THE BEHAVIOR OF D-GLUCURONIC, D-GALACTURONIC, AND D-MANNURONIC ACID IN DILUTE AQUEOUS SODIUM HYDROXIDE

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ABSTRACT

Two percent aqueous sodium hydroxide at room temperature, preferably for 24 hours, was used to obtain the Lobry de Bruyn – van Ekenstein transformation products. The transformations proceeded much more slowly than in the case of the hexoses; complex changes in specific rotation occurred, and equilibrium among the products was not attained, probably because acidic non-reducing substances formed at a comparable rate. After 24 hours, 39% of D-glucurone was recovered unchanged, together with 9% of D-mannuronic acid and 2% of keto acid probably based on fructose. D-Mannurone gave the same products, but unchanged starting material once more predominated. D-Galacturonic acid was recovered in 58% yield together with 12% of D-taluronic acid, a new substance characterized as a crystalline monohydrated brucine salt. A trace of a keto acid, presumably derived from D-tagatose, was also formed.

INTRODUCTION

During a recent study (1) it was incidentally noticed that D-galacturonic acid appeared to be much more resistant to the oxidizing action of chlorous acid, and also to the Lobry de Bruyn – van Ekenstein transformation in 1% aqueous sodium hydroxide, than was an aldohexose. It was also known (2) that glycosides of uronic acids were unusually resistant to acid hydrolysis. These observations suggested that the chemical behavior of the aldehyde group in uronic acids should be studied in its own right, and this article is concerned with the behavior of uronic acids in cold, dilute alkali. Applications of the Lobry de Bruyn – van Ekenstein transformation to reducing carbohydrates and a few polyuronides have been reviewed (3, 4); more drastic conditions were necessary to epimerize the aldonic acids (3, 5, 6) and galactaric acid (7). D-Galacturonic acid with aqueous calcium or strontium hydroxide was reported to yield some 5-keto-L-galactonic acid, but other reducing substances were produced with baryta. D-Glucuronic acid behaved similarly (8), but lime water converted the 4-O-methyl derivative into a dicarboxylic acid (2).

Preliminary work, carried out by our former colleague E. W. H. Böhme, involved keeping D-glucurone in 1% aqueous sodium hydroxide at room temperature for various times, then removing cations on an ion-exchange resin and chromatographing the effluent on paper. Glucuronic acid or glucurone appeared to be almost completely stable under the conditions cited. The present research therefore employed 2, 6, and 12% aqueous sodium hydroxide at room temperature for periods of 2, 5, 7, and 24 hours. When the chromatograms were developed with naphthoresorcinol a faint blue spot indicated the presence of a keto acid, while a subsequent treatment with water vapor at 70° brought out two new blue spots. This behavior was characteristic of uronic acids (9), and control experiments showed that the greater of these two spots was unchanged glucuronic acid. The acidic nature of these products was substantiated by their negligible movement on paper chromatograms eluted by a basic solvent system. After the glucurone had been in

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the alkali for a week, the naphthoresorcinol and *o*-aminodiphenyl sprays failed to develop any spots on the paper chromatograms; other chromatograms developed with a bromophenol blue spray, however, contained five or six spots corresponding to non-reducing acids of lower molecular weight. It appeared, therefore, that the Lobry de Bruyn – van Ekenstein transformation was accompanied by a rather rapid sequence of degradation reactions. These reactions were not investigated in the present research.

Since the experiments showed that 2% aqueous sodium hydroxide at room temperature for about 24 hours produced maximum amounts of the two new spots, these conditions were used in subsequent work. D-Mannurone produced a chromatographic pattern superposable on that from D-glucurone, with the exception that the major component was unchanged mannuronic acid. Thus the results suggested that glucurone and mannurone did not yield the same equilibrium mixture of glucuronic, mannuronic, and fructuronic acids, probably because side reactions competed on approximately equal terms with the Lobry de Bruyn – van Ekenstein transformation.

Under the same conditions, D-galacturonic acid yielded a chromatographic spot corresponding to the starting material, together with a minor spot. D-Galacturonic acid was then isomerized on a larger scale and preparative paper chromatography was used to recover from the product both unchanged galacturonic acid (58%) and D-taluronic acid (12%), the former being isolated in a crystalline condition and the latter, a new compound, as a crystalline, monohydrated brucine salt. The crude brucine taluronate contained traces of another brucine salt which gave the naphthoresorcinol test for the salt of a keto acid, presumably the unknown D-tagaturonic acid. To investigate this possibility, the isomerized product was converted to a methyl ester methyl glycoside mixture which was then reduced with lithium aluminum hydride and finally hydrolyzed with aqueous acid. When separated by paper chromatography, the product yielded crystalline D-galactose and an uncrystallized D-talose which was characterized as the crystalline methylphenylhydrazone. The chromatogram also yielded a substance responding to the naphthoresorcinol test and having an R_F value in the range expected for keto sugars. Only a trace, however, could be isolated, and its identity with D-tagatose was not definitely proved.

A larger-scale isomerization of D-glucurone, followed by quantitative paper chromatography, yielded 39%, 8.5%, and 1.9% of glucuronic, mannuronic, and presumably fructuronic acid, the mole ratio being approximately 20:5:1. Reduction and subsequent hydrolysis of the corresponding mixture of methyl glycoside methyl esters gave a mixture of neutral sugars from which paper chromatography recovered D-glucose and D-mannose. The former, which crystallized, was also identified as N-*p*-nitrophenyl-β-D-glucosylamine dihydrate; the latter, which failed to crystallize, was characterized as the corresponding crystalline derivative of mannose. Fructose was recovered in amounts too small for positive identification.

Since control experiments showed that glucose readily yielded both mannose and fructose when kept dissolved in 1% sodium hydroxide for 2 hours, glucuronic, mannuronic, and galacturonic acids differed from the hexoses by failing to yield substantial quantities of keto isomers. Fischer and Schmidt (10), however, recently showed that when sodium salts of the above uronic acids were heated in aqueous solution at pH 7 and 100° for 2 hours or more, the Lobry de Bruyn – van Ekenstein transformation at C_2 was suppressed in favor of isomerism at C_5, D-glucuronic acid, for example, yielding 40% or more of L-iduronic acid. The influence of temperature and hydrogen ion concentration on the uronic acids thus appears to be complex, and worthy of further study.

EXPERIMENTAL

The following mixtures (v/v ratios) were used for paper chromatograms: (A) ethyl acetate:acetic acid:water (9:2:2); (B) *n*-butanol:formic acid:water (500:115:383); (C) ethyl acetate:pyridine:water (8:2:1); (D) ethyl acetate:acetic acid:water (3:3:1). Reducing sugars were detected with A, an *o*-aminodiphenyl spray (11); keto sugars with B naphthoresorcinol; after development, the latter chromatograms were treated with water vapor at 70° to reveal uronic acids (9). Spray C, bromophenol blue in 95% ethanol, was used to detect acids (12). To remove formic or acetic acid completely, the chromatograms were dried *in vacuo* at 110° for 8 hours before being sprayed. Whatman No. 3MM paper with a wick of No. 1 paper was used in preparative paper chromatography, the sheets measuring 46.5 cm by 57 cm.

Optical rotations were measured on a photoelectric precision polarimeter to 0.005° and were equilibrium values unless otherwise stated. All specific rotations were measured at 25±1° and at the sodium D line wavelength. Evaporations were carried out at 35° in a rotary film evaporator. All the uronic acid samples used for control purposes were chromatographically pure. Amberlite IR-120 resin (Rohm and Haas Company) was used to deionize the solutions.

Preliminary Experiments

Samples, 25 mg, of D-glucurone were kept dissolved in 10-ml volumes of 2%, 6%, and 8% aqueous sodium hydroxide at room temperature. After 2, 5, 7, and 24 hours, 1-ml aliquots were mixed with 2 ml of the ion-exchange resin and were then spotted on chromatograph paper. The use of solvents A and B with spray A in each case revealed D-glucuronic acid as the major component, and small amounts of two other substances ($R_{glucurone}$, 0.47, 0.38 in solvent A; 0.50 and 0.42 in solvent B). Spray B detected only the component of $R_{glucurone}$ 0.47, 0.50 and this reaction for a fructuronic acid was very weak; subsequent development with water vapor at 70° yielded blue spots for all the components but that for the keto acid was still weakest. No movement occurred in the basic solvent C. When the action of the alkali was prolonged for 7 days, paper chromatography showed the presence of no reducing products whatever. Solvent B and spray C yielded faint spots of R_F 0.26, 0.34, 0.40, 0.45, 0.55, and 0.65, which were thought to be those of non-reducing acids of lower molecular weight. The maximum production of new reducing substances occurred when the 2% sodium hydroxide acted for 24 hours.

A similar treatment of D-mannuronic acid ended in paper chromatograms with spots superposable on those in similar chromatograms from D-glucurone, with the difference that D-mannuronic acid instead of D-glucuronic acid was the major component. An examination of D-galacturonic acid under the same conditions gave chromatograms showing much unchanged starting material and a small amount of a compound with $R_{glucurone}$ 0.49 in solvent A, a blue spot being developed by spray B followed by the water vapor treatment. The accompanying changes in specific rotation (Table I) were complex.

TABLE I

Specific rotation of uronic acids in alkali

D-Glucurone*												
Hours	0.16	0.5	2	5	7	24						
$[\alpha]_D^{25}$	+11.5°	+10.1°	+7.9°	+6.8°	+5.3°	−0.5°						
D-Glucurone†												
Hours	0.16	0.4	0.55	0.75	1	2.2	3	4	7.5	9.3	11	21
$[\alpha]_D^{25}$	+9.8°	+9.5°	+8.5°	+8.5°	+9.0°	+8.7°	+7.9°	+7.6°	+6.3°	+5.9°	+3.3°	0
D-Mannurone*												
Hours	0.16	0.5	2	4	6	24						
$[\alpha]_D^{25}$	−10.3°	−9.2°	−8.9°	−7.1°	−6.3°	−3.5°						
D-Galacturonic*												
Hours	0.5	2	4	5	7	24						
$[\alpha]_D^{25}$	+14.3°	+9.3°	+8.7°	+6.3°	+4.3°	−0.9°						

*One percent solutions in 2% aqueous sodium hydroxide at room temperature.
†Four percent solution in 3% sodium hydroxide.

Isolation of Talose and Taluronic Acid

(*a*) A solution of D-galacturonic acid, 1 g, in 100 ml of 2% aqueous sodium hydroxide, was kept at room temperature, the specific rotation decreasing from +14.3 after 30 minutes to −0.9 after 24 hours. The solution was then deionized with 50 ml of Amberlite IR-120 resin and was evaporated to a syrup, 900 mg. This syrup was separated on eight sheets of chromatographic paper with solvent A and elution of the appropriate strips gave 454 mg of galacturonic acid and 95 mg of the uronic acid of $R_{glucurone}$ 0.49. These products were separately heated under reflux for 8 hours with 2% methanolic hydrogen chloride, and the resulting methyl ester methyl glycosides were separately reduced with an excess of lithium aluminum hydride (13) in tetrahydrofurane. The methyl glycosidic groups were hydrolyzed from the products in *N* sulphuric acid for 10

hours at 100°, and paper chromatography of the two syrups yielded one spot with the R_F and staining properties of galactose in one case and of talose in the other. Crystallization from 95% ethanol gave D-galactose, m.p. 167–170°, and specific rotation +85.5° (*c*, 4.17 in water).

Since the recovery of talose was too small to permit identification, the experiment was repeated with 5 g of D-galacturonic acid. After hydrolysis of the mixture of methyl hexosides, 2.2 g, the product was separated on 12 sheets of paper to yield 1.6 g of D-galactose, 0.15 g of uncrystallized talose, and traces of ketose. A solution of all of the talose fraction in 1 ml of water was mixed with 1 ml of a solution made from 25 g of 1-methyl-1-phenylhydrazine, 100 ml of ethanol, and 3 ml of glacial acetic acid (14). After being kept for 12 hours at 37°, the mixture was cooled to 0°, and 24 hours later 7 mg of crystals was removed. These crystals had m.p. 154–155° and a specific rotation of −4.6° (*c*, 0.63 in methanol), whereas the recorded melting points of the methylphenylhydrazone of talose were 154° (15) and 220–222° (16).

The trace of a ketose recovered from the chromatograms had an R_F value and staining properties appropriate to a ketohexose and therefore appeared to be tagatose.

(*b*) A second 5-g sample of D-galacturonic acid was treated with aqueous sodium hydroxide, the solution was freed of cations, all as described above, and was then chromatographed on 36 sheets of paper, using solvent A. Elution of the appropriate strips gave 2.74 g of galacturonic acid and 0.56 g of taluronic acid. After allowing 6% for locating the strips, these yields corresponded to 58% and 12% respectively. The galacturonic acid had m.p. 103–107° and specific rotation +50.5° (*c*, 1.97 in water), after crystallization from aqueous ethanol, whereas the original sample had m.p. 103–107° and a rotation of 47.9° (*c*, 2.94 in water).

Attempts to crystallize the taluronic acid were unsuccessful. A 200-mg sample was therefore heated at 100° for 10 minutes in 60 ml of water containing 480 mg of brucine. After cooling the solution, excess brucine was removed by three extractions with chloroform, the aqueous residue was concentrated to about 10 ml, and was diluted with acetone. A slight turbidity was removed on the centrifuge, and concentration of the clear liquor yielded a syrup which was dissolved in aqueous ethanol. The slow evaporation of this solution deposited crystals of brucine taluronate monohydrate; m.p. (decomp.) 151–154° with softening at 145°; specific rotation −20.5° (*c*, 1.02 in water). Anal. Calc. for $C_{29}H_{36}O_{11}N_2,H_2O$: C, 57.2; H, 6.2; N, 4.62%. Found: C, 57.0; H, 6.14; N, 4.76%.

The minute amount of acetone-insoluble material removed from the above brucine salt, when chromatographed on paper with naphthoresorcinol as the spray, yielded a single blue spot. This behavior suggested that a trace of the unknown tagaturonic acid was present.

Products from the Transformation of D-*Glucurone*

(*a*) A solution of 1 g of D-glucurone in 100 ml of 2% aqueous sodium hydroxide was decationized after 24 hours and was concentrated to a syrup, 1.1 g, which was quantitatively chromatographed on 16 sheets of paper using solvent A. Fraction 1, 284 mg, was glucuronic acid; fraction 2, 87 mg, mannuronic acid; fraction 3, 18 mg, an unidentified keto acid; fractions 4 and 5, traces of unidentified material; fraction 6, 80 mg, glucurone. After correction for a 6% loss in the guide strips of the chromatograms, the yields were: glucuronic acid plus glucurone, 39%; mannuronic acid, 9.2%; unknown keto acid, 1.9%.

Fractions 4 and 5 were not investigated. The others were separately converted to the methyl ester glycosides, reduced, and hydrolyzed to the free sugars as previously described. Chromatography in solvents A, C, and D with sprays A and B showed that fractions 1 and 6 contained glucose; fraction 2, mannose with traces of glucose and a ketose; fraction 3, like the trace of ketose in fraction 2, had staining properties (naphthoresorcinol) and an R_F value identical with those of fructose, and a trace of mannose was also present.

The combined syrup, 150 mg, from fractions 1 and 6 was heated under reflux for 15 minutes with 3 ml of a solution made from 9 g of *p*-nitroaniline, 200 ml of methanol, and 0.14 ml of concentrated hydrochloric acid. After being filtered while hot, the solution deposited crystals which were twice recrystallized from methanol. The product, m.p. 184–185° and specific rotation −215° (*c*, 1.09 in dry pyridine), was N-*p*-nitrophenyl-β-D-glucopyranosylamine dihydrate, the reported constants (17) of which were m.p. 184° and $[\alpha]_D^{20}$ −201° (*c*, 1.0 in dry pyridine).

(*b*) To identify mannose, a 5-g sample of D-glucurone was treated as described in (*a*) to yield 2 g of the neutral sugars. Of this mixture, 1.2 g was separated on 12 sheets of paper (solvent D) to yield 0.52 g of glucose and 70 mg of uncrystallized mannose, together with a minute amount of fructose. The glucose crystallized and after recrystallization from aqueous ethanol had the correct melting point and specific rotation. The syrupy mannose fraction, 70 mg, was converted to the crystalline N-*p*-nitrophenyl-β-D-mannosylamine dihydrate, which had the specific rotation −335° (*c*, 0.185 in dry pyridine) and m.p. 219°. The recorded rotation was $[\alpha]_D^{20}$ −325° (*c*, 0.16 in dry pyridine) and m.p. 219°. A mixed melting point with an authentic sample was not depressed.

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