[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Reduction of 5-Keto-D-gluconic Acid with Sodium Borohydride[1]

BY J. K. HAMILTON AND F. SMITH

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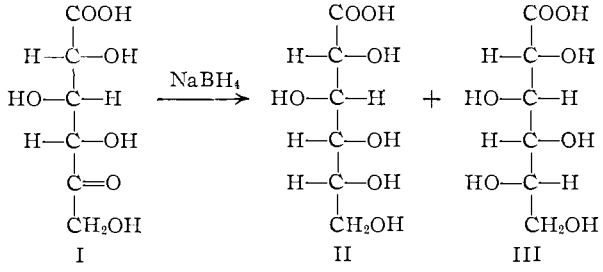
Calcium 5-keto-D-gluconate trihydrate has been reduced with sodium borohydride and the two diastereoisomers so formed D-gluconic acid and L-idonic acid, have been identified as their phenylhydrazides.

Treatment of reducing sugars with sodium borohydride has been shown to give the corresponding sugar alcohols.[2,3] Other investigators have shown that, under controlled conditions, sugar acid lactones may be converted into reducing sugars.[4]

In an investigation of new routes to the L-series of sugar compounds and of a new source of L-idose in particular we have examined the reduction of 5-keto-D-gluconic acid, a product that is readily obtained from D-glucose by a fermentation process.[5]

It is shown herein that reduction of 5-keto-D-gluconic acid (I) affords the two possible isomeric acids, D-gluconic acid (II) and L-idonic acid (III).



The two acids II and III, separated by chromatography on filter paper using phenol saturated with water as the irrigating solvent, appeared to be produced in about equal amounts. Separation and characterization of II and III also was achieved by fractional crystallization of the corresponding phenylhydrazides.

Fischer and Fay[6] did not report the crystallization of either D- or L-idonic acid phenylhydrazide while Micheel and Kraft[7,8] report that it crystallizes with difficulty. Our product corresponded to the compound described by Micheel and it was found to be the enantiomorph of the phenylhydrazide obtained from D-gulonic acid by epimerization.[6,9,10] The work described herein showed that the phenylhydrazide of L-idonic acid is not crystalline and does not constitute a good derivative for either the separation or identification of L-idonic acid.

Experimental

Reduction of D-Gluconic Acid with Sodium Borohydride.—When a solution of calcium 5-keto-D-gluconate trihydrate (3.76 g.) in water (700 ml.), was treated with sodium borohydride (1.0 g.) at room temperature the specific rotation changed from $[\alpha]^{26.5}D$ −9° to +19° in 3 hours. A further addition of sodium borohydride (0.5 g.) did not effect any change in rotation and the solution was non-reducing to Fehling solution when tested after keeping overnight.

Separation of D-Gluconic Acid (II) and L-Idonic Acid (III). Experiment A.—A portion of the above reduction mixture, containing product from 100 mg. of the original calcium 5-keto-D-gluconate, was passed successively through a cation ("Amberlite IR-120")[11] and an anion ("Duolite A_4")[12] exchange column. The acids were displaced from the anion column with a saturated solution of barium hydroxide. The alkaline eluate was neutralized with carbon dioxide and the insoluble barium carbonate removed by filtration. The filtrate was passed over the cation exchange resin, concentrated *in vacuo* to a sirup, methanol was added and the concentration repeated in order to lactonize the acids.

Paper chromatographic analysis of the sirupy product showed that two components were present having R_f values of 0.27 and 0.34 tested with butanol-1, ethanol, water (4:1:5) and of 0.52 and 0.75, tested with phenol saturated with water. The components were located by the hydroxamic acid test spray reagents.[13]

The sirupy mixture of acids was separated by paper chromatography using Whatman No. 3 filter paper ($22\frac{1}{2}'' \times 18\frac{1}{4}''$) and phenol saturated with water as the irrigating solvent. One vertical strip ($\frac{1}{2}''$ wide) was cut from each edge and another ($\frac{1}{4}''$ wide) was cut from the center of the chromatogram. The position of the two components was determined by spraying these strips with the hydroxamic acid test reagents.[13] The areas of the filter paper sheet containing each component were cut out and eluted with water (25 ml.). The eluate in each case was filtered and concentrated *in vacuo* to a sirup of constant weight. The component with R_f 0.52 corresponding to D-glucono-γ-lactone amounted to 21.3 mg. and showed $[\alpha]^{21.5}D$ +10.5° in water (*c* 0.7). The component with R_f 0.73 corresponding to L-idono-γ-lactone amounted to 20.8 mg. and showed $[\alpha]^{21.5°}D$ +4.5° in water (*c* 0.7). Attempts to crystallize the separated compounds as free acids using amyl alcohol–ethanol–water failed.

Experiment B.—A large sample of calcium 5-keto-D-gluconate trihydrate (3.76 g.) was reduced with sodium borohydride as described above. The reduction mixture was acidified with acetic acid (glacial) and passed through a cation-exchange column (Amberlite IR-120) and the eluate concentrated to a sirup *in vacuo*. The sirup was dissolved in 1.2% methanolic hydrogen chloride (35 ml.) and refluxed for 5.5 hours to decompose any boric acid complexes.[2,14-16] The hydrogen chloride was neutralized with silver carbonate, the solution was filtered, passed over the cation-exchange resin (Amberlite IR-120) and concentrated *in vacuo* to a sirup. In order to lactonize the sugar acids the above sirup was heated at 55–60° for 3–4 hours. Several attempts were made to form the phenylhydrazides at this stage but borate impurities interfered with the reaction. The sirup was dissolved in a large volume of ethanol and acetone was added. The white precipitate so formed was removed by centrifugation. The filtrate was freed from solvent and the sirupy residue was dissolved in ethanol and the solution treated with ether to remove the remaining borate impurity. After removal of the solvent *in vacuo* and repeated addition

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(6) E. Fischer and I. W. Fay, *Ber.*, **28**, 1975 (1895).

(7) F. Micheel and K. Kraft, *Z. physiol. Chem.*, **222**, 235 (1933).

(8) F. Micheel and K. Kraft, *ibid.*, **218**, 280 (1933).

(9) J. U. Nef, *Ann.*, **403**, 267 (1914).

(10) O. F. Hedenburg and L. H. Cretcher, THIS JOURNAL, **49**, 478 (1927).

(11) Obtained from Rohm and Haas Co., Philadelphia, Pa.

(12) Obtained from Chemical Process Co., Redwood City, Calif.

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(14) R. F. Nystrom and W. G. Brown, *ibid.*, **69**, 1197, 2548 (1947).

(15) W. S. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

(16) L. P. Zill, J. X. Khym and G. M. Cheniae, *ibid.*, **75**, 1339 (1953).

and distillation of ethanol to remove any further borate impurity[16] the sirupy product was heated at a temperature of 55–60° for 4–5 hours to effect lactonization (yield 1.35 g.).

Separation of the Phenylhydrazides of D-Gluconic and L-Idonic Acid.[7-9,17]—The sirupy mixture of the lactones (1.35 g.) was dissolved in ethanol (15 ml.) and phenylhydrazine (0.75 ml.) was added. The reaction mixture was refluxed on a boiling water-bath for 40 minutes. The solution was allowed to cool slowly at room temperature whereupon D-gluconic acid phenylhydrazide separated in the form of fine white crystals. The crystals were filtered, washed with ethanol and dried (yield 280 mg.). The filtrate and washings were combined and concentrated to a small volume *in vacuo* and left overnight to crystallize. A further amount (0.20 g.) of D-gluconic acid phenylhydrazide was obtained in this way. This procedure was carried out five times until no more crystals separated. The total yield of the phenylhydrazide of D-gluconic acid was 0.504 g., and after a further crystallization from water it showed $[\alpha]^{21.5}D$ +11.5° in water (*c* 0.7), m.p. and mixed m.p. 198–201°. These values are in good agreement with those (m.p. 198–201°, $[\alpha]^{20}D$ +12° in water) recorded for D-gluconic acid phenylhydrazide.[14]

Anal. Calcd. for $C_{12}H_{18}O_6N_2$: C, 50.4; H, 6.39; N, 9.8. Found: C, 50.46; H, 6.21; N, 9.67.

After the isolation of the D-gluconic acid phenylhydrazide the concentrated mother liquors were kept at 2° for 24 hours, whereupon a mass of crystalline-like material was deposited. The mother liquor was removed and the solid residue triturated with ice-cold ethanol. Recrystallization of this material from ethanol yielded L-idonic acid phenylhydrazide (0.73 g.), m.p. 115–117° with previous sintering at 100–105°, $[\alpha]^{22}D$ +12.5° in water (*c* 1.6). One sample showed m.p. 117–120° with previous sintering at 107°. Crystallization from other solvents, such as methanol, 1,4-dioxane, ethanol–ethyl ether or benzene, produced no change in the m.p. of the product. When observed under the polarizing microscope the product did not appear to be crystalline. Micheel recorded m.p. 115° with previous sintering at 102°, and $[\alpha]^{20}D$ +10.5 (water) for L-idonic acid phenylhydrazide,[7,8] while Nef reported m.p. 100–110° for the enantiomorphic compound which showed $[\alpha]^{20}D$ −12.4° in water.[9]

Anal. Calcd. for $C_{12}H_{18}O_6N_2$: C, 50.4; H, 6.39; N, 9.8. Found: C, 50.05; H, 6.6; N, 9.3.

Epimerization of D-Gulono-γ-lactone.—A solution of D-gulono-γ-lactone (2 g.) in water (12.5 ml.) containing pyridine (0.9 g.) was heated in a sealed glass tube for 97 hours in a boiling water-bath. The solution was decolorized (charcoal), diluted with water and evaporated *in vacuo* to give a sirupy product. The latter was heated *in vacuo* for 2 hours at 50–55° in order to lactonize the mixture of D-gulonic and D-idonic acids. The sirup was dissolved in hot methanol (10 ml.) and the cooled solution seeded with a crystal of D-gulono-γ-lactone. When the crystallization at room temperature was complete the supernatant liquid was removed, concentrated and a further crop of the crystalline D-gulono-γ-lactone removed. Crystallization was carried out until no more crystals appeared upon cooling to 0°. The sirupy product obtained upon removal of the solvent was chiefly D-idono-γ-lactone (yield 1.18 g.).[cf. 6,10]

Formation of the Phenylhydrazide of D-Idonic Acid.—When a small portion of the above sirup was treated with phenylhydrazine in boiling ethanol as described above, the phenylhydrazide of D-idonic acid was obtained. After "crystallization" from ethanol the product had m.p. 113–114° with previous sintering at 97°.

Attempts to "recrystallize" the phenylhydrazide from methanol, 1,4-dioxane, ethyl ether and water failed to raise the m.p. and in all cases the material appeared to be amorphous.

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ST. PAUL, MINNESOTA

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

2-*O*-(D-Galactopyranosyluronic Acid)-L-rhamnose from Okra Mucilage[1]

BY ROY L. WHISTLER AND H. E. CONRAD

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Okra mucilage on partial acid hydrolysis yields a mixture of oligosaccharides which can be separated by chromatography on carbon and cellulose columns. Among the acidic oligosaccharides, there are an aldobiouronic acid and two aldotriouronic acids. By the methylation method the first of these is shown to be 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose. The aldotriouronic acids are shown to be galactosyl → (galactosyluronic acid) → rhamnose and a (galactosyluronic acid) → rhamnosyl → galactose. The occurrence of these oligosaccharides as hydrolytic fragments of okra mucilage suggests that the linkages involved are present in the polysaccharide.

Okra mucilage,[2] obtained by water extraction of defatted okra pods is a polysaccharide composed of D-galactose, L-rhamnose and D-galacturonic acid units. A previous investigation of the structure of this mucilage[2] has shown that partial hydrolysis gives rise to three galactobioses, one of which has been proved to be 4-*O*-D-galactopyranosyl-D-galactose. Here is reported the isolation and characterization of an aldobiouronic acid obtained upon incomplete acid hydrolysis of okra mucilage. Two aldotriouronic acids are also isolated in low yields from the hydrolyzate.

A mixture of acidic oligosaccharides is obtained from the mucilage hydrolysate by use of carbon chromatography.[3] Separation of the mixture into individual acidic components is achieved by rechromatography on a column of cellulose.[4]

Determination of physical constants and monosaccharide components for each of these oligosaccharides indicates that one is an aldobiouronic acid composed of D-galacturonic acid and L-rhamnose and that the other two are aldotriouronic acids, each of which is composed of galacturonic acid, galactose and rhamnose.

Upon complete methylation of the aldobiouronic acid, followed by reduction with lithium aluminum hydride and hydrolysis of the reduced material, there are obtained 3,4-di-*O*-methyl-L-rhamnose and 2,3,4-tri-*O*-methyl-D-galactose. The latter compound arises from the reduced galacturonic acid part of the molecule. From the isolation of these methylated sugars it can be directly concluded

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