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# PREPARATION OF (ARYL α-L-IDOPYRANOSID)URONIC ACIDS*

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ABSTRACT

The earlier preparation of cyclohexylammonium (phenyl α-L-idopyranosid)-uronate has been improved, and (4-methylumbelliferyl α-L-idopyranosid)uronic acid (**14**), a more sensitive substrate for α-L-iduronidase, has been synthesized by an analogous route. Zinc chloride-catalyzed condensation of 4-methylumbelliferone with 1,2,3,4,6-penta-*O*-acetyl-α-L-idopyranose (**4**) in 1,2-ethanediol diacetate gave crystalline 4-methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl-α-L-idopyranoside (**7**). *O*-Deacetylation and catalytic oxidation gave **14**, characterized as a cyclohexylammonium salt. The starting material **4** was prepared, in 21% yield from L-glucose, by conversion of the intermediate 1,2,3,4,6-penta-*O*-acetyl-β-L-glucopyranose to 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyl chloride and acetoxonium ion rearrangement, as described for the D-series.

## INTRODUCTION

(Phenyl α-L-idopyranosid)uronic acid[1] (**11**) has rapidly taken its place as a valuable test substrate for demonstrating the occurrence[2] and assaying the activity[3–5] of α-L-iduronidase. This mammalian hydrolase is of particular interest because its genetic deficiency is now known to be the biochemical defect underlying Hurler's syndrome[6,7], a long-studied mucopolysaccharidosis in which heparan sulfate and dermatan sulfate accumulate[8]. This finding obviously also implicates α-L-iduronidase in catabolism of these two polysaccharides. Because of the relative inefficiency of our earlier preparation[1], however, the phenyl glycoside **11** has remained difficultly accessible. We now report a much improved synthesis. Moreover, we describe an analogous synthesis for (4-methylumbelliferyl α-L-idopyranosid)uronic acid (**14**), a fluorogenic substrate[9]. As will be reported elsewhere, introduction of this new substrate should permit some 1000-fold increase of sensitivity in assays of α-L-iduronidase, an enzyme whose activity in tissues and body fluids is generally meager.

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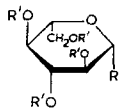

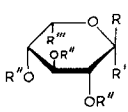

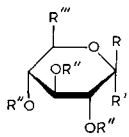

1 R = OH, R′ = H
2 R = OAc, R′ = Ac
3 R = Cl, R′ = Ac

4 R = OAc, R′ = H, R″ = Ac, R‴ = $CH_2OAc$
5 R = OPh, R′ = H, R″ = Ac, R‴ = $CH_2OAc$
6 R = OPh, R′ = R″ = H, R‴ = $CH_2OH$
7 R = OMu, R′ = H, R″ = Ac, R‴ = $CH_2OAc$
8 R = OMu, R′ = R″ = H, R‴ = $CH_2OH$
9 R = H, R′ = OPh, R″ = Ac, R‴ = $CH_2OAc$
10 R = H, R′ = OPh, R″ = H, R‴ = $CH_2OH$
11 R = OPh, R′ = R″ = H, R‴ = $CO_2H$
12 R = OPh, R′ = R″ = H, R‴ = $CO_2Am$
13 R = H, R′ = OPh, R″ = H, R‴ = $CO_2H$
14 R = OMu, R′ = R″ = H, R‴ = $CO_2H$
15 R = OMu, R′ = R″ = H, R‴ = $CO_2Am$

16 R = OPh, R′ = R″ = H, R‴ = $CO_2H$
17 R = H, R′ = OPh, R″ = H, R‴ = $CO_2H$
18 R = OMu, R′ = R″ = H, R‴ = $CO_2H$
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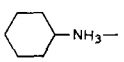
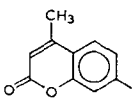
Am = 

Mu = 

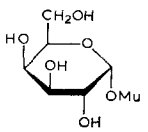


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TABLE I

PLATINUM-CATALYZED OXIDATION OF THE MIXTURE OF PHENYL L-IDOPYRANOSIDES **6** AND **10**[a]

| *Run no.* | *Temp. (°)* | *pH* | *$NaHCO_3$ consumed (% theory)* | *Time (h)* | *Yield (%) and color ratio*[b] | | |
|---|---|---|---|---|---|---|---|
| | | | | | **6+10** *(Peak I)* | **13+16+17** *(Peak II)* | **11** *(Peak III)* |
| 1 | 79 | 7.5 | 100 | 2.1 | [c] | 3.9 (0.50) | 7.0 (0.22) |
| 2 | 79 | 7.5 | 130 | 4.0 | 51 | 7.5 (0.63) | 6.2 (0.23) |
| 3 | 79 | 6.5 | 130 | 1.1[d] | 14.7 | 15.0 (0.24) | 50.0 (0.22) |
| 4 | 79 | 5.5 | 115 | 4.0[d] | 12.8 | 11.6 (0.25) | 50.8 (0.21) |
| 5 | 74 | 6.5 | 130 | 5.9 | 21 | 5.7 (0.24) | 15.2 (0.21) |

[a]Analysis of reaction mixtures by ion-exchange chromatography was monitored by the orcinol test, the idoside[1] **10** being used as a color standard for Peak I, and L-iduronic acid for Peaks II and III. The numerical values in parentheses refer to ratios of molar color yield in the carbazole and orcinol tests, where a ratio of 1.00 is defined for a glucuronic acid standard and a measured ratio of 0.22 is observed for the iduronic acid standard. [b]Color ratio in parentheses. [c]Not determined. [d]Runs 3 and 4 were performed with a new oxidation vessel, the stirring efficiency of which was apparently superior to that used for runs 1,2, and 5. The manner and degree in which the results were influenced by this technical change are difficult to evaluate.

## DISCUSSION

In the present work, α-L-idose pentaacetate (**4**), an intermediate common to

both syntheses, was prepared from the commercially available L-glucose (**1**) by reactions already reported for the D-series. These include the preparation[10] of β-L-glucose pentaacetate (**2**), conversion[11] of this compound to 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyl chloride (**3**), and transformation of this glycosyl chloride to **4** by the elegant acetoxonium ion rearrangement of Paulsen *et al.*[12]. The appreciable cost of **1** introduces far greater constraint for economy, however, than required in the D-series. Accordingly, the substantial improvements that have been effected in this reaction sequence after some trials are described.

As in the earlier synthesis of **11**, zinc chloride-catalyzed condensation of the pentaacetate **4** with phenol gave a syrupy mixture of phenyl 2,3,4,6-tetra-*O*-acetyl-α- and β-L-idopyranosides[1] (**5** and **9**). The previous, laborious chromatographic separation was now avoided, deferring separation of anomers to a later stage of the reaction sequence. Instead, the mixture of phenyl α- and β-L-idopyranosides (**6** and **10**) produced by *O*-deacetylation was directly subjected to catalytic oxidation. Because of earlier difficulties with this step, a limited study of the oxidation was undertaken, the results of which are summarized in Table I. Since the content of β-L-idoside **10** in similar anomeric mixtures of **6** and **10** is[13] about 25%, the maximum yield to be expected of the desired α-L-idosiduronic acid **11** is about 75%. The quantity of hydrogencarbonate required to neutralize the acid formed is listed in the Table as a rough indicator of oxygen uptake. As seen (run 1), for an approximately theoretical oxygen uptake, a temperature of 79°, and pH 7.5, the yield of **11** was 7%; the yield of (phenyl β-L-idopyranosid)uronic acid (**13**) was correspondingly small. The yield of **11** actually decreased somewhat when the oxygen uptake was increased to 130% (run 2), although half of the starting material remained. These findings suggest a secondary oxidation of **11** and **13** to be an unavoidable feature of the reaction, less pronounced at lower values of pH (runs 3 and 4). A slight decrease of temperature prolongs reaction time considerably, with deleterious effect on yield (run 5). The reaction conditions of run 3 were applied for preparative purposes. In preparative runs, the overall yield of pure cyclohexylammonium (phenyl α-L-idopyranosid)uronate (**12**) from L-glucose was some 8%. Although modest, this represents improvement over the earlier production of **12** from 1,2-*O*-isopropylidene-D-glucose[1] in overall yield of 0.2%.

The sensitivity of **11** and **13** to further catalytic oxidation is conspicuous, since phenyl glycosides of glucuronic and galacturonic acid have been prepared by catalytic oxidations at pH 8–10 and 90° in excellent yields[14]. Another noteworthy feature of the oxidations is the partial inversion at C-5 incidental to oxidation, indications of which are seen in the present results at higher pH values, as in those already reported. Thus, oxidation of the α- or β-L-idosides **6** or **10** had earlier been shown to produce, in part, the corresponding (phenyl β- or α-D-glucopyranosid)uronic acids[1] **16** or **17**. By use of the carbazole–orcinol colorimetric ratios, previously established for the pure substances[1], it may be estimated from Table I that, at pH 7.5, the combined yield of the D-glucosiduronic acids **16** and **17** from oxidation of the L-idosides **6** and **10** was 2% in run 1, 4% in run 2, but small or negligible at pH values of 6.5 or less. Since it has already been ascertained that neither the idosides **6** or **10** nor the

idosiduronic acids **11** or **13** are epimerized under typical reaction conditions[1], the decreased epimerization with decreased pH must pertain to an intermediate stage of the oxidation. These observations would, for example, be consistent with a mechanism entailing the base-induced, reversible enolization of a transient 6-aldehydo intermediate.

It appeared reasonable to extend the synthetic route just described to a synthesis of the (4-methylumbelliferyl idosid)uronic acid **14**, especially since successful Helferich condensations[15] of 4-methylumbelliferone with D-galactose pentaacetate[16] and D-mannose pentaacetate[17] had been reported. Zinc chloride-catalyzed condensation of L-idose pentaacetate (**4**) with 4-methylumbelliferone gave unexpected difficulties, however. In reactions attempted under a variety of conditions, much formation of 4-methylumbelliferyl acetate and tarry products was noted, but u.v.-absorbing carbohydrate products with mobility, in t.l.c., resembling that of 4-methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**20**) could not be detected. Introduction of 1,2-ethanediol diacetate as a solvent for both the sugar acetate and the highly insoluble 4-methylumbelliferone soon led to the isolation of crystalline 4-methylumbelliferyl 2,3,4,6-tetra-*O*-acetyl-α-D-idopyranoside (**7**) from reaction mixtures in moderate yield. This solvent, the use of which in this condensation is apparently novel, also has a favorable boiling point, in that it serves as a "chaser" for the continuous distillation of the acetic acid formed in the reaction[15]. Coumarin was another favorable reaction solvent (highest yield of **7** obtained in trials, 15%). The procedure described employs 1,2-ethanediol diacetate. It is necessary to note the persistence in this condensation of an uncontrolled factor, which remains unidentified despite numerous pilot runs, and which has given rise to variable yields. The procedure described gave 15% and 28% yield of **7** in two runs, but a yield of 37% was attained in a single, small-scale pilot run, not successfully duplicated.

*O*-Deacetylation of **7** gave crystalline 4-methylumbelliferyl α-L-idopyranoside (**8**), catalytic oxidation of which gave the desired L-idosiduronic acid **14**, but in an impure state. The substance was characterized as its readily crystallizing cyclohexylammonium salt **15**. Epimerization was not observed in the oxidation, which was conducted at pH 5.

## EXPERIMENTAL

*General methods.* — Silicic acid (Code 923) from the Davison Chemical Co. was used for chromatography. For t.l.c. Silica gel G plates with fluor (Merck No. 5765) were developed with 1:1 (v/v) ethyl acetate–hexane. Spots were visualized under u.v. illumination, where applicable; sugar spots were charred by spraying with methanolic sulfuric acid and heating. 4-Methylumbelliferone showed $R_F$ 0.24; 4-methylumbelliferyl acetate, 0.34; **4**, 0.28; **7**, 0.17; and **20** [obtained by acetylation of 4-methylumbelliferyl β-D-glucopyranoside (**19**), purchased from Koch-Light Laboratories Ltd., Colnbrook SL3 OBZ, Bucks., England], 0.17. N.m.r. spectra were recorded on a Varian T60A instrument with an internal tetramethylsilane standard.

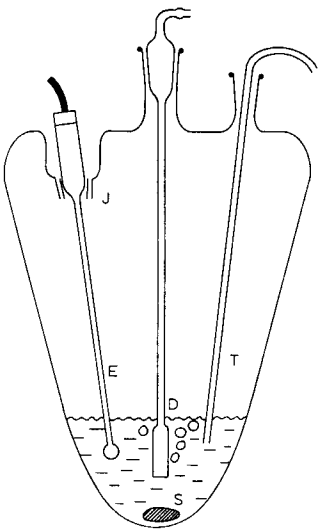


Fig. 1. Vessel used for catalytic oxidations, built from a standard Erlenmeyer flask of appropriate capacity. Oxygen is delivered by a fritted dispersion tube (D). Stirring by an oval magnetic stirring bar (S) keeps the catalyst in suspension. The pH is continuously determined by the combination glass-reference electrode (E), which is rigidly positioned by an adapted ground joint (J). Sodium hydrogencarbonate solution is delivered into the reaction medium from a graduated cylinder through the plastic capillary tube (T), as required, by use of a peristaltic pump.

Measurements of pH during oxidations were made with a Corning 476022 glass electrode, calibrated at temperature of use. Vessels of the design illustrated in Fig. 1, heated in a constant-temperature water bath, were particularly advantageous for the catalytic oxidations, in which rapid settling of catalyst and extensive frothing proved irksome. Solutions were evaporated under reduced pressure, generally at bath temperatures below 40°. Microanalyses in duplicate were performed by Micro-Tech Laboratories. For other procedures, including orcinol and carbazole colorimetric determination of uronic acid derivatives, see ref. 1.

*Penta-O-acetyl-β-L-glucopyranose* (**2**). — Acetylation of L-glucose 180 g, (Pfanstiehl Laboratories, Waukegan IL 60085) with acetic anhydride (1.2 l) and sodium acetate (90 g) was conducted in a 4-liter Erlenmeyer flask, essentially as described[10]. After cooling the reaction mixture to about 40°, ice (1.0 kg) was added. On stirring for 1 h, all dissolved with sudden warming. Crystallization was then induced by addition of water (1.4 l) and completed by chilling at ~10° for several hours. Filtration and washing with ice-cold 25% aqueous acetic acid and water gave nearly pure **2** (229 g), m.p. 130–131°, $[\alpha]_D^{25}$ −4.4° (*c* 2, chloroform); reported[18] for the D-enantiomer of **2**, m.p. 135°, $[\alpha]_D$ +3.8° (chloroform). Additional solid fractions having a more negative rotation, obtained on successive additions of water to the filtrate, were processed[19] by transformation into 2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl bromide (not isolated) and treatment with mercuric acetate to give 75 g of additional pure **2** (total yield 78%).

*2,3,4,6-Tetra-*O*-acetyl-β-*L*-glucopyranosyl chloride* (**3**). — Following essentially the procedure reported for the D series[11], but on larger scale, **2** (300 g) was shaken for 35 min at room temperature with dry, alcohol-free chloroform (1.2 l) and anhydrous aluminum chloride (60 g); slight warming was observed. Following addition of chromatographic silicic acid (40 g) and benzene (2.4 l), and brief additional shaking, the mixture was filtered through a bed of filter aid. To the filtrate and benzene washings (0.5 l) was added alcohol-free chloroform (0.5 l) to promote rapid settling. The solution was washed with ice-cold, 10% aqueous sodium sulfate solution, briefly dried (sodium sulfate), and evaporated to a thin syrup. This was rapidly crystallized at room temperature from a mixture of dry ether (900 ml) and pentane (about 400 ml) to give nearly pure **3** (170 g), m.p. 97–99°, $[\alpha]_D^{23}$ +6.2° (*c* 2.9, chloroform); reported[20] for the D-enantiomer of **3**, m.p. 99–100°, $[\alpha]_D^{17}$ −13° (chloroform). Additional **3** (51 g, total yield 78%), m.p. 95–97°, $[\alpha]_D^{23}$ +4.8° was obtained by evaporation of the filtrate and treatment with ether and pentane.

*1,2,3,4,6-Penta-*O*-acetyl-α-*L*-idopyranose* (**4**). — A flask fitted with thermometer, separatory funnel, gas inlet, and magnetic stirring bar was charged with **3** (229 g) and dichloromethane (600 ml). While maintaining an argon atmosphere, the solution was stirred in a dry ice-acetone bath maintained at −20°. When the internal temperature had fallen to −10°, a solution of antimony pentachloride (187 g) in dichloromethane (200 ml) was added in a thin stream at a rate regulated to maintain an internal temperature of −9° to −10°. The addition was complete in 25 min. After 5 min more, the cooling bath was removed and, when no rapid warming was observed, the mixture was warmed in an aqueous ethanol bath to room temperature. After about 10 min, when the internal temperature was +11°, precipitation of solid from the now deep-yellow solution commenced. In 6 min more, when the internal temperature was 23°, heavy precipitation had stopped the stirring. About 30 min from start of precipitation, the now almost immobile slurry was filtered off with moderate suction on a large, fritted-glass Buchner funnel, which was kept under an atmosphere of argon. The transfer was facilitated by forcing the thick slurry through the neck of the flask by application of slight argon pressure. Careful washing of the funnel with dichloromethane (250 ml) and abs. ether (650 ml) gave a light-colored solid. Darker, partially gummy solid (22 g), precipitated from the filtrate by ether was discarded. The funnel and its contents were dried in a desiccator under 20 mm Hg for ~6 h over sodium hydroxide, and the friable cake of antimony complex (377 g) was broken up in a dry bag under argon.

On the day of its preparation, about one-third of the solid antimony complex was rapidly stirred in a chilled blender with ice-cold 1.2M sodium potassium tartrate solution (400 ml). Chloroform was added while blending was in progress. The fluid suspension was decanted, and the residual paste was blended again with additional tartrate solution and chloroform. For treatment of the entire batch of antimony complex (in three portions), 1850 ml of tartrate solution and 1200 ml of chloroform were used. The resulting suspension was stirred for 30 min and kept overnight at 4°. The solid was removed by vacuum filtration and washed on the

filter with chloroform (3.6 l). The pooled chloroform layers of the filtrate were dried and evaporated. The amber, syrupy residue[12] (130 g) dissolved when shaken for 3 h with 1:1 acetic anhydride–pyridine (200 ml), while chilling in ice. After being kept for 3 days at 4° and treated with ice–water, the mixture was processed in the usual way. The resulting amber syrup crystallized, from a solution in warm abs. ethanol, on seeding to give **4** (106 g, 45% yield, based on **3**), m.p. 94–95.5°, $[\alpha]_D^{23}$ −56.3° (*c* 2.7, chloroform); lit.[21] m.p. 95–96°, $[\alpha]_D^{25}$ −57° (chloroform).

Aside from the larger scale of operation, the procedure described differs from that reported for the D-series[12] principally in the use of less dichloromethane in the treatment of **3**, in the use of an argon atmosphere, in a more explicitly designated temperature schedule, and in the technique for hydrolysis of the antimony complex.

*Phenyl α- and β-L-idopyranosides*[1] (**6** and **10**). — A flask provided with magnetic stirring, heated by an oil bath, and arranged for vacuum distillation was charged with **4** (35.7 g) and phenol (120 g). To the homogeneous melt resulting at 90°, a solution of zinc chloride (3 g) in 19:1 acetic acid–acetic anhydride (10 ml) was added. After distillation for 20 min at a bath temperature of 90° and a pressure of 18–20 mm Hg to remove added acetic acid, the bath temperature was quickly raised. On maintaining the mixture at 121–125° and 38 mm Hg for 30 min more, additional acetic acid and some phenol (~20 ml) distilled. The light-brown residue was dissolved in chloroform, washed with 10% sodium sulfate solution, with ice-cold 2M NaOH (600 ml), and with several portions of sodium sulfate solution. Drying with sodium sulfate and removal of solvent left a syrup, from the earlier work[1] known to be a mixture of the phenyl 2,3,4,6-tetra-*O*-acetyl-α- and β-L-idopyranosides (**5** and **9**). A solution in ether—pentane, kept for several months, crystallized slowly despite seeding, to give only 0.9 g of crude **9**, m.p. 76–80°. The materials in the mother liquor were *O*-deacetylated[1] to give the mixture of **6** and **10** (75 mmol, based on u.v. spectrum[1], 84%, total yield from **4**) used in the following oxidation.

*Cyclohexylammonium (phenyl α-L-idopyranosid)uronate* (**12**). — A 250-ml oxidation vessel (Fig. 1) was immersed in a water-bath maintained at 79° and charged with a solution of **6** and **10** (4.0 mmol) in water (10 ml). Platinum black, freshly prepared by hydrogen reduction of Adam's platinum oxide catalyst (1.5 g, Engelhard Industries, Union, NJ 07083) in aqueous suspension, was added. Oxygen was passed through the vigorously stirred suspension (initially 20 ml), while M sodium hydrogencarbonate was added continuously to maintain a pH 6.5 ±0.2. The reaction was terminated 70 min from the starting time, when 5.2 mmol of hydrogencarbonate had been consumed. The suspension was filtered through a bed of filter aid, and the brown filtrate was adjusted to pH 5 by addition of acetic acid.

The filtered reaction mixture from two such runs, containing 5.1 mmol of uronic acid (orcinol colorimetry, iduronic acid standard), was applied to a column (5 × 83 cm) of Dowex 1 × 8 ($HCO_3^-$, 200–400 mesh), which had been prewashed with 0.05M formic acid. On elution of the column with water, starting material (**6** and **10**, 1.05 mmol based on orcinol colorimetry, **10** as standard) was recovered in the effluent (8.8–15.6 l, Peak I). Elution was continued with 1.2M formic acid, and

fractions were monitored by orcinol colorimetry (iduronic acid standard). A peak (Peak II, at 43–49 l, containing 1.0 mmol of uronic acid), which had by earlier calibration with authentic specimens been shown to contain **13**, **16**, and **17** when present, was set aside. The major peak immediately following (Peak III, 49–61 l) had been shown to contain **11**. Discarding a small zone of overlap with Peak II, the fractions of Peak III were pooled (3.64 mmol of uronic acid).

Formic acid was removed by pumping these pooled fractions through a short, wide column containing 1:1 Norite A–Celite 535 (60 g, Johns Manville Corp.) The charcoal column was first washed with water, and the absorbed **11** was then eluted with 20% aqueous pyridine (1.8 l). Following evaporation *in vacuo* of the pyridine eluate to a small volume, the remaining pyridine was removed by passage through Dowex 50 ($H^+$, 5 ml). The resin was copiously washed with water (75 ml). The effluent and washings, containing 3.11 mmol of **11** (u.v. $\varepsilon_{267}$ 890), were treated with an equimolar amount of freshly distilled cyclohexylamine and acetic acid (0.15 mmol). Evaporation left a solid residue, which was further dehydrated by addition and vacuum distillation of abs. ethanol. The residue was crystallized from abs. ethanol–ether at room temperature to yield 1.13 g (36%, based on the mixture of **6** and **10**) of the cyclohexylammonium salt **12**, m.p. 194–195° (dec.), $[\alpha]_D^{25}$ −57.5° (*c* 2.4, water); lit.[1] m.p. 181–183° (dec.), $[\alpha]_D^{23}$ −56.8° (water).

The pilot-oxidation runs summarized in Table I were carried out on a 4-mmol scale with procedures analogous to those described for the preparative run.

*4-Methylumbelliferyl 2,3,4,6-tetra*-O-*acetyl*-α-L-*idopyranoside* (**7**). — A 200-ml vacuum distilling flask of short vapor-path equipped with thermometer (dipping into liquid phase) and magnetic stirring bar was heated by an oil bath while the pressure was maintained at 110 mm Hg. The flask was charged with **4** (15 g), 4-methylumbelliferone (9 g), and 1,2-ethanediol (50 ml) diacetate. The bath temperature was adjusted to ensure slow distillation of the solvent when the melt temperature was 135°. When the melt had become homogeneous, the vacuum was interrupted momentarily for introduction of a solution of zinc chloride (1.6 g) in 19:1 acetic acid–acetic anhydride (5 ml), and then resumed. Vigorous distillation, chiefly of acetic acid, occurred with transient fall of melt temperature. After 8 min, 10 ml of distillate had been collected and the internal temperature had again reached 135°. This temperature was maintained for 55 min longer, with collection of an additional 15 ml of distillate at a steady rate.

The reaction mixture was now chilled and treated for 1 h in an ice-bath with chloroform (100 ml) and 10% sodium sulfate solution (100 ml). Filtration left unreacted 4-methylumbelliferone (3.4 g). The chloroform layer was dried (sodium sulfate) and concentrated. A solution of the residue in 1:1 ethyl acetate–hexane was applied to a silica gel column (5 × 40 cm), which was eluted with the same solvent. The chromatography was followed by t.l.c., and fractions containing material of the mobility expected for **7** were pooled. (Initially, the corresponding β-D-glucoside **20**, which, as anticipated, has the same mobility as **7**, was used as a standard). The column was deliberately overloaded, and the fractions of interest contained small amounts

of 4-methylumbelliferyl acetate and other unidentified minor by-products, but no 1,2-ethanediol diacetate. The pooled fractions were evaporated. Crystallization of the residue from ethanol (60 ml) gave 5.4 g (28%) of pure **7**, m.p. 137–138°, $[\alpha]_D^{23}$ −125° (*c* 1.0, chloroform), $\lambda_{max}^{H_2O}$ 317 nm (ε 14 800), m.p. and optical rotation unchanged on recrystallization.

*Anal.* Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.18. Found: C, 56.82; H, 5.24.

In a previous run on the same scale using a procedure apparently identical to that described, the yield was 15%. The mother liquors from the crystallization of **7** regularly contained much noncrystalline material of identical $R_F$ on silica gel. Isolated as a chromatographically homogeneous syrup in one case, this material had $[\alpha]_D^{25}$ −27° (chloroform), suggesting that it might in large part be the β-anomer of **7**.

The D-enantiomer of **7**, which had been prepared in pilot experiments under analogous reaction conditions from 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose[12], had m.p. 137–138°, $[\alpha]_D^{25}$ +125° (*c* 0.5, chloroform). This preparation was examined by n.m.r.

*Anal.* Calc. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.18. Found: C, 56.63; H, 5.18.

The n.m.r. spectrum in chloroform-*d* showed complex signals for aromatic protons. These included a narrow doublet (τ 7.62) for the ring methyl group and a narrow unresolved quartet (τ 3.79, $J \sim 1.8$ Hz) for the vinyl proton H-3′; the latter became a sharp singlet on irradiation of the methyl signal at τ 7.62. (Similar signals for aromatic protons were found for 4-methylumbelliferyl acetate and for **20**). A broad singlet (τ 4.37) was attributed to the anomeric proton, corresponding to the H-1 signal previously[13] noted for phenyl α-L-idopyranoside (**6**; τ 4.47, in acetone-$d_6$). The methyl protons of the acetoxy groups displayed signals at τ 7.87 (9 H) and at τ 8.1 (3 H); the latter was thought to originate from protons of the C-3 acetoxy group, which are shielded by the ring current effect of the aromatic nucleus. No attempt was made to attribute signals observed in the region τ 4.67–5.94, which showed correct integration values for H-2, H-3, H-4, H-5, and H-6.

The α-L-pyranoside structure of **7** is in addition supported by the periodate oxidation study reported in the section which follows.

*4-Methylumbelliferyl α-L-idopyranoside* (**8**). — A solution of **7** (10 g) in chloroform (50 ml) was boiled briefly to remove any water. After addition of dry methanol (40 ml), the solution was again brought to boiling, 3M sodium methoxide (0.2 ml) was added, and boiling was continued for 2 min longer. Crystals separated from the hot solution. The mixture was allowed to cool, neutralized with acetic acid, and filtered to yield 6.0 g of pure **8**, m.p. 208–209°, $[\alpha]_D^{25}$ −132° (*c* 0.6, pyridine), $\lambda_{max}^{H_2O}$ 316 nm (ε 14 800), unchanged on recrystallization from water. The compound is sparingly soluble in cold water, but is soluble in hot water.

Concentration of the filtrate gave a second crop of **8** (0.43 g, total yield 96%).

*Anal.* Calc. for $C_{16}H_{18}O_8$: C, 56.78; H, 5.36. Found: C, 56.54; H, 5.43.

The α-L-idoside **8** (10.3 mg) was dissolved at 40° in 0.14M sodium metaperiodate (2.00 ml); after 10 min, when the solution was allowed to cool, no solid precipitated. In a parallel experiment, the α-D-galactoside **21** (Koch-Light Laboratories) was

identically treated. After 4 h at room temperature, when the optical rotations had become constant, oxidized **8** showed $[\alpha]_D^{25}$ −170.8° (based on weight of **8** taken) and oxidized **21** $[\alpha]_D^{25}$ +171.2°. This correspondence is the result expected[22] from the structure proposed for **8**, thus confirming the identity, ring size, and anomeric configuration.

*Cyclohexylammonium (4-methylumbelliferyl α-L-idopyranosid)uronate* (**15**). — Proceeding as in the preparation of **12**, at a bath temperature of 71°, oxygen was passed through a stirred suspension of platinum black (2 g) in a solution of **8** (1.00 g, 2.96 mmol) in water (40 ml), while adding sodium hydrogencarbonate as required to maintain pH 5.0. After 2 h, an additional 2 g of platinum was added, but without much effect on the slow rate of hydrogencarbonate consumption. After an additional 1 h (3.15 mmol, total, of hydrogencarbonate consumed), the reaction was terminated. The reaction mixture (1.07 mmol of uronic acid by orcinol colorimetry, iduronic acid standard) was applied to a Dowex 1 × 8 column (2.5 × 45 cm, $HClO^-$); unreacted **8** (0.23 mmol, based on u.v. spectrum), was eluted with water (8 l). The column was then eluted with 1.25M formic acid, and fractions were determined by measurements at 318 nm. A peak appearing at 13.2 l could be shown to contain free 4-methylumbelliferone (0.23 mmol from u.v. spectrum measurements). The fractions of a second peak appearing at 18.3 l were pooled for isolation of **14**. The β-D-glucosiduronic acid **18** was not detected. In pilot experiments with a smaller column, 4-methylumbelliferone was eluted at 65 bed-volumes of 1.25M formic acid, **14** at 96 bed-volumes, and **18** (obtained from Koch-Light Laboratories) at 55 bed-volumes.

The pooled idosiduronic acid fractions were adsorbed on a column containing Norite A (5 g) and Celite 535 (5 g), which was then washed with water and eluted with 40% aqueous pyridine. The pyridine eluate (600 ml) was evaporated and the residue, after repeated codistillations with toluene and abs. ethanol, was crystallized from abs. ethanol, to give impure (4-methylumbelliferyl α-L-idopyranosid)uronic acid (**14**) (224 mg), m.p. 112–113° (dec.), $[\alpha]_D^{23}$ −94.5° (*c* 0.5, water). Recrystallization from abs. ethanol, with some loss, gave a sample having m.p. 117° (dec.), $[\alpha]_D^{23}$ −94.0° (*c* 0.5, water), the constants and analyses of which remained unchanged on repeated further recrystallizations.

*Anal.* Calc. for $C_{16}H_{16}O_9$: C, 54.54; H, 4.58; O, 40.88. Found: C, 52.59; H, 5.49; O, 41.88.

The unsatisfactory elemental analyses of this and similar samples were reflected by colorimetric analyses for uronic acid (carbazole and orcinol, iduronic acid standard), which gave values about 10% lower than those expected for the structure assigned. The apparent molar extinction coefficient at 317 nm was also 10% low.

A solution of a similar preparation of impure **14** (100 mg) in warm mM acetic acid (10 ml) was treated with an alcoholic solution of freshly distilled cyclohexylamine (0.250 mmol) and the cooled solution (pH 5) was evaporated. The residue, after codistillation with abs. ethanol, crystallized rapidly from a solution in a little abs. ethanol to give the pure cyclohexylammonium salt **15** (99 mg), m.p. ~160° (with

darkening and gas evolution), $[\alpha]_D^{23}$ −71.8° (*c* 0.8, water), $\lambda_{max}^{H_2O}$ 317 nm (ε 14 900), unchanged on recrystallization from abs. ethanol.

*Anal.* Calc. for $C_{22}H_{29}NO_9$: C, 58.52; H, 6.47; N, 3.10. Found: C, 58.30; H, 6.54; N, 2.94.

*Enzymic tests for purity.* — β-D-Glucopyranosiduronic acid contaminants (**16** or **18**) (in phenyl and 4-methylumbelliferyl α-L-idopyranosid)uronic acid preparations (**12** and **15**), which would be a matter for concern in their use as α-L-iduronidase substrates, were tested as follows. Overnight incubation of 10mM **12** in 0.05M sodium acetate buffer (pH 5) with 0.05 I.U.B. enzyme units/ml of rat preputial gland β-D-glucuronidase[23] liberated no detectable phenol in the digest, corresponding to less than 0.2% of impurity in **12**. Incubation of mM **15** for 1 h under similar conditions resulted in the liberation of 4-methylumbelliferone, measured fluorimetrically[24], corresponding to less than 0.02% of hydrolysis. When, in both cases, the digests were supplemented with appropriate traces of the corresponding **16** or **18**, essentially the expected increment of phenol was liberated on incubation with the enzyme, thus excluding the possibility of inhibition effects.

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