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.

Acknowledgment.—We wish to acknowledge the generosity of the Northern Regional Research Laboratories (Peoria, Illinois) for the calcium 5-keto-D-gluconate trihydrate used in this work.

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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

RECEIVED JANUARY 14, 1954

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

(1) Journal Paper No. 774 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

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TABLE I

ACIDIC OLIGOSACCHARIDES FROM OKRA MUCILAGE HYDROLYSATE

Component	Yield, %	R, gal.[a]	Monosaccharide components	$[\alpha]^{25}D$	Equiv. wt. of Ba salt Calcd.	Found
I, aldobiouronic acid	1.7	0.97	D-Galacturonic acid L-Rhamnose	+15.1°	407	446
II, aldotriouronic acid	1.0	.42	D-Galacturonic acid L-Rhamnose D-Galactose	.....	570	489
III, aldotriouronic acid	0.5	.19	D-Galacturonic acid L-Rhamnose D-Galactose	+52.2	570	576

[a] Ratio of distance traveled by unknown on a paper chromatogram to distance traveled by D-galactose in solvent C.

that the original acidic disaccharide is 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose. This disaccharide has been isolated previously from hydrolyzates of slippery elm mucilage,[5] flaxseed mucilage[6] and *Plantago ovato Forsk* mucilage.[7]

The two aldotriouronic acids recovered from the cellulose column have been partially characterized. Monosaccharide units on the reducing ends of these trisaccharides are identified by oxidizing the aldotriouronic acids with bromine water to convert the terminal reducing sugar units to glyconic acids. On hydrolysis of the oxidized oligosaccharides followed by chromatographic separation of the components and spraying with *p*-anisidine reagent, the sugars giving rise to the glyconic acids are found to be uncolored. This evidence suggests that a galactose unit is at the reducing end of one aldotriouronic acid and that a rhamnose unit is at the reducing end of the other. Further structural evidence is obtained by chromatographic identification of the sugar derivatives obtained by hydrolysis of the methylated and hydrogenated trisaccharides. Together these data suggest that one of the acids is a galactosyl → (galactosyluronic acid) → rhamnose and that the other is a (galactosyluronic acid) → rhamnosyl → galactose.

Experimental

Paper Chromatography.—Chromatographic separations were made on filter paper strips and cellulose columns at room temperature using one of the following solvents in the volume ratios indicated: (A) butanol-1, pyridine, water (6:4:3); (B) butanol-1, acetic acid, water (2:1:1); (C) ethyl acetate, acetic acid, formic acid, water (18:3:1:4); (D) butanol-1, ethanol, water (5:1:4, top layer); and (E) benzene, ethanol (170:50), saturated with water. *p*-Anisidine hydrochloride[8] was used to detect the sugars and their derivatives on paper chromatograms.

Hydrolysis of Okra.—Fifty grams of okra mucilage was homogeneously dispersed in 2 l. of water at 80° and aqueous sulfuric acid added to a 5% concentration. The mixture was hydrolyzed at 80° with stirring for 4 hours. On cooling, the mixture was neutralized with 750 g. of barium hydroxide and the barium sulfate removed by filtration. Barium ions in salt formation with the acidic oligosaccharides were removed by passing the solution through a column of Amberlite IR-120 resin. Effluent was then added to the top of a 44 × 265 mm. column[3] of Darco; Celite (1:1) and monosaccharides were removed by washing the column with 8 l. of water and were discarded. Acidic oligosaccharides were removed with 5 l. of 10% ethanol. After concentrating the eluate to a sirup (3.5 g.) barium hydroxide was added to neutrality and the mixture was chromatographed on a 44 × 265 mm. column of cellulose. Solvent A was used to elute the neutral oligosaccharides. Barium salts of the acidic oligosaccharides were washed from the column with water, concentrated to a sirup (2 g.) and rechromatographed on another cellulose column of the same size using solvent B. Since the irrigating solution was acidic, the acidic oligosaccharides were removed from the column in the free acid form. Fractions were collected through use of an automatic fraction collector[9] and analyzed qualitatively by paper chromatography. Chromatographically similar fractions were combined. By this means there were obtained three acidic oligosaccharides whose characteristics are given in Table I.

Methylation of Aldobiouronic Acid (Component I).—Chromatographically pure sirup (1.05 g.) was dissolved in water (8 ml.) and dimethyl sulfate (8 ml.) was added. While stirring this mixture at 0°, aqueous sodium hydroxide (12 ml., 40%) was added dropwise over an 8-hour period. The reaction mixture was stirred an additional 15 hours during which it warmed to room temperature. It was then cooled again to 0°, solid sodium hydroxide (7.2 g.) added and dimethyl sulfate introduced dropwise with stirring as before. After a final reaction period of 15 hours, the solution was acidified with dilute hydrochloric acid and extracted continuously for 16 hours with chloroform. The chloroform extract was concentrated *in vacuo* to a sirup (0.57 g.) which was twice methylated with methyl iodide (5 ml.) and silver oxide (2 g.) to give a fully methylated sirup (0.52 g.) with $[\alpha]^{25}D$ +54.4° (*c* 1.74 in water).

Reduction and Hydrolysis of Methylated Sirup.—The sirup was dissolved in anhydrous ether (20 ml.) and the solution was added dropwise to a vigorously stirred slurry of lithium aluminum hydride (1.2 g. in 40 ml. of anhydrous ether) at room temperature. After a reaction time of 1 hour, excess hydride was destroyed by cautious addition of water. On evaporation of the ether the aqueous mixture was acidified with dilute hydrochloric acid and extracted continuously for 15 hours with hot chloroform. Removal of the chloroform left a sirup (0.50 g.) which was refluxed with methanolic hydrogen chloride (20 ml., 4%) for 4 hours. Water (20 ml.) was added, methanol evaporated and the resulting aqueous solution heated at 100° for 2 hours. Neutralization with Amberlite IR-4B resin and concentration of the hydrolysate gave a sirup (0.50 g.) which on paper chromatography was found to contain two components with R_g (g = 2,3,4,6-tetra-*O*-methyl-D-glucose) values of 0.90 and 0.75 in solvent D. These values suggested the presence of 3,4-di-*O*-methyl-L-rhamnose and 2,3,4-tri-*O*-methyl-D-galactose.

Identification of Hydrolysis Products.—The above hydrolysate was separated on 4 large sheets (18.5 × 22.5 in.) of Whatman No. 1 paper with solvent D. Upon paper ionophoresis in borate buffer at *p*H 10, the rhamnose component moved toward the anode, which suggested that it was 3,4-di-*O*-methyl-L-rhamnose.[10] Oxidation with bromine water to 3,4-di-*O*-methyl-L-rhamnonolactone gave crystals which on recrystallization from ether–heptane had m.p. 78–79° and $[\alpha]^{25}D$ −154° (*c* 0.21, water) → −116° (48 hours).

Anal. Calcd. for $C_8H_{14}O_5$: C, 50.5; H, 7.4. Found: C, 50.5; H, 7.3.

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2,3,4-Tri-*O*-methyl-D-galactose was identified as its characteristic anilide derivative. After recrystallization from absolute ethanol, the m.p. was 167–168°.

Anal. Calcd. for $C_{15}H_{23}O_5N$: N, 4.7. Found: N, 4.7.

An X-ray diffraction pattern of the crystals was identical with that of an authentic specimen.

Test for Reversion.—On paper chromatography of a solution of D-galactose and L-rhamnose in equimolar concentrations which had been subjected to the same conditions used in the hydrolysis of okra mucilage no oligosaccharide spots were observed, showing that the aldobiouronic acid arising upon hydrolysis of okra mucilage is not a reversion product.

Characterization of Aldotriouronic Acids.—Bromine oxidation of a small amount of component II followed by acid hydrolysis and paper chromatography of the hydrolysate indicated that galactose is at the reducing end of this trisaccharide since galactose, having been oxidized to galactonic acid, did not appear on the chromatograms of the hydrolysate when sprayed with *p*-anisidine hydrochloride. Component II was methylated and reduced as described for the aldobiouronic acid. Paper chromatography in solvent D of a hydrolysate of a small amount of the reduced product showed the presence of two trimethylgalactoses and a dimethylrhamnose. The main quantity of the reduced sirup was remethylated and then hydrolyzed to yield tetramethylgalactose, a trimethylgalactose and a dimethylrhamnose as shown by paper chromatography in solvents D and E. Attempts to isolate these derivatives were unsuccessful because of the small amounts present.

By using the bromine oxidation procedure described above, rhamnose was shown to be at the reducing end of component III. Paper chromatography of a hydrolysate of completely methylated component III indicated the presence of a dimethylrhamnose, a dimethylgalacturonic acid and tetramethylgalactose.

Acknowledgment.—The authors wish to express their thanks to Dr. L. Hough for supplying the authentic specimen of 2,3,4-tri-*O*-methyl-D-galactose anilide used for X-ray comparison.

LAFAYETTE, INDIANA

[CONTRIBUTION FROM DEFENCE RESEARCH CHEMICAL LABORATORIES, OTTAWA]

Organic Phosphorus Compounds. III. Reactions of Methanephosphonyl Dichloride with Diols[1]

BY A. F. MCKAY, R. A. B. BANNARD, R. O. BRAUN AND R. L. BENNESS

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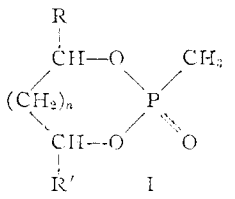
Glycols combined with methanephosphonyl dichloride to give heterocyclic structures containing the phosphonate group. Methanephosphonyl dichloride on treatment with D-(−)-2,3-butanediol in the presence of ether and pyridine gave 2,4,5-trimethyl-2-oxo-1,3-dioxa-2-phosphacyclopentane and an optically active compound. The latter compound was identified by degradation with phosphorus pentachloride as 1-methyl-2-hydroxypropyl 1-methyl-1-propenyl methanephosphonate.

A continuation of the studies[2] of the reaction of glycols with methanephosphonyl dichloride has shown that 2,4-pentanediol, 1,3-butanediol and 1,4-butanediol give the cyclic structures 2,4,6-trimethyl-2-oxo-1,3-dioxa-2-phosphacyclohexane (I, R and R′ = CH_3, n = 1), 2,6-dimethyl-2-oxo-1,3-dioxa-2-phosphacyclohexane (I, R = CH_3, R′ = H,

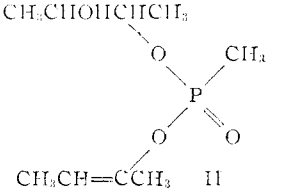


I

n = 1) and 2-methyl-2-oxo-1,3-dioxa-2-phosphacycloheptane (I, R and R′ = H, n = 2), respectively. In each run some methanephosphonic acid and/or methanephosphonic anhydride was also produced.

Ethylene chlorohydrin combined with methanephosphonyl dichloride giving a mixture of β-chloroethyl hydrogen methanephosphonate and di-β-chloroethyl methanephosphonate.

Recently[2] it was shown that D-(−)-2,3-butanediol and methanephosphonyl dichloride in methylene chloride and in the presence of pyridine gave a 75% yield of the cyclic product 2,4,5-trimethyl-2-oxo-1,3-dioxa-2-phosphacyclopentane (I, R and R′ = CH_3, n = 0). Now it has been found that replacement of the solvent methylene chloride by ether lowered the yield of cyclic product to 53%. Moreover a new optically active compound was formed in 38% yield. This new compound had a rotation of −103° and it gave analytical values in good agreement with the empirical formula $C_9H_{19}O_4P$. Its infrared spectrum has a band at 3400 cm.$^{-1}$ due to O–H stretching vibrations. A medium band at 1710 cm.$^{-1}$ is assigned to C=C stretching vibrations or an associated ester group because C–O stretching vibrations would be expected to give a stronger absorption band. The strong absorption band at 1282 cm.$^{-1}$ with an absorption band on the shoulder at 1265 cm.$^{-1}$ is assigned to stretching vibrations of the P=O group[3-5] and 983 cm.$^{-1}$ is assigned[4,5] to the P–O–C linkage. Since the infrared spectrum of the optically active compound indicated the presence of a double bond, a hydroxy group and P–O–C linkages and the absence of the group C=O, structure II was assigned to it. This structure was confirmed by degradation with phosphorus pentachloride. Previously[6] the structure of dichlorophosphorylmethanephosphonyl



II

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