. On circulating air the pale yellow-to-orange solutions very quickly became a dark brown. After air had been passed through for two hours the solutions were allowed to stand over two days. Both mixtures that were being aerated then contained a flesh-pink precipitate, and the supernatant solution in the guanidine experiment

[9] Witzemann, *J. Biol. Chem.*, **45**, 1 (1920).

was almost colorless The solution in the potassium hydroxide experiment was light brown. When air was again passed through the potassium hydroxide mixture, it darkened more quickly than the other. After two hours both had become equally dark red-brown. Decolorization was almost complete in 0.5 hour. The potassium hydroxide solution decolorized faster than the guanidine solution. These solutions after standing in the decolorized condition for some time showed a much darker color at the top of the solution where it was in contact with air. After the solution had been thus darkened several times a day for a week the decolorization became quite slow, indicating that the system was behaving differently. The solutions were then analyzed as before. The results were approximately the same as in Expt. 2. About 10% of the glucose was oxidized in both cases and guanidine seemed to function a little better than potassium hydroxide. The impression was obtained that the oxidation was considerably accelerated by the presence of the manganous hydroxide, considering the time during which air was circulated, but no satisfactory quantitative data were obtained. In any case the manganous hydroxide, besides functioning as an oxygen acceptor, served as an interesting indicator of the changes occurring in the solution. At first, compounds were present that took up oxygen rapidly, but the transformation of these compounds into others (presumably polyhydroxy acids), that are not readily oxidizable under these conditions, takes place much more rapidly than oxidation, and the solution then ceases to utilize appreciable amounts of oxygen. The same result was obtained with both potassium hydroxide and guanidine and is typical for such bases as sodium and potassium hydroxide.

Summary

The results described in this paper show that guanidine, which is an organic base that is comparable to sodium and potassium hydroxide, so far as the velocity of saponification of ethyl acetate[5] is concerned, is also comparable to these bases in its action upon glucose, in both the presence and the absence of oxygen or hydrogen peroxide. Guanidine causes a polymerization accompanied by yellowing more readily than potassium hydroxide, but this reaction was much less influenced by temperature than in the case of potassium hydroxide. The latter effect was the only difference in the behavior of these two bases that was large enough to be suspected of being specific.

CHICAGO, ILLINOIS