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THE OXIDATION OF *d*-GLUCOSE BY MEANS OF COPPER IN SODIUM CARBONATE SOLUTION (SOLDAÏNI'S REAGENT)

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Introduction

The oxidation of the simple hexose and pentose sugars in alkaline solution by means of various oxidizing agents has been extensively studied by Nef[2] and his students. In all of these experiments the oxidations were carried out in the presence of an excess of sodium hydroxide, usually six equivalents, in an initial concentration of approximately 5% sodium hydroxide. Nef[3] and also Anderson[4] have studied the oxidation of sugars by means of ordinary Fehling's solution.

The general theory of the mechanism of the oxidation of sugars in the presence of alkalies by various oxidizing agents has been fully developed by Nef[2] and is given also in papers by Anderson,[4] Spoehr[5] and Glattfeld.[6]

The present paper gives an account of the quantitative examination of the products formed in the oxidation of *d*-glucose by means of copper carbonate in sodium carbonate solution. The oxidizing medium is a modification of the Soldaïni reagent.[7] The oxidation was therefore carried out in the presence of a less active alkali than that employed in any of the experiments referred to above. Nevertheless we were able to establish qualitatively the presence of the same oxidation products that are formed in the presence of the more active alkali.

Experimental Part

Two hundred g. of glucose was oxidized in eight portions of 25 g. each. For each 25g. portion 275 g. of copper chloride and 275 g. of anhydrous sodium carbonate were used. This amount of sodium carbonate gave an excess of eight equivalents of sodium carbonate based on the glucose, over the amount necessary to react with the copper chloride. The amount of copper chloride was slightly in excess of that necessary to cause complete disappearance of the glucose, as determined by separate experiment. The required amount of copper chloride in solution was added to the sodium carbonate, also in solution, the glucose was then added and the whole at once made up to 2500 cc. in a large flask. The flask was kept immersed in boiling water throughout the experiment and the mixture was vigorously stirred by means of a mechanical stirrer. Cuprous oxide began to form after about one-half hour and the oxidation was complete

[1] The abstract of a thesis presented to the University of Nebraska by Fred W. Jensen in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Nef, (a) *Ann.*, **357**, 214 (1907); (b) **403**, 205 (1914).

[3] Ref. 2 a, p. 271.

[4] Anderson, *Am. Chem. J.*, **42**, 407 (1909).

[5] Spoehr, *ibid.*, **43**, 241 (1910).

[6] Glattfeld, *ibid.*, **50**, 135 (1913).

[7] Soldaïni, *Gazz. chim. ital.*, **6**, 322 (1876).

after approximately eight hours. The oxidation, therefore, proceeds much more slowly than with Fehling's solution. Anderson found that the reaction with Fehling's solution was complete in about 12 minutes.

After completion of the oxidation the cuprous oxide was filtered out, the filtrate acidified with hydrochloric acid and the excess of copper removed as sulfide. Thereafter the filtrate was subjected to vacuum distillation at a temperature of 50–70° and a pressure of 20–30 mm. The salt residue was freed from volatile acids by several distillations to dryness after additions of 100cc. portions of distilled water.

Formic Acid.—The total distillates from each oxidation were made up to definite volume and an aliquot portion was titrated for total acid with standard alkali solution. The filtrates always contained some hydrochloric acid which was determined by titration with silver nitrate solution. The formic acid produced in one oxidation of 25 g. of glucose varied between 3.21 g. and 3.56 g., the total from 200 g. of glucose amounting to 30.86 g. Of this amount 3.8 g. came from the acid extraction of the combined salt residues of all the oxidations as explained below.

Non-Volatile Acids.—The combined salt residues from the 8 oxidation experiments were repeatedly extracted with 95% alcohol, yielding 95.85 g. of non-volatile acid gums. The salt residue was then extracted thrice with concd. hydrochloric acid. From this hydrochloric extract upon evaporation there separated, in addition to sodium chloride, 9.4 g. of glycolic acid in long crystals which could be picked out mechanically from the sodium chloride residue. After separation of the glycolic acid there remained 15.3 g. of gummy acids and the filtrate was found to contain 3.8 g. of formic acid as noted above. There were thus obtained 30.86 g. of formic acid, 9.4 g. of glycolic acid and 111.15 g. of non-crystalline acids or a total from 200 g. of glucose of 151.1 g. of oxidation products.

The 111.15 g. of non-crystalline acid mixture, completely soluble in 95% alcohol, was resolved into three fractions as follows. It was first extracted repeatedly with hot ethyl acetate until no further solution took place. There remained after this process 9.1 g. of material, designated Fraction 1. The soluble portion after removal of the ethyl acetate was repeatedly extracted with ether free from alcohol, yielding a fraction of 33.9 g., designated Fraction 3. The portion insoluble in ether but soluble in ethyl acetate, 56.25 g., was designated Fraction 2. There was a loss of 11.9 g. due in the main to volatilization of glycolic acid.

Gluconic and Mannonic Acids.—Fraction 1 on treatment with phenylhydrazine gave 3.41 g. of crystalline phenylhydrazides from alcohol. The alcohol-soluble hydrazides were hydrolyzed by means of barium hydroxide under toluene. The use of toluene in this process serves to prevent decomposition of the phenylhydrazine, thus facilitating its subsequent removal by means of ether. After removal of the phenylhydrazine by means of ether and exact precipitation of the barium as sulfate, the gum remaining was added to Fraction 2.

The crystalline phenylhydrazides obtained as described above melted at 195–197°. When they were recrystallized from hot water, 0.45 g. of an extremely insoluble hydrazide, m. p. 214–215°, was obtained. This is the melting point given by Fischer[8] for *d*-mannonic-phenylhydrazide. This hydrazide was converted first to the free acid by the usual process and then into the brucine salt. There was obtained 0.43 g. of brucine *d*-mannonate; m. p., 212°; $[\alpha]_D^{20}$, —27.4° (0.4169 g. in 12.8115 g. of H_2O gave α = —0.87°, in a 1-dcm. tube). The constants for brucine *d*-mannonate given by Nef[9] are m. p. 212°; $[\alpha]_D^{20}$, —26.73°.

From the more soluble hydrazides there was obtained 2.5 g. of *d*-gluconic-phenylhydrazide in the form of snow-white, flaky crystals; m. p., 200–201°; $[\alpha]_D^{20}$, +12.9°

[8] Fischer, *Ber.*, **22**, 3221 (1889).

[9] Ref. 2 b, p. 306.

(0.5104 g. in 16.0048 g. of H_2O gave, in a 1-dcm. tube, $\alpha = +0.04°$). These constants correspond exactly with those given by Nef and by Fischer for *d*-gluconic-phenylhydrazide.

A portion of the hydrazide was converted first to the free acid and then into the brucine salt. There was obtained 1 g. of recrystallized brucine *d*-gluconate; m. p., 120–122°. On drying in a vacuum, 0.7640 g. of this salt lost 0.0451 g. of water, or 5.9%; m. p. (anhyd.), 155–157°; $[\alpha]_D^{20}$, —18.9° (0.6300 g. in 15.3727 g. of H_2O gave $\alpha = -0.75°$, in a 1-dcm. tube). These constants agree closely with those of brucine *d*-gluconate as given by Nef.[10]

***d*-Arabonic Acid.**—Fraction 2, 56.25 g., soluble in ethyl acetate and described above was converted directly into brucine salts in the usual way. The dry salts obtained on removal of the water by vacuum distillation were recrystallized from 95% alcohol, yielding three crops of crystalline salts; Crop A, 29.3 g., m. p. 112–113°; Crop B, 63.5 g., m. p. 195–215°; and Crop C, non-crystalline, gummy brucine salts.

The brucine salt of Crop A, Fraction 2, after recrystallization melted at 166–168°; $[\alpha]_D^{20}$, —26.6° (0.9582 g. in 22.0677 g. of H_2O gave, in a 1-dcm. tube, $\alpha = -1.12°$). These constants agree with those given for brucine *d*-arabonate by other investigators.

Part of the brucine salt, 13.2 g., was decomposed by means of barium hydroxide in hot water solution, and after removal of the brucine by filtration and extraction with benzene and exact precipitation of the barium as sulfate gave 3.5 g. of arabonic lactone in the form of a gum. The substance titrated like a lactone and the results agreed with those required for a lactone with 5 carbon atoms.

Anal. Subs., 0.4781: 25.95 cc. of 0.1267 *N* NaOH. Calcd. for $C_5H_8O_5$: 25.5 cc.

On crystallization of the gum from ethyl acetate, there was obtained 0.93 g. of *d*-arabonic lactone in the form of fine, transparent crystals; m. p., 96°. A determination of rotation gave $[\alpha]_D^{20} = +73.3°$ (0.5032 g. in 8.6540 g. of H_2O gave, in a 0.5-dcm. tube, $\alpha = +2.05°$. These constants agree with those given by Ruff[11] and others for arabonic lactone.

Anal. Subs., 0.3822: 20.5 cc. of 0.1267 *N* NaOH. Calcd. for $C_5H_8O_5$: 20.38 cc.

A portion of the gum was treated with phenylhydrazine, yielding finally 0.85 g. of crystalline hydrazide; m. p., 213°; $[\alpha]_D^{20}$, —13.8° (0.7552 g. in 16.5461 g. of H_2O gave, in a 1-dcm. tube, $\alpha = -0.61°$). Spoehr[12] records —13.9° and Hudson[13] —14.5° as the specific rotation of this substance. These constants, together with those of the crystalline lactone and the brucine salt, prove beyond doubt that arabonic lactone is one of the products of the oxidation of glucose under the conditions of our experiment.

***d*-Erythronic Acid.**—The high-melting brucine salts, Crop B of Fraction 2 mentioned above, on recrystallization from alcohol yielded 16.4 g. of material in the form of hard, shining crystals characteristic of brucine *d*-erythronate; m. p., 212–213°; $[\alpha]_D^{20}$, —24.4° (0.6409 g. in 13.7291 g. of H_2O gave, in a 1-dcm. tube, $\alpha = -1.1°$). These constants agree with those given by other investigators for this brucine salt.

The brucine salt was converted to the lactone in the usual way. On recrystallization of the lactone from a mixture of ether and ethyl acetate, beautiful, colorless crystals in the form of radiating clusters were obtained; m. p., 102°; $[\alpha]_D^{20}$, —72.8° (0.1457 g. in 13.6670 g. of H_2O gave, in a 1-dcm. tube, $\alpha = -0.77°$). The melting point of *d*-erythronic lactone is recorded by Ruff as 102–103° and the rotation as —73.3°.

Anal. Subs., 0.0849: 26.8 cc. of 0.0272 *N* NaOH. Calcd. for $C_4H_6O_4$: 26.45 cc.

[10] Ref. 2 b, p. 305.

[11] Ruff, *Ber.*, **32**, 557 (1899).

[12] Ref. 5, p. 240.

[13] Hudson, THIS JOURNAL, **39**, 467 (1917).

These results prove that the substance isolated was *d*-erythronic lactone.

Glycolic Acid.—Fraction 3, 33.87 g. of original gums soluble in ether, was heated at 100° with an excess of strychnine. After filtration from undissolved strychnine and removal of the water under reduced pressure, 82.8 g. of strychnine salts was obtained. This was crystallized from alcohol giving two fractions of crystalline salts; Crop A, 34.2 g., m. p. 160–165°; Crop B, 25.7 g., m. p. 200–230°. The residual strychnine salts, Crop C, which did not crystallize amounted to 22.9 g.

Crop A strychnine salts because of the melting point 160–165° was considered to be nearly pure strychnine glycolate. It was converted directly to the calcium salt by heating in water solution with an excess of calcium hydroxide. After filtration from the strychnine thus liberated the filtrate was concentrated to small volume and alcohol added to the solution. When this was cooled, 10.1 g. of calcium glycollate crystallized.

Anal. Subs., 1.0041 (air dry): loss of H_2O at 110°, 0.2815 g. Calcd. for $Ca(C_2H_3O_3)_2.4H_2O$: H_2O, 27.48. Found: 28.04.

Subs., 0.7226 (anhyd.): CaO, 0.2135. Calcd. for $Ca(C_2H_3O_3)_2$: CaO, 29.47. Found: 29.55.

Ten and one-tenth g. of calcium glycolate is equivalent to 5.9 g. of glycolic acid. The amount of glycolic acid isolated from the oxidation mixture was thus 5.9 g. + 9.4 g. obtained as free glycolic acid as previously described, or a total of 15.3 g. of glycolic acid.

Glycerinic Acid.—The brucine salts of Crop B, Fraction 2, remaining after removal of brucine erythronate were converted to the free acid in the usual way and united with gums obtained from the strychnine salts of Crop B, Fraction 3. An attempt to separate glycerinic acid from these gums in the form of quinine salt proved unsuccessful. The brucine salts of these gums were again formed and yielded on recrystallization from alcohol 29.3 g. of crystalline salt; m. p., 198–200°; $[\alpha]_D^{20}$, —29.3° (0.4471 g. in 15.2644 g. of H_2O gave, in a 1dcm. tube, α = —0.84°).

The brucine salts were resolved in the following manner. The salt was first extracted with boiling absolute alcohol, after which 20.2 g. remained; m. p., 210–212°; $[\alpha]_D^{20}$, —30.6° (0.3732 g. in 14.9904 g. of H_2O gave, in a 1dcm. tube, α = —0.75°). Four recrystallizations from 90% alcohol gave finally 9 g. of crystalline brucine salt; m. p., 218–219°; $[\alpha]_D^{20}$, —31.5° (0.7493 g. in 17.8801 g. of H_2O gave, in a 1dcm. tube α = —1.28°). The constants as given by Anderson[14] for brucine *d*-glycerinate are m. p. 222°; $[\alpha]_D^{20}$, —33.2°. Our product was, therefore, nearly pure brucine *d*-glycerinate. From the 9 g. of brucine salt was obtained 1.55 g. of free acid, which was analyzed.

Anal. Subs., 0.0776: 26.2 cc. of 0.0272 *N* NaOH. Calcd. for $C_3H_6O_4$: 26.9 cc.

The free acid was converted to the calcium salt in the usual way. Upon recrystallization the salt melted at 137°; $[\alpha]_D^{20}$, —8.8° (0.6548 g. in 15.1398 g. of H_2O gave, in a 1dcm. tube, α = —0.37°). According to Anderson the constants for calcium *d*-glycerinate are m. p. 137°; $[\alpha]_D^{20}$, —10.2°. Our rotation as well as that of Anderson is obviously low due to incomplete resolution.

Anal. Subs., 0.3604 (air-dried): loss of H_2O at 105–110°, 0.0472. Calcd. for $Ca(C_3H_5O_4)_2$: H_2O, 12.59. Found: 13.1.

Subs., 0.3132 (anhyd.): CaO, 0.0687. Calcd. for Ca $(C_3H_5O_4)_2$: CaO, 22.4. Found: 21.94.

The treatment of the brucine salts as described above accomplished therefore a very nearly complete separation of the *d*-glycerinic acid. Brucine *l*-glycerinate to the extent of 3.8 g. was isolated from the mother liquors obtained in the alcoholic extraction of the mixed brucine salts as described above. This salt melted at 218–219° and gave

[14] Ref. 4, p. 423.

$[\alpha]_D^{20}$, —23.0° (0.4992 g. in 12.8505 g. of H_2O gave, in a 1-dcm. tube, α = —0.87°. Anderson gives for this salt, m. p. 222°; $[\alpha]_D^{20}$, —22.02°.

The brucine salt was converted first to the free acid and then to the calcium salt, yielding 0.5 g.; m. p., 134°.

Anal. Subs., 0.4336: loss of H_2O at 105°, 0.0525. Calcd. for $Ca(C_3H_5O_4)_2$: H_2O, 12.59. Found: 12.1.

Subs., 0.3811 (anhyd.): 8.4792 g. of H_2O, 0.5-dcm. tube; α, +0.29°; $[\alpha]_D^{20}$, +13.3°.

Anderson found the rotation of calcium *l*-glycerinate to be +15.5°. The calcium in the solution used for the rotation was precipitated as the oxalate and weighed as CaO.

Anal. Subs., 0.3811: CaO, 0.0829. Calcd. for $Ca(C_3H_5O_4)_2$: CaO, 22.4. Found: 21.74.

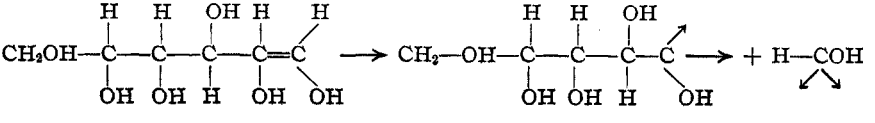
The results of the experiments recorded above indicate that partial resolution of the racemic glycerinic acid was accomplished by treatment of its brucine salt as described before, and further that both *d*- and *l*-glycerinic acid were present in the products of oxidation of glucose under the conditions of our experiment.

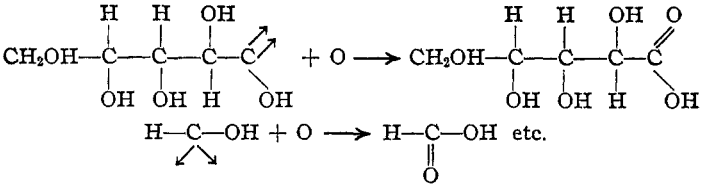
d-Threonic Acid.—The brucine salts in the filtrate obtained from the original brucine *dl*-glycerinate contained a small amount of *d*-threonic brucine. From the salt there was obtained after conversion to the free acid and treatment with phenylhydrazine, a small amount of hydrazide possessing the properties of *d*-threonic phenylhydrazide; m. p., 156–160°; $[\alpha]_D^{20}$, —23° (0.0672 g. in 13.7568 g. of H_2O gave, in a 1dcm. tube, α = —0.11°). The constants as given by Anderson are m. p. 158°; $[\alpha]_D^{20}$, —31.17°. While the rotation of our product is low the results indicate without doubt the presence of threonic acid in the oxidation mixture. No other substance could have been present the phenylhydrazide of which possesses a negative rotation.

Attempts to isolate ribonic acid first as the cadmium salt and then as the phenylhydrazide proved unsuccessful.

Discussion

Nef accounts in the following manner for the formation of the various products obtained in the oxidation of the sugars in the presence of alkalies. The sugar is first converted to the various 1,2-, 2,3- and 3,4-dienols which then under the influence of the alkali undergo splitting to give the methylenenols containing from one to five carbon atoms. These latter then undergo oxidation directly to give the various oxidation products, carbon dioxide, formic acid, glycolic acid, *dl*-glycerinic acid, and the isomeric trioxybutyric and tetraoxyvaleric acids. In addition a part of the sugar in the case of hexoses, oxidizes to the corresponding hexonic acids, when copper is the oxidizing agent but not when hydrogen peroxide or air is used. Thus the 1,2-dienol of glucose dissociates to give active formaldehyde, H—C=O, which oxidizes directly to formic acid and then to carbon dioxide, and *d*-arabinose in its active form, which then oxidizes to arabonic acid.





The oxidation of glucose directly leads to the formation of both gluconic and mannonic acids. Nef[15] explained the formation of the latter substance as due to preliminary oxidation of the glucose dienol to the osone without breaking of the carbon chain. The osone then, by the benzilic acid rearrangement, is converted into mannonic acid. We feel, however, that a better explanation is to be found in the Lobry de Bruyn-Van Ekenstein equilibrium. As shown by their investigation, mannose is one of the products formed from glucose in alkaline solution through molecular rearrangement. Mannose is then in all probability converted by direct oxidation to mannonic acid. A similar explanation accounts for the formation of *d*-ribonic and *d*-threonic acids when these are found in the oxidation products from glucose. Thus, in the splitting of the 1,2-dienol as noted above, the arabinose formed may in part undergo rearrangement to ribose which then is oxidized to ribonic acid.

Summary of Results

Oxidation of *d*-glucose by means of copper in the presence of sodium carbonate leads to the formation of the same products as in the other copper oxidations. While hexonic acids are definitely present in the oxidation mixture they were isolated in much smaller amounts than in the oxidations with Fehling's solution. As regards the other products of oxidation there was little difference between our experiments and those with Fehling's solution.

The oxidation products from 200 g. of *d*-glucose, excluding carbon dioxide, amounted to 142 g. in which the following substances were identified: formic acid, 30.86 g.; glycolic acid, 15.3 g.; gluconic acid, 1–2 g.; mannonic acid, small amount; arabonic acid, 6 g., isolated as the brucine salt; erythronic acid isolated in the form of brucine salt and identified as the lactone, 3.6 g.; *dl*-glycerinic acid, 6.2 g., isolated in the form of brucine salts; *d*-threonic acid, trace.

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[15] Ref. 2 a, p. 231.