Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF METHYL D-GALACTURONATE FROM D-GALACTOSE OR D-GALACTURONIC ACID: PREPARATION OF METHYL-^{14}C D-GALACTURONATE USING METHYL-^{14}C IODIDE

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(Received December 10th, 1966; in revised form, February 13th, 1967)

Although tracer studies have provided evidence for direct utilization of D-galacturonic acid (**1**) or its methyl ester (**2**) as galactosyluronic acid residues in pectic substance of detached ripening strawberries[1], they did not provide information on the fate of the methyl carbon of **2**. To investigate this aspect, a method was sought that would permit the synthesis of methyl-^{14}C-labeled **2** of high specific radioactivity. Preparative methods have been reported previously[2,3], but the procedures described did not meet the needs of a synthesis involving the use of a ^{14}C-labeled methylating agent where high radiochemical yield, efficient use of a ^{14}C-labeled reagent, and simple recovery of product were additional considerations.

This paper describes two synthetic routes to **2**, one from D-galactose *via* benzyl 2,3-di-*O*-benzyl-*β*-D-galactopyranosiduronic acid (**4**), the other from **1** *via* 1,2:3,4-di-*O*-benzylidene-D-galactopyranuronic acid (**7**). The sodium salt of **4** or **7**, dissolved in *N*,*N*-dimethylformamide, reacted smoothly with methyl iodide to form the corresponding methyl ester **6** or **9**. Hydrogenolysis of **6** or **9** gave **2** in good yield. When a slight excess of **5** was methylated with methyl-^{14}C iodide, the final product, methyl-^{14}C-labeled **2**, accounted for nearly all ^{14}C present and had a specific radioactivity equivalent to that of the methylating agent.

RESULTS AND DISCUSSION

In the first synthetic sequence, benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside (**3**)[4-8] was oxidized with oxygen in the presence of platinum-on-carbon[9] to give the uronic acid derivative **4**. Reaction of the sodium salt **5** of **4** with 30% excess of methyl iodide in *N*,*N*-dimethylformamide for 7 h at 75° gave the methyl ester derivative **6** as a syrup in 93% yield. The infrared spectrum showed peaks at 3550 cm^{-1} for a hydroxyl group and at 1725 cm^{-1} for an ester carbonyl group. In one experiment, the syrup crystallized from chloroform–hexane as a waxy solid that had a carbon, hydrogen, and methoxyl content corresponding to the expected structure **6**. Benzyl groups were removed from **6** by hydrogenolysis at room temperature and atmospheric pressure in the presence of palladium-on-carbon catalyst. The product, **2**, was obtained as a syrup, which showed on paper chromatography in benzene–butyl alcohol–pyridine–water (1:5:5:3, v/v) a single spot (R_G 1.52) and on thin-layer chromatography on cellulose a single spot corresponding to that of authentic methyl D-galacturonate[3] (**2**).

In the preparation of methyl-^{14}C-labeled **2**, a 20% excess of **5** over that of methyl-^{14}C iodide was employed to insure quantitative conversion of the labeled reagent into ester. Otherwise, reaction conditions were similar to those used in experiments with unlabeled methyl iodide. Thin-layer chromatography of the labeled product **2** showed a major radioactive spot which corresponded to that of authentic **2**. In addition, a second, minor, radioactive spot that moved faster than **2** was detected. This product had not been detected in runs made with unlabeled methyl iodide. The nature of the labeled methyl group in this minor constituent appeared to be similar to that in **2**, since treatment of the developed thin-layer plate with ammonia vapor removed all radioactivity from both spots. The possibility exists that dehydration occurred through elimination of water from C-4–C-5 during methylation. Further efforts are under way to determine the chemical nature of this minor product.

An alternate sequence for the synthesis of **2** from **1** was also investigated with unlabeled methyl iodide and shown to be a practical method for the preparation of methyl-^{14}C-labeled **2** starting from the uronic acid. Zinner and Thielebeule[10] have reported a synthesis of 1,2:3,4-di-*O*-benzylidene-D-galactopyranuronic acid (**7**) for which they listed a melting point of 212°. In the synthesis reported in this paper, D-galacturonic acid monohydrate was employed instead of anhydrous[10] **1**. The infrared spectrum of the product showed peaks for aromatic groups and a carbonyl. It had a broad melting point range of 190° to 200° that could not be sharpened by additional crystallization. When converted into its sodium salt (**8**) with one equivalent of sodium hydrogen carbonate, there was a shift in the infrared spectrum from 1725 cm^{-1} to 1620 cm^{-1}. Methylation with 2 equivalents of methyl iodide in *N,N*-dimethylformamide gave the methyl ester (**9**) in near quantitative yield. The presence of two carbonyl peaks in the infrared, one near 1740 cm^{-1}, the other near 1760 cm^{-1}, suggested that more than one isomer of **9** were present. Theoretically, four isomers of **7**, **8**, and **9** are possible, due to two asymmetric centers created in the formation of the dibenzylidene derivative **7**. In the course of one run, syrupy methyl ester **9** partially crystallized from methanol–petroleum ether. The crystalline product gave correct elemental analysis for **9**; it had a melting point of 148–150° and its specific rotation was −126°. Apparently the crystalline ester **9** described here is different from the one prepared by Zinner and Thielebeule[10], who reported m.p. 165° and $[\alpha]_D^{18}$ −168°. Following removal of this crystalline ester, the mother liquors were evaporated to a syrup. Attempts to recover additional crystals from this syrup were unsuccessful; however, after purification by chromatography on silica gel, a syrup was recovered that corresponded to **9**.

Gas chromatography of the crystalline ester gave a single peak from a six foot column packed with silicone polymer-coated particles. The syrup from which this crystalline ester was recovered gave four peaks; based on the retention time of hexakis-*O*-trimethylsilyl-*myo*-inositol as unity, their retention times were 1.78, 1.82, 1.88, and 1.92. Addition of a small amount of crystalline **9** to the syrup enhanced the first peak.

Hydrogenolysis of **9** in the presence of palladium-on-carbon catalyst gave syrupy ester **2** which showed, by t.l.c. on cellulose, a single spot having the same R_F value as

authentic methyl D-galacturonate[3]. The syrupy **2** was crystallized from methanol–dioxane. The melting point (141–144°) and the specific rotation, +86.2° (10 minutes) → +30.5° (equilibrium), agreed well with the reported values[2,3]. Thin-layer chromatography gave a major spot corresponding to authentic **2**. In addition, a faint spot moving slightly ahead of **2** was also observed.

EXPERIMENTAL

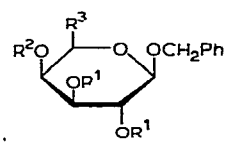
All melting points were determined on a Fisher–Johns melting-point apparatus. Melting points reported in this paper have been corrected. Infrared spectra were determined on a Perkin–Elmer Model 137 "Infracord" spectrophotometer. Optical rotations were determined with a Perkin–Elmer Model 141 Polarimeter. Thin-layer chromatography was performed on cellulose powder MN-300 from Brinkmann Instruments, Inc. Unless otherwise noted, thin-layer plates were developed in butyl alcohol–acetic acid–water (3:1:1, v/v) and sprayed with silver nitrate in acetone followed by potassium hydroxide in methanol[11]. Radioactive areas on the plates were detected with a Packard Model 7201 radiochromatogram scanner. Column chromatography was performed with silica gel, grade 923 or 950, from Will Scientific Corp. Methyl-^{14}C iodide was purchased from New England Nuclear Corp. Elementary analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. For gas chromatography a Packard gas chromatograph, Model 7821, equipped with a flame ionization detector and temperature programmer was used. Separations were made on a six foot, coiled, glass column packed with 3% silicone polymer, JXR, on Gas Chrom Q, 100–200 mesh (Applied Science Labs., State College, Pa.). Solutions of the samples in chloroform were injected into a stream of nitrogen (60 cc per min) and programmed from 140 to 240° at 3° per min. *myo*-Inositol was injected as a solution of its hexakis-*O*-trimethylsilyl derivative[12] in heptane.

*Benzyl 2,3-di-*O*-benzyl-β-*D*-galactopyranosiduronic acid* (**4**). — A modification of a method previously used to oxidize benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside[9] was employed. To a suspension of benzyl 2,3-di-*O*-benzyl-β-D-galactopyranoside[4–8] (**3**) (1.9 g) and 10% platinum-on-carbon catalyst (1.7 g) in water (275 ml) was added 2.5% aqueous sodium hydrogen carbonate (5 ml). A continuous stream of oxygen (35 l/h) was passed through the suspension for 10 h, while the temperature was maintained at 95°. Additional portions of 2.5% aqueous sodium hydrogen carbonate were added as necessary to maintain the pH between 7–8 (total, 20.9 ml). After 4.5 h, a second portion of platinum-on-carbon catalyst (1.7 g) was added. After 10 h, no further change in pH was observed, and the suspension was filtered through Hyflo Super Cel and washed with water. The combined filtrates were evaporated to dryness, and the residue was extracted with chloroform. After the chloroform extract had been evaporated to dryness under reduced pressure, the residue was dissolved in acetone (100 ml) and water (50 ml), and adjusted to pH 2 with N HCl. Further removal of the solvent, extraction with acetone, and evaporation to a syrup at reduced pressure gave a product that crystallized from chloroform–heptane;

yield, 1.33 g (67%); m.p. 139–144°. Two recrystallizations from the same solvent gave 0.87 g (44%), m.p. 150–152°; $[\alpha]_D^{25}$ −33.2° (*c* 0.72, chloroform); ν_{max}^{KBr}: 3700 (weak, OH), 1720 (C=O), 1500 (weak), 1450, 750, 730, and 690 (aromatic) cm^{-1}.

Anal. Calc. for $C_{27}H_{28}O_7$: C, 69.82; H, 6.08. Found: C, 69.56; H, 5.99.

Sodium (benzyl 2,3-di-O-benzyl-β-D-galactopyranosid)uronate (**5**). — To a solution of **4** (0.107 g, 0.23 mmoles) in acetone (5 ml) was added powdered sodium hydrogen carbonate (0.019 g, 0.23 mmoles) and water (5 ml). After 1 h at 25°, the clear solution was evaporated and dried by successive evaporations with acetone (10 ml), acetone–benzene (1:1 v/v; 10 ml), and benzene (10 ml). The residue was triturated with heptane and filtered to give **5**, yield 0.097 g (87%); $[\alpha]_D^{25}$ −16.3° (*c* 0.75, chloroform); ν_{max}^{KBr}: 3500 (OH), 1610 (COO^-), 1490, 1440, 725, and 690 (aromatic) cm^{-1}.



(3) R^1 = CH_2Ph; R^2 = H; R^3 = CH_2OH
(4) R^1 = CH_2Ph; R^2 = H; R^3 = COOH
(5) R^1 = CH_2Ph; R^2 = H; R^3 = COONa
(6) R^1 = CH_2Ph; R^2 = H; R^3 = COOMe

Methyl (benzyl 2,3-di-O-benzyl-β-D-galactopyranosid)uronate (**6**). — Methyl iodide (20 µl; 31% excess) was added to a solution of **5** (0.119 g; 0.25 mmole) in *N,N*-dimethylformamide (0.25 ml). This solution was heated for 7 h at 75°. The solvent was removed by evaporation with xylene (2 × 3 ml). The residue was extracted with chloroform (3 ml), and the extract was added to a column (1 cm diameter) of silica gel (10 g) packed with chloroform. The column was developed with chloroform. Compound **6** appeared in fractions eluted between 100–140 ml. Removal of chloroform from these fractions gave a syrup; yield, 0.109 g (93%); ν_{max}^{film}: 3550 (OH), 1725 (C=O), 1490, 1440, 1430, 740, 725, and 685 (aromatic) cm^{-1}. In a similar run, the syrup crystallized from chloroform–hexane to give a waxy solid, m.p. 110–111°; $[\alpha]_D^{25}$ −28.4° (*c* 0.63, chloroform).

Anal. Calc. for $C_{28}H_{30}O_7$: C, 70.28; H, 6.32; OCH_3, 6.49. Found: C, 69.80; H, 6.27; OCH_3, 6.87.

For the synthesis of methyl-^{14}C labeled **6**, a 20% excess of **5** was employed. Methyl-^{14}C iodide (0.2 mmole) had 5 mc per mmole of ^{14}C. Radiochemical conversion into **6** was quantitative.

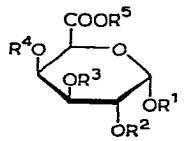
1,2:3,4-Di-O-benzylidene-D-galactopyranuronic acid (**7**)[10]. — **1**-Monohydrate was converted into **7**, using the procedure of Zinner and Thielebeule[10], in 33% yield; m.p. 199–201° (mainly at 198–201°) [lit.[10] m.p. 212°]; ν_{max}^{KBr} 3000–2500 (dimeric COOH), 1725 (C=O), 750 and 690 (aromatic) cm^{-1}.

Anal. Calc. for $C_{20}H_{18}O_7$: C, 64.85; H, 4.90. Found: C, 64.66; H, 5.03.

Sodium 1,2:3,4-di-O-benzylidene-D-galactopyranuronate (**8**). — Sodium hydrogen carbonate (2.74 g, 32.6 mmoles) was added to a solution of **7** (12.08 g, 32.6 mmoles) in acetone (200 ml) and water (200 ml). After 13.5 h, the solvents were removed and the residue was dissolved in water (125 ml). A small amount of insoluble matter was removed by filtration and the filtrate was evaporated to dryness. The

salt was dried over phosphorus pentoxide for several h at 0.05 mm; yield, 10.5 g (82%) ν_{max}^{KBr}: 1620 (COO^-), 760 and 700 (aromatic) cm^{-1}.

Methyl 1,2:3,4-di-O-benzylidene-D-galactopyranuronate (**9**). — To a suspension of the sodium salt **8** (4.98 g, 12.7 mmoles) in *N,N*-dimethylformamide (25 ml) was added methyl iodide (1.58 ml; 25.4 mmoles); the dissolution of the salt was immediate. The solution was heated in a water bath for 3.5 h at 95°. The solvent was removed by evaporation at 60° under reduced pressure, and finally by evaporation with xylene (4 × 25 ml). The residue was extracted with chloroform (15 ml) and then the extract was poured onto a column (2 cm diameter) of silica gel (50 g) packed in chloroform. The column was eluted with chloroform and the fractions eluted between 50–200 ml were combined. Removal of the solvent afforded a syrup; yield, 4.60 g (94%); ν_{max}^{film}: 1760 and 1740 (C=O), 750 and 690 (aromatic) cm^{-1}.



(7) $R^1R^2 = R^3R^4 =$ >CHPh, $R^5 = H$
(8) $R^1R^2 = R^3R^4 =$ >CHPh, $R^5 = Na$
(9) $R^1R^2 = R^3R^4 =$ >CHPh, $R^5 = Me$

Crystallization of the syrup from methanol–petroleum ether gave colorless crystals (1.55 g, 32%), m.p. 143–146°. An analytical sample, m.p. 148–150° (lit.[10] m.p. 165°), was obtained in another experiment by recrystallization from methanol; $[\alpha]_D^{25}$ −126° (*c* 1.06, chloroform) {lit.[10] $[\alpha]_D^{18}$ −168° (chloroform)}; ν_{max}^{KBr} 1760 (C=O), 760 and 700 (aromatic) cm^{-1}.

Anal. Calc. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24; OCH_3, 8.07. Found: C, 65.50; H, 5.21; OCH_3, 8.15.

A second crop of crystals, m.p. 110–125°, was obtained from the mother liquor; g.l.c. showed it to be a mixture of 4 isomers; yield, 0.65 g (13%).

The mother liquor, after the removal of the second crop, was evaporated to a syrup which could not be induced to crystallize further; yield, 2.07 g (43%). In another experiment, this syrup was chromatographed on silica gel to obtain the analytical sample; ν_{max}^{film} 1760 and 1740 (C=O), 760 and 700 (aromatic) cm^{-1}.

Anal. Calc. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24; OCH_3, 8.07. Found: C, 65.37; H, 5.35; OCH_3, 8.30.

In gas chromatography, the crystalline isomer (m.p. 148–150°) gave rise to a single peak, while the syrupy product gave 4 peaks, the first of which corresponded to that of crystalline **9**.

Methyl D-galacturonate (**2**) *from methyl (benzyl 2,3-di-O-benzyl-β-D-galactopyranosid)uronate* (**6**). — A solution of **6** (0.109 g) in absolute ethanol (125 ml) was hydrogenated slightly above atmospheric pressure, in the presence of 10% palladium–on–carbon catalyst (150 mg) until hydrogen uptake ceased. After removal of the catalyst and the solvent, a pale yellow syrup (**2**) remained in nearly quantitative yield; ν_{max}^{film}: 3500 (OH), 1750 (ester C=O) cm^{-1}. Paper chromatography on Whatman no. 1

paper with benzene–butyl alcohol–pyridine–water (1:5:5:3, v/v) gave a single spot (R_G 1.52). Thin-layer chromatography also gave a single spot, corresponding to authentic[3] **2**. Compound **2** (3.3 mg) was saponified with 0.03N sodium hydroxide (0.7 ml) for 2 h at 45°, and then neutralized by passage through a column of Dowex-50 (H^+) ion-exchange resin. The column was flushed with water, and the combined eluates were evaporated to dryness. Thin-layer chromatography of the residue showed a single spot with an R_F corresponding to that of authentic **1**. The saponification product and **1** also had the same electrophoretic mobility on paper (0.1M formate buffer, pH 3.8, 2500 volts, 1.25 h, Whatman No. 1 paper).

When methyl-^{14}C labeled **6** (0.10 g) was hydrogenated under the conditions described above, the yield of labeled ester was quantitative. Thin-layer chromatography showed the presence of a major radioactive spot corresponding to **2** and of a minor radioactive spot near the solvent front. When the thin-layer plate was exposed to ammonia vapor for a brief period and then re-scanned for radioactivity, no ^{14}C was found to be present on the plate.

(*b*) *From methyl 1,2:3,4-di-*O*-benzylidene-*D*-galactopyranuronate* (**9**). — A solution of syrupy **9** (0.842 g), shown to be a mixture of four isomers by g.l.c., in absolute ethanol (100 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of 10% palladium–on–carbon catalyst (400 mg) until hydrogen uptake stopped. The catalyst was removed by filtration through Celite. Removal of the solvent under reduced pressure gave a nearly colorless syrup (0.52 g) which showed a single spot, having the same R_F value as authentic **2**, by t.l.c. on cellulose; the saponification product was identical with authentic **1**.

The syrup was crystallized from methanol–dioxane (1:3.5, v/v). The colorless crystals were collected by filtration and washed successively with dioxane–anhydrous ether (1:1, v/v) and anhydrous ether; yield, 0.134 g (29.3%); m.p. 141–144° [Morell and Link[2] reported a m.p. of 146–148°]; $[\alpha]_D^{25}$ +86.2° (10 min)→+30.5° (equilibrium, 2 days; *c* 1.08, methanol) [lit.[2] $[\alpha]_D^{25}$ +75.5° (3 min) →+38° (equilibrium, 90 min; methanol). Jansen and Jang[3] reported $[\alpha]_D^{25}$ +94° (initial) →+34° (equilibrium, 3 days; methanol)]; ν_{max}^{KBr} 3450 (OH), 1750 (ester C=O) cm^{-1}.

An additional crop of crystals (0.098 g; 21.4%), m.p. 129–131°, was obtained from the mother liquor, by crystallization of the residue from methanol–dioxane–anhydrous ether (1:6:6, v/v). The infrared spectrum of the second crop was superimposable on that of the first crop.

A nearly colorless syrup was obtained by evaporation of the mother liquor, which could not be crystallized further. Thin-layer chromatography of each of the two crops of crystals and of the residual syrup revealed a major spot identical with that of authentic **2**; in each case, a trace of a constituent moving slightly faster than **2** was observed.

ACKNOWLEDGMENTS

The authors thank the National Institutes of General Medicine, National

Institutes of Health, United States Public Health Service for a Grant (No. GM 12422) in support of this work. The authors are indebted to Dr. M. Schwartz for use of a polarimeter.

SUMMARY

Methyl D-galacturonate was prepared by two different routes; one from D-galactose by way of benzyl 2,3-di-*O*-benzyl-*β*-D-galactopyranosiduronic acid, and the other from D-galacturonic acid *via* its 1,2:3,4-di-*O*-benzylidene derivative. The first of these methods was employed for the preparation of methyl-^{14}C D-galacturonate, the radiochemical yield being nearly quantitative. Ester formation was achieved by reaction of the sodium salt of either D-galacturonic acid derivative (**5** or **8**) with methyl iodide in *N*,*N*-dimethylformamide. Benzyl and benzylidene groups were removed from the esters (**6** and **9**) by hydrogenolysis in ethanol solution. Either of the above methods gave methyl D-galacturonate chromatographically indistinguishable from authentic ester **2**.

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