THE PHENYL α- AND β-L-IDOPYRANOSIDURONIC ACIDS AND SOME OTHER ARYL GLYCOPYRANOSIDURONIC ACIDS*

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ABSTRACT

Condensation of 1,2,3,4,6-penta-*O*-acetyl-α-L-idopyranose (**1**) with phenol yielded phenyl 2,3,4,6-tetra-*O*-acetyl-α- (**2**) and β-L-idopyranoside (**4**) Deacetylation of **2** and **4** afforded phenyl α- and β-L-idopyranosides (**3** and **5**), respectively, the structures of which were verified by periodate oxidation studies A platinum-catalyzed oxidation of **3** and **5** produced the amorphous phenyl α- and β-L-idopyranosiduronic acids (**9** and **11**), respectively, which were isolated as the crystalline cyclohexylammonium salts Phenyl β- and α-D-glucopyranosiduronic acids are apparent minor byproducts of the catalytic oxidations, resulting from an inversion at C-5 *p*-Nitrophenyl α-D-mannopyranosiduronic acid and *p*-nitrophenyl α- and β-D-galactopyranosiduronic acids are also described

INTRODUCTION

In 1957, it was briefly reported that extracts of skin catalyzed the hydrolysis of a tetrasaccharide derived from dermatan sulfate[1] A more recent note described the enzymic release of iduronic acid from desulfated dermatan sulfate by action of extracts of human tissue, supplemented with β-glucuronidase and hyaluronidase[2] The present work was undertaken to provide substrates suitable for the study of α-L-iduronidase activity, whose occurrence was indicated by the reports cited and whose existence would have important implications for the catabolism of dermatan sulfate and other glycosaminoglycans The availability, from the chemical syntheses described here, of aryl glycosides of L-iduronic acid and related glycosides has indeed permitted a demonstration of α-L-iduronidase as a distinct mammalian enzyme[3]

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RESULTS AND DISCUSSION

Zinc chloride-catalyzed condensation[4,5] of 1,2,3,4,6-penta-*O*-acetyl-α-L-idopyranose (**1**) with phenol gave syrupy phenyl 2,3,4,6-tetra-*O*-acetyl-α-L-idopyranoside (**2**) and a small quantity of the crystalline β-anomer (**4**), which were isolated chromatographically. Deacetylation of **2** and **4** yielded phenyl α-L-idopyranoside (**3**) and the β-anomer **5**, respectively. Although both **3** and **5** were amorphous, it was possible to confirm their ring size, anomeric configuration, and identity by a periodate oxidation study, the results of which are shown in Table I. The consumption of periodate and the production of formic acid are correct for pyranosides. Moreover, since the periodate oxidation products from the α-L-idoside **3** and from phenyl α-D-glucopyranoside (**6**) are enantiomers, their optical rotation values should be equal in magnitude but differ in sign, a like relationship should be observed for the periodate oxidation products from the β-L-idoside **5** and from phenyl β-D-glucopyranoside (**7**). These criteria[6] are satisfied within accuracy reasonable for the circumstances, as seen in Table I.

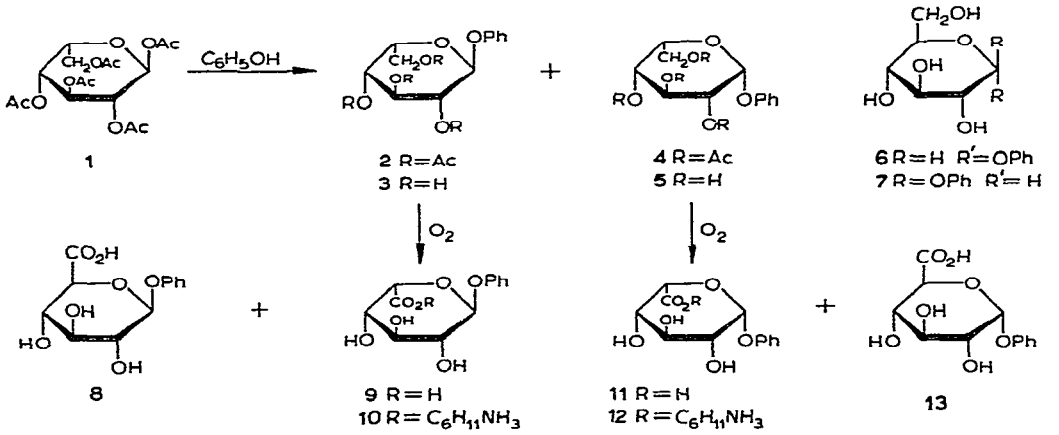


TABLE I

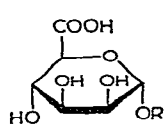
PERIODATE OXIDATION OF PHENYL L-IDOPYRANOSIDES AND D-GLUCOPYRANOSIDES

Phenyl glycoside	*Periodate consumed (mole)*	*Formic acid produced (mole)*	*Final* $[M]_D \times 10^{-3}$ (°)
α-L-Idopyranoside (**3**)	2 17	0 98	−32 6
α-D-Glucopyranoside (**6**)	2 20	0 93	+31 0
β-L-Idopyranoside (**5**)	1 90	0 89	+30 0
β-D-Glucopyranoside (**7**)	2 04	1 02	−35 1

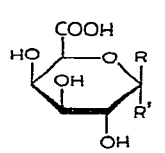
Catalytic oxidations[7] of the idosides **3** and **5** produced phenyl α-L-idopyranosiduronic acid (**9**) and phenyl β-L-idopyranosiduronic acid (**11**), both amorphous These were isolated by chromatography and analyzed as their crystalline cyclohexylammonium salts **10** and **12** The pure, anomeric free acids **9** and **11**, regenerated from the salts, showed a difference in molar rotation values ($\Delta M_D = 2A$) of −54,000 As predicted from Hudson's rules, this is opposite in sign from values of 2A calculated for known phenyl glycosides of the D-series but, for reasons not yet understood, corresponds only roughly in magnitude. As calculated from data in the literature, 2A for the anomeric phenyl α- and β-D-glucopyranosiduronic acids (**13** and **8**)[8] is +68,100, for the anomeric phenyl α- and β-D-glucopyranosides[9] (**6** and **7**), it is +64,600

The purity of **10** and **12** can, in part, be verified by enzymic tests, since, as previously mentioned[3], the occurrence in rat liver lysosomes of an α-L-iduronidase was demonstrated by use of the α-L-iduronide **9** as substrate This enzyme is distinct from β-D-glucuronidase The latter has no action on **9**, indicating the absence of detectable amounts (2%) of the β-D-glucuronide **8** in **9** Although purification of α-L-iduronidase, an enzyme possessing weak activity, has just been begun at time of writing, it has been possible to demonstrate with crude rat liver preparations an enzymic hydrolysis of **9** as extensive as 91% (unpublished observations) Since neither the β-L-iduronide **11** nor the α-D-glucuronide **13** are attacked by the crude enzyme, substantial contamination of **9** with these compounds is also excluded by this result The same experiments show the absence of **8** or **9** from preparations of the β-L-iduronide **11**

In the catalytic oxidation of the α-L-idoside **3**, a small quantity of crystalline byproduct, which was identified as phenyl β-D-glucopyranosiduronic acid (**8**) by comparisons with an authentic specimen, was isolated Also, in the products from the catalytic oxidation of the β-L-idoside **5**, evidence from colorimetry suggests the second crop material isolated in the crystallization of the principle product **12** to be the impure salt of a phenyl glucosiduronic acid By analogy to the isolation of **8** from the oxidation product of **3**, this byproduct, which was not characterized, is probably the α-D-pyranoside **13** Attempts to demonstrate the production of **8** from **9** or of **9** from **8** under the conditions of the catalytic oxidation were unsuccessful This interesting, but presently unexplained, inversion at C-5 is apparently incidental to the oxidation It may be speculated that the unidentified byproducts that result from the catalytic oxidation of the methyl α- and β-D-idopyranosides[10] may be derivatives of L-glucuronic acid, in analogy to the present findings

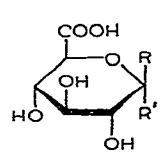


14 R=*p*-$NO_2C_6H_4$



15 R=H, R=*p*-$NO_2C_6H_4O$

16 R=*p*-$NO_2C_6H_4O$, R'=H



17 R=H, R=*p*-$NO_2C_6H_4O$

18 R=*p*-$NO_2C_6H_4O$, R'=H

Catalytic oxidation of the corresponding *p*-nitrophenyl glycopyranosides, currently available commercially, readily afforded *p*-nitrophenyl α-D-mannopyranosiduronic acid (**14**) and the anomeric p-nitrophenyl α- and β-D-galactopyranosiduronic acids (**15** and **16**) The value of 2A calculated for **15** and **16**, +101,700, is in satisfactory agreement with the value of +98,300 calculated for the anomeric *p*-nitrophenyl α- and β-D-glucopyranosiduronic acids (**17** and **18**) *o*-Nitrophenyl β-D-galactopyranosiduronic acid has been described previously[7]

The differential response of derivatives of the various hexuronic acids to colorimetry by the carbazole[11] and orcinol[12] (100°, 30 min[13]) methods, which permitted the detection of L-iduronic acid in polysaccharides[14], can be exploited for verification of the gross identity of uronic acid components of glycosides, as illustrated in Table II

TABLE II

U V ABSORPTION AND COLORIMETRIC RESPONSE OF ARYL GLYCOPYRANOSIDURONIC ACIDS

Aryl glycopyranosiduronic acid[a]	λ_{max} (*nm*)	$\varepsilon_{max} \times 10^{-3}$	*Molar color yield*[b]		
			Carbazole	*Orcinol*	*Ratio*
Ph α-L-ido[c] (**10**)	266 2	0 93	0 29	1 49	0 19
Ph β-L-ido[c] (**12**)	266 7	0 93	0 29	1 50	0 19
PNP α-D-manno (**14**)	305	10 6	0 10	1 28	0 08
PNP α-D-galacto (**15**)	305	10 5	1 21	1 44	0 84
PNP β-D-galacto (**16**)	304	10 5	1 22	1 49	0 82
PNP α-D-gluco (**17**)	304	10 5	1 01	0 97	1 04
PNP β-D-gluco (**18**)	303	10 9	1 01	0 98	1 03

[a]Abbreviations used, Ph, phenyl, PNP, *p*-nitrophenyl [b]The molar color yields in the carbazole and orcinol reactions and the ratio between these are taken by definition as 1 00 for D-glucopyranurono-3,6-lactone, used as the reference standard Corresponding values measured for 1,2-isopropylidene-L-idopyranurono-3,6-lactone are 0 29, 1 46, and 0 20, for D-galacturonic acid, 1 14, 1 41, and 0 81 These trends roughly follow those reported by Hoffman, Linker, and Meyer[14], who reported values of 0 17, 1 28, and 0 13 for D-mannuronic acid [c]Cyclohexylammonium salts

In the u v spectrum, a triplet peak centered at 266–267 nm is characteristic of phenyl glycosides, for example, the phenyl α- and β-D-glucopyranosides and the phenyl 2-acetamido-2-deoxy-α- and β-D-glucopyranosides (λ_{max} 266 3, 265 7, 266 5, and 265 8 nm, ε_{max} 890, 880, 870, and 830, respectively), ε_{max} 890 was assumed for the amorphous idosides **3** and **5** in measuring their concentrations Based on measurements made with the cyclohexylammonium salts **10** and **12**, shown in Table II, ε_{max} 930 was assumed for the amorphous idopyranosiduronic acids **9** and **11** For *p*-nitrophenyl glycosides, a broad absorption maximum at about 304 nm (ε_{max} 10,500) is characteristic, as exemplified by *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (λ_{max} 303 nm, ε_{max} 10,400) and the *p*-nitrophenyl glycosides listed in Table II

EXPERIMENTAL

General methods — Melting points are corrected. U.v. spectra were determined in aqueous solution with a Cary Model 15 spectrophotometer and i.r. spectra on potassium bromide pressings with a Perkin–Elmer Model 137 spectrophotometer. Optical rotations were measured in a 1-dm tube with a Perkin–Elmer Model 141 automatic polarimeter. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Chromatography on dimethyl sulfoxide–silicic acid[15] — Silicic acid (Silicar CC-7, 300–325 mesh, Mallinckrodt Chemical Works, 675 g) was poured as a slurry in toluene to form a column of bed volume of 1550 ml. The column was prewashed with 1 l. dimethyl sulfoxide–toluene (7.5 l), ethyl ether–dimethyl sulfoxide[15] (9 l), dry ether (0.8 l), and 2-isopropoxypropane–dimethyl sulfoxide[15] (1 l), which also was used as the developer. The syrupy product isolated from the condensation of idose pentaacetate with phenol was applied as a solution in the developer (300 ml), supplemented with ethyl ether (50 ml). Following development, small aliquots of the effluent fractions were analyzed for hexose by the anthrone method[16], after prior evaporation of the solvent. A major peak (A) at 5.04 l. was followed by a slightly overlapping smaller peak (B) at 7.20 l. Fractions within each peak were pooled, washed with a 10% sodium sulfate solution, dried, and concentrated to syrups *in vacuo*.

The result of a model experiment in which 50 mg each of the phenyl 2,3,4,6-tetra-*O*-acetyl-α- and β-D-glucopyranosides were chromatographed on a similar column of 13.5-ml bed volume, illustrates further the excellent resolving power of this technique. Two peaks, at 43 ml and 93 ml, were completely separated.

1,2,3,4,6-Penta-O-*acetyl*-α-L-*idopyranose* (**1**) — This compound was prepared (*cf.* Ref. 17) *via* 1,2-*O*-isopropylidene-5,6-di-*O*-tosyl-α-D-glucofuranose[18], which was prepared from 1,2-isopropylidene-α-D-glucofuranose (30% yield) and treated[19] to form 3,5,6-tri-*O*-acetyl-1,2-isopropylidene-β-L-idofuranose (64% yield). This was transformed into **1** without isolation of additional intermediates[17,19,20] in 24% yield. Thus, hydrolysis for 7 h in a large volume of boiling M sulfuric acid, neutralization with barium hydroxide, and concentration *in vacuo* gave a syrup, acetolysis[17] of which gave **1**, m.p. 94.5–95.5°, $[\alpha]_D^{23}$ −56.8° (*c* 1, chloroform), lit.[17] m.p. 95–96°, $[\alpha]_D^{25}$ −57° (chloroform).

Reaction of penta-O-*acetyl*-α-L-*idopyranose* (**1**) *with phenol* — In preliminary experiments of this Helferich condensation, conditions reported as optimal for the preparation of phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[5] were tried. However, for the isolation of the products obtained from **1**, a greatly decreased zinc chloride concentration and a shortened heating interval proved to be essential. A flask arranged for vacuum distillation was charged with **1** (12 g), molten phenol (40 ml), and a solution of zinc chloride (1.3 g) in 19 l. acetic acid–acetic anhydride (3 ml). The flask was heated in an oil bath at 88° with occasional shaking, while the pressure was maintained at 38 mm. After 20 min, the temperature was raised and

the mixture was maintained for 33 min more at 122–124° and 25 mm After cooling, the residue was dissolved in chloroform, washed twice with a 10% sodium sulfate solution, once with a 2M sodium hydroxide solution (170 ml) containing ice, and four times with a 10% sodium sulfate solution The syrup (14 g) obtained by drying with sodium sulfate and removal of the solvent *in vacuo* was subjected to chromatography on a column of dimethyl sulfoxide–silicic acid, as described in the preceding paragraphs The syrup from peak A (7 4 g), $[\alpha]_D^{23}$ −80° (*c* 1 23, chloroform), was identified by subsequent reactions as phenyl 2,3,4,6-tetra-*O*-acetyl-α-L-idopyranoside (**2**) The syrup from peak B crystallized readily on dilution with ethanol Recrystallization from ethanol gave 1 67 g of phenyl 2,3,4,6-tetra-*O*-acetyl-β-L-idopyranoside (**4**), m p 103 5–104°, $[\alpha]_D^{23}$ +64 2° (*c* 1 1, chloroform)

Anal Calc for $C_{20}H_{24}O_{16}$ C, 56 6, H, 5 66 Found C, 56 7, H, 5 68

*Phenyl α-(**3**) and β-L-idopyranoside* (**5**) — Sodium methoxide (0 04 mmol) was added to a boiling solution of **2** (6 3 g) in chloroform–methanol After heating for an additional 2 min, the solution was kept for 2 h, neutralized with acetic acid, and evaporated *in vacuo* This gave 3 3 g of syrupy **3**, $[\alpha]_D^{23}$ −125° (*c* 0 54, water), calculated from a concentration established by u v measurements Similar deacetylation of **4** gave amorphous **5**, $[\alpha]_D^{23}$ +55° (*c* 1 0, water), calculated from a molar concentration based on the weight of crystalline **4** from which it was produced

Periodate oxidation studies were performed with the idosides **3** and **5**, whose initial concentrations (about 4mM) were established by u v measurements The plateau values of the periodate consumption and formic acid generation[21] shown in Table I were observed after reaction with unbuffered 0 02M sodium metaperiodate for 24 h at 25° Also shown are plateau values (4 h) of molar optical rotation measured for reaction mixtures at 25° initially containing 0 02M phenyl glycoside and 0 065M sodium metaperiodate In this experiment, the molar concentration of **3** was measured by u v absorption and that of **5** from calculations based on the weight of crystalline tetraacetate **4**, from which it was produced

Oxidation of phenyl α-L-idopyranoside (**3**) — Adams' platinum oxide catalyst (Engelhardt Industries), suspended in water, was reduced with hydrogen at about atmospheric pressure for 20 min on a Parr shaker The resulting platinum black, after repeated washing with water, was used for oxidations on the day of preparation A solution of **4** (3 2 g) in water (125 ml) was treated with Norite A to remove some minor turbidity About 0 5 g of catalyst was suspended by vigorous stirring in this solution, which was heated at 70–72° in a water bath while a stream of oxygen was passed through it As the oxidation progressed, 0 5M sodium hydrogen carbonate was added in portions to maintain a pH value of 6 4–7 6 Portions (0 5 g) of fresh catalyst were added after 1,2, and 3 h Based on experience in earlier experiments, the reaction was terminated after 4 5 h After removal of the catalyst and adjustment of the pH to 5 with acetic acid, the solution was concentrated *in vacuo* to 10 ml and applied to a 250-ml (bed volume) column of Dowex 1(X-8, HCO_2^-) Orcinol-positive[13] peaks observed on elution with 0 75M formic acid included a small one at 0 8 l, which was not investigated (presumably unchanged **3**), and another small peak (G) at

9 5 l, which was well resolved from the major peak (I) at 10 7 l Peaks G and I accounted for 8% and 77% of the orcinol-positive material applied to the column The pooled fractions of the peaks were freed of formic acid by continuous extraction with ether, and concentrated *in vacuo* to syrups

Hydrolyzates of aliquots of the materials from peaks G and I (0 8M hydrochloric acid, 1 h, 100°) were subjected to paper chromatography in several solvent systems[22,23] that separate iduronic acid[24] and glucuronic acid Hydrolyzates from peak G showed only spots for glucuronic acid, and hydrolyzates from peak I showed only spots for iduronic acid

The syrup from peak I failed to crystallize On treatment with cyclohexylamine (10 ml) and addition of ether, a crystalline solid separated Recrystallization from ethanol–ether gave 0 61 g of cyclohexylammonium (phenyl α-L-idopyranosid)-uronate (**10**), m p 181–183° (dec), $[\alpha]_D^{23}$ −56 8° (*c* 0 9, water)

Anal Calc for $C_{18}H_{26}NO_7$· C, 58 7; H, 7 11 Found C, 58 4, H, 7 29

The pure, free acid **9** was regenerated from a solution of **10** by passage through a column of Dowex 50 (H^+), its concentration was calculated from u v measurements; $[\alpha]_D^{23}$ −86° (*c* 0 2, water)

The syrup obtained from peak G of the Dowex-1 chromatogram soon crystallized when diluted with a little water The m p of the crystals (158 5–159 5°) was not depressed on admixture with authentic phenyl β-D-glucopyranosiduronic acid[25], and the i r. spectrum was identical with the spectrum of this compound

Oxidation of phenyl β-L-idopyranoside (**5**) — A solution of **5** (prepared from 0 64 g of **4**) in water (12 ml) was vigorously stirred with freshly prepared platinum black (0 2 g) at 85° while oxygen was passed through the suspension A 0 5M sodium hydrogen carbonate solution was added in 0 1-ml portions, and the mixture was tested between additions with short range pH indicator paper The pH of the mixture remained below 7 at 24 min from the start, when the theoretical quantity of sodium hydrogen carbonate (3 0 ml) had been added. The mixture was cooled after an additional 2 min After removal of the catalyst, the deep-orange solution was applied to a column of diethylaminoethylcellulose (12-ml bed volume, Whatman DE 52), which had previously been equilibrated with 0 01M tris(hydroxymethyl)aminomethane acetate buffer of pH 7 1 and washed with water Following the addition of the load, the column was washed with water (these washings were discarded) and eluted with 0 075M sodium acetate (120 ml), a procedure found in model experiments with **8** to yield an aryl glycosiduronic acid fraction[26] The eluate was passed through Dowex 50 (H^+) to remove sodium ions and repeatedly concentrated *in vacuo* with addition of water to remove acetic acid A solid separated at once on treatment of the final residue, a yellowish syrup, with cyclohexylamine (0 2 ml) in ethanol (2 ml) This was filtered off and washed sparingly with ethanol and copiously with ether In the filtrate, a second crop of solid (G′) separated

The first crop of material (76 mg), was subjected to colorimetry for uronic acid and u v spectrophotometry The carbazole–orcinol ratio was 0 22, the value expected for a derivative of iduronic acid The orcinol color value and the u v absorption both

corresponded to a content of 48 mg of a phenyl idosiduronic acid (59 mg of salt), the impurity was believed to be the carbamate of cyclohexylamine A solution of the solid in dilute acetic acid was concentrated to dryness *in vacuo*, and the residue was recrystallized from ethanol–ethyl acetate containing a little dilute acetic acid, to give 55 mg of pure cyclohexylammonium (phenyl β-L-idopyranosid)uronate (**12**), m p 219° (dec), $[\alpha]_D^{23}$ +73 2° (*c* 0 2, water)

Anal Calc for $C_{18}H_{26}NO_7$ C, 58 7; H, 7 11 Found C, 58 5; H, 6 81

The pure, free acid **11**, regenerated from **12** as for the α-anomer **9**, had $[\alpha]_D^{23}$ +115° (*c* 0 2, water)

The second crop of material (G′, 44 mg), had a carbazole–orcinol ratio of 0 85, nearly correct for a derivative of glucuronic acid The orcinol color value and u v absorption both corresponded to a content of 15 mg of a phenyl glucosiduronic acid (as free acid)

p-*Nitrophenyl* α-D-*mannopyranosiduronic acid* (**14**) — This compound was prepared by oxidation of *p*-nitrophenyl α-D-mannopyranoside (0 14 g, Pierce Chemical Co) as described for **5** With 0 1 g of catalyst, the theoretical amount of sodium hydrogen carbonate reacted after an oxidation of 11 min Crystallization of **14** (0 07 g) occurred during concentration of the eluate from the Dowex-50 column The compound was recrystallized from water for analysis, m p 234° (dec), $[\alpha]_D^{23}$ +113 3° (*c* 0 2, water)

Anal Calc for $C_{12}H_{13}NO_9$ C, 45 7, H, 4 16, N, 4 44 Found C, 45 8, H, 4.10, N, 4 45

p-*Nitrophenyl* α-D-*galactopyranosiduronic acid* (**15**) — This compound was similarly prepared by catalytic oxidation of *p*-nitrophenyl α-D-galactopyranoside (0 15 g, Pierce Chemical Co) The product crystallized (0 08 g) during concentration of the eluate from the Dowex-50 column and was recrystallized from aqueous ethanol for analysis, m p 260°, $[\alpha]_D^{23}$ +186 4° (*c* 0 2, water)

Anal Calc for $C_{12}H_{13}NO_9$· C, 45 7; H, 4 16, N, 4 44 Found. C, 45 8; H, 4 21, N, 4 44

p-*Nitrophenyl* β-D-*galactopyranosiduronic acid* (**16**) — This compound was similarly prepared by oxidation of *p*-nitrophenyl β-D-galactopyranoside (0 45 g, Sigma Chemical Co) It crystallized on being kept overnight (0 08 g) after evaporation of the Dowex 50-column eluate and was recrystallized from water to give a monohydrate, m p 165–167° (dec) $[\alpha]_D^{23}$ −122 2° (*c* 0 2, water), calc loss 5 4, loss at 60°, found 6 0)

Anal Calc for $C_{12}H_{13}NO_9$· C, 45 7, H, 4 16, N, 4 44 Found· C, 45 5, H, 4 21; N, 4 34

Additional optical rotation measurements — They are reported on an anhydrous basis and were measured for *p*-nitrophenyl α-D-glucopyranosiduronic acid monohydrate (**17**), prepared as described[7], $[\alpha]_D^{23}$ +184 8° (*c* 0 2, water); and for *p*-nitrophenyl β-D-glucopyranosiduronic acid monohydrate (**18**), $[\alpha]_D^{23}$ −127 2° (*c* 0 2, water), purchased from Calbiochem (Los Angeles, California)

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