Note

An Improved Method for the Preparation of Phenyl α -L-Arabinofuranoside and Its Isomers

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Phenyl glycosides are generally used as substrates to determine the enzyme activity and kinetic parameters or to study the substrate specificity of glycosidases. For instance, Tsujisaka,¹⁾ and Chiba and Shimomura²⁾ have compared in detail substrate specificity and kinetic parameters of various α -glucosidases, using phenyl α -D-glucoside and a series of malto-oligosaccharides, and classified the enzymes according to these results.

In α -L-arabinofuranosidases (AFase), however, there have been only a few reports studying the substrate specificity or kinetic parameters^{3~5)} in comparison with other glycosidases. The difficulty of a detail study on substrate specificity or kinetic parameters seems to be one reason why P α LAF is not commercially available and why preparation of P α LAF or arabino-oligosaccharides is not particularly easy.

P α LAF is generally synthesized according to the method reported by Börjeson *et al.*⁶⁾ which is a preferential method for the synthesis of P α LAF of mp 63~66°C, $[\alpha]_D -159^\circ$ ($c=2.0$, water), Found C, 58.1; H, 6.19; O, 35.5, C₁₁H₁₄O₅ requires C, 58.4; H, 6.24; O, 35.4%. Many researchers have synthesized P α LAF by this method, but presented no data for the purity or the structural confirmation of P α LAF.

This paper deals with a modification of the method of Börjeson *et al.* and the isolation of P α LAF by the method of Austin *et al.*⁷⁾

EXPERIMENTAL

General method. Gas liquid chromatography was performed by a Shimadzu GC-6A gas chromatograph with a glass column (3 mm $\times$ 1.5 m) packed with 3% ECNSS on Gas Chrome Q. Methylation analysis was carried out by the method of Hakomori,⁸⁾ and methylated arabinitol acetate was analyzed with a Shimadzu GCMS 7000. IR spectra were recorded with a Shimadzu IR-420 Spectrophotometer and optical rotations were determined with a JASCO DIP-140 Digital polarimeter. Elementary analysis was performed by the Chemical Analysis Center of The University of Tsukuba.

1,2,3,5-Tetra-O-acetyl-L-arabinofuranose (I). To a solution of L-arabinose (50 g) in DMF were added acetic anhydride (125 ml) and pyridine (100 ml), and the mixture was heated for 50 hr at 50°C. The reaction mixture was poured into ice-cooled water (2500 ml), extracted with chloroform (3 $\times$ 250 ml), and the extract concentrated to a syrup. This syrup was dissolved in carbon tetrachloride (700 ml), washed with water (2 $\times$ 100 ml), dried with anhydrous magnesium sulfate, and further concentrated to a syrup. This syrup was then applied to a column of silica gel (2 $\times$ 50 cm), and chromatographed with a solvent system of ethyl acetate-chloroform (1:1, v/v). The fractions containing sugars were collected and concentrated to a syrup.

In this mild condition, the yield of acetylated arabinose was not decreased, and there was no difference in the ratio

FIG. 1. Gas Liquid Chromatography of Acetylated Arabinose.

A series with DMF: (1) 150°C for 5 min. (3) 50°C for 50 hr. A series without DMF: (2) 150°C for 5 min. (4) 50°C for 50 hr. Peak 1, 1,2,3,4-tetra-O-acetyl L-arabinopyranose; peaks 2 and 3, 1,2,3,5-tetra-O-acetyl L-arabinofuranose.

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Abbreviations: AFase, α -L-arabinofuranosidase; P α LAF, phenyl α -L-arabinofuranoside; P β LAF, phenyl β -L-arabinofuranoside; P α LAP, phenyl α -L-arabinopyranoside; P β LAP, phenyl β -L-arabinopyranoside; DMF, *N,N*-dimethylformamide.

of arabinofuranose tetraacetate, peaks 2 and 3, as determined by GLC and GCMS and shown in Fig. 1. Furthermore, the production of colored materials was reduced. From L-arabinose (50 g), 85 g of (I) was yielded as a pale yellow syrup (89.1%).

Phenyl 2,3,5-tri-O-acetyl- α -L-arabinofuranoside (II). The whole amount of (I) was mixed with phenol (100 g). *p*-Toluenesulfonic acid (0.8 g) was added as a catalyst, and the mixture heated at 100°C for 25 min under reduced pressure. Benzene (600 ml) was added to the reaction mixture, which was then washed with 1 N sodium hydroxide (6 $\times$ 40 ml), washed with water (until neutral pH), dried with anhydrous magnesium sulfate, and concentrated to a syrup. In this step, 61 g of (II) was synthesized (64.8%).

Phenyl α -L-arabinofuranoside (P α LAF). The whole amount of (II) was dissolved in dry methanol (1500 ml) and sodium methoxide (1.5 g) added. After standing for 3 hr at room temperature, the reaction mixture was concentrated to a syrup, dissolved in water (100 ml), desalted with Dowex 50, and concentrated to a small volume. The concentrate was washed with chloroform (3 $\times$ 25 ml), extracted with ethyl acetate (8 $\times$ 100 ml), and concentrated to a syrup. This syrup was decolorized by silica gel column chromatography as described above, and the fractions containing sugars were collected, and concentrated to a syrup. In this step, 8.4 g of P α LAF was yielded as a colorless syrup (21.5%).

Isolation of the major component C. The resulting colorless syrup of P α LAF contained a major component C and three minor components A, B and D as shown in the GLC data (Fig. 2-1). Isolation of component C was performed according to the method of Austin *et al.* A column of Dowex 1 $\times$ 2 (free base, 2 $\times$ 50 cm) was activated with 0.5 N sodium hydroxide, and washed with distilled water (free from carbon dioxide). One and half grams of the colorless syrup was applied to this column and chromatographed with distilled water (free from carbon dioxide). The phenyl L-arabinoside in each fraction was monitored by the measurement of absorbance at 266 nm, and also checked by GLC. As shown in Fig. 3, two fractions (Fr-I and Fr-II) were obtained; Fr-II contained only component C (1.0 g), which was identified as P α LAF, and Fr-I was a mixture of components A, B and D (Figs. 2-2 and 2-3).

Fractionation of the minor components A, B and D. The sample, Fr-I (1.96 g) was applied to a column of silica gel (2 $\times$ 50 cm), which was previously equilibrated with ethyl acetate, chromatographed with the same solvent, and fractionated. As shown in Fig. 4, three fractions (Peaks 1, 2 and 3) were obtained. Peak 1 contained only component B, and peak 2 contained component D with a trace amount of components A and B. Rechromatography on the silica gel column with the same procedure, and crystal-

FIG. 2. Gas Liquid Chromatography of Phenyl L-Arabinosides.

(1) Synthesized phenyl L-arabinosides mixture; (2) Fr-II on Dowex 1 column chromatography; (3) Fr-I on Dowex 1 column chromatography.

FIG. 3. Column Chromatography of Phenyl L-Arabinosides Mixture on Dowex 1.

FIG. 4. Column Chromatography of Phenyl L-Arabinosides Mixture on Silica Gel.

lization of these two fractions gave components B and D as pure materials, but component A could not be successfully isolated.

Component C (P α LAF). Colorless syrup, $[\alpha]_D^{20} -144^\circ$ ($c=0.85$, water). Methylation analysis gave 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl arabinitol. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 690, 750, 1220 (>C-O-C<), 1490, 1590, 1600 (C_6H_5), 3320 (-OH). GC-MS m/z : 43 ($\text{H}_3\text{C-C}\equiv\text{O}^+$), 94 ($\text{C}_6\text{H}_5\text{-OH}$), 97, 139, 199, 259 ($\text{M}^+ - 94$), 279 ($\text{M}^+ - \text{CH}_2\text{-O-CO-CH}_3$). *Anal.*, Found: C, 58.25; H, 6.19; O, 35.56. Calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.40; H, 6.23; O, 35.37%.

Component B (P β LAF). White needles, mp $82\sim 85^\circ\text{C}$, $[\alpha]_D^{20} +148^\circ$ ($c=0.99$, water). Methylation analysis gave 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl arabinitol. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 690, 750, 1220 (>C-O-C<), 1490, 1590, 1600 (C_6H_5), 3320 (-OH). GC-MS m/z : 43 ($\text{H}_3\text{C-C}\equiv\text{O}^+$), 94 ($\text{C}_6\text{H}_5\text{-OH}$), 97, 139, 199, 259 ($\text{M}^+ - 94$), 279 ($\text{M}^+ - \text{CH}_2\text{-O-CO-CH}_3$). *Anal.*, Found: C, 58.23; H, 6.24; O, 35.53. Calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.40; H, 6.23; O, 35.37%.

Component D (P α LAP). White needles, mp 158°C , $[\alpha]_D^{20} +8.8^\circ$ ($c=0.67$, water). Methylation analysis gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl arabinitol. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 690, 750, 1220 (>C-O-C<), 1490, 1590, 1600 (C_6H_5), 3300 (-OH). GC-MS m/z : 43 ($\text{H}_3\text{C-C}\equiv\text{O}^+$), 94 ($\text{C}_6\text{H}_5\text{-OH}$), 97, 139, 199, 259 ($\text{M}^+ - 94$). *Anal.*, Found: C, 58.21; H, 6.21; O, 35.58. Calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.40; H, 6.23; O, 35.37%.

P β LAP (commercial product). White needles, mp $173\sim 175^\circ\text{C}$, $[\alpha]_D^{20} +246^\circ$ ($c=0.51$, water). Methylation analysis gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl arabinitol. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 690, 755, 1220 (>C-O-C<), 1480, 1590, 1600 (C_6H_5), 3400 (-OH). GC-MS m/z : 43 ($\text{H}_3\text{C-C}\equiv\text{O}^+$), 94 ($\text{C}_6\text{H}_5\text{-OH}$), 97, 139, 199, 259 ($\text{M}^+ - 94$). *Anal.*, Found: C, 58.21; H, 6.21; O, 35.58. Calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5$: C, 58.40; H, 6.23; O, 35.37%.

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