AN ALTERNATIVE SYNTHESIS OF 5-*O*-METHYL-L-ARABINOSE[1]

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ABSTRACT

5-*O*-Methyl-L-arabinose has been synthesized by an unambiguous route in which *O*-benzyl groups were used to block positions 2 and 3 in the L-arabinose molecule. In addition to the usual indirect proofs of structure, a direct proof is described involving isolation of the product from periodate oxidation of the 5-*O*-methyl-L-arabinose. Two new derivatives, the phenylhydrazide and amide of 5-*O*-methyl-L-arabonic acid, were prepared.

The probable occurrence of 5-*O*-methyl-L-arabinose among the hydrolysis products of methylated wheat bran hemicellulose (1) prompted the synthesis of this compound by Dutton *et al.* (2). These authors used *O*-acetyl groups to block positions 2 and 3 in ethyl α-L-arabinofuranoside during methylation by Purdie's reagents and stated that "although sugar acetates may undergo acetyl migration even in the presence of such a weak base as silver oxide there was no indication of such migration in the preparation described here" (2). This result indicated that *O*-acetyl migrations, which are known to occur in the hexopyranoside series (3–6) and in D-glucose diethyl thioacetal tetracetate (7), may not happen in the pentofuranoside series.

Authentic samples of mono- and di-*O*-methyl ethers of L-arabinose were required in connection with another problem (8) and were obtained from various laboratories. Examination of the methyl glycosides of these compounds by gas–liquid partition chromatography (9, 10) revealed that all of the samples that were syrups contained more than one component. These samples had been obtained by chromatography on cellulose and the results showed that pure methyl ethers of arabinose may be difficult to isolate by that method.

Because of the unexpected absence of *O*-acetyl migration in the synthesis of Dutton *et al.* (2), the apparent difficulties in purification of these compounds by cellulose chromatography, and the urgent need for an authentic sample of this important reference sugar, an alternative synthesis of 5-*O*-methyl-L-arabinose seemed desirable. The present paper reports such a synthesis together with a proof of structure and two new derivatives of this sugar, namely the phenylhydrazide and the amide of 5-*O*-methyl-L-arabonic acid.

The route followed in this synthesis was similar to that used by Dutton *et al.* (2) except that *O*-benzyl rather than *O*-acetyl groups were used to block positions 2 and 3 in the L-arabinose molecule. The sequence of reactions was as follows. Ethyl α-L-arabinofuranoside was tritylated and the product was benzylated to yield ethyl 2,3-di-*O*-benzyl-5-*O*-trityl-α-L-arabinoside. This compound was successively detritylated, methylated, debenzylated, and hydrolyzed to yield 5-*O*-methyl-L-arabinose. Purities of the compounds obtained in each step of the synthesis were checked by analysis and, where possible, by gas–liquid partition chromatography and paper chromatography.

Confirmation that the syrupy mono-*O*-methyl-L-arabinose from the above synthesis was 5-*O*-methyl-L-arabinose was obtained from the following evidence. Complete methylation of the methyl glycosides, prepared by refluxing the mono-*O*-methyl-L-arabinose in methanolic hydrogen chloride, yielded methyl 2,3,5-tri-*O*-methyl α- and β-L-arabinosides,

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readily distinguished from the methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinose by gas–liquid partition chromatography (8). Oxidation of the mono-*O*-methyl-L-arabinose by bromide yielded a lactone that crystallized from the chloroform extract of the reaction mixture. This lactone showed a very slow change in rotation in aqueous solution and had carbonyl absorption at 1773 cm^{-1} in the C=O stretching frequency of its infrared spectrum. Barker *et al.* (11) have shown that γ-lactones of aldonic acids have their carbonyl absorption bands in the range 1765–1790 cm^{-1} while those of δ-lactones are between 1726 and 1760 cm^{-1}. This was confirmed here by observation of the infrared spectra of the following lactones, which showed carbonyl absorption as indicated: D-gluconic acid δ-lactone (1720 cm^{-1}); 2,5-di-*O*-methyl-L-arabonic acid-γ-lactone (1777 cm^{-1}); and 3,5-di-*O*-methyl-D-galactonic acid-γ-lactone (12) (1786 cm^{-1}). The results of methylation and the isolation of the γ-lactone showed that the parent mono-*O*-methyl-L-arabinose was capable of existing in the furanose form and therefore that the *O*-methyl group could not be located at C_4.

The mono-*O*-methyl-L-arabonic acid-γ-lactone provided a crystalline phenylhydrazide and a crystalline amide on reaction with phenylhydrazine and ammonia respectively. The amide gave a positive Weerman (13) reaction showing the presence of a free hydroxyl at C_2. This conclusion was supported by isolation of an osazone from the mono-*O*-methyl-L-arabinose without loss of methoxyl and by the large M_G value of the parent sugar on paper electrophoresis in borate buffer (14).

On oxidation by periodate the methyl glycosides of the mono-*O*-methyl-L-arabinose consumed one molar proportion of oxidant and released neither formic acid nor formaldehyde. This result could not have been obtained from methyl 3-*O*-methyl-L-arabinoside, which would not be oxidized in either the furanoside or pyranoside form, and showed that C_3 in the mono-*O*-methyl-L-arabinose must carry a free hydroxyl.

The foregoing evidence showed that free hydroxyls existed on C_2, C_3, and C_4 of the mono-*O*-methyl-L-arabinose and constituted indirect proof that the *O*-methyl group was located on C_5. On periodate oxidation the mono-*O*-methyl-L-arabinose yielded methoxyacetaldehyde, isolated by azeotropic distillation with water and identified as its *p*-nitrophenylhydrazone (15, 16). The isolation of this product, which could have arisen only from the 5-*O*-methyl isomer, constituted a direct proof of structure.

The physical constants of the 5-*O*-methyl-L-arabinose, 5-*O*-methyl-L-arabonolactone, and 5-*O*-methyl arabinosazone isolated in the present study are in reasonable agreement with the values reported for these derivatives by Dutton *et al.* (2) except for the specific rotations of the free sugar and of its osazone. Although certain partially methylated reducing sugars are known to give anomalous results when oxidized by periodate (17, 18), Dutton *et al.* (2) have shown that 5-*O*-methyl-L-arabinose behaves normally under these conditions, a conclusion supported by the present work.

EXPERIMENTAL

Paper chromatograms were run by the descending method on Whatman No. 1 paper using the organic phase of butanone saturated with water containing 2% of concentrated ammonium hydroxide. Paper electrophoresis was done on Whatman 3MM paper at 700–800 v for 2 hours using 0.2 *M* borate buffer, pH 10. Sugars were detected on the papers by the *p*-anisidine hydrochloride spray reagent (19). Infrared spectra were measured in the frequency range 3800–699 cm^{-1} either on chloroform solutions or by the Nujol mull technique. Gas–liquid partition chromatograms were done on a Pye Argon

Chromatograph using straight, 4-ft columns with the following packings:

(*a*) butanediol succinate polyester on Chromosorb W, 60–80 mesh (1:9 w/w),

(*b*) polyphenyl ether, *m*-bis(*m*-phenoxyphenoxy)-benzene, on Celite 545, 80–100 mesh (1:6 w/w).

Flow rates and temperatures were as cited for individual analyses. Evaporations were carried out at 35° C under diminished pressure unless otherwise stated and melting points are corrected.

Ethyl 5-O-Trityl-α-L-arabinoside

Ethyl α-L-arabinofuranoside (10.8 g, m.p. 48–49° C, $[\alpha]_D^{26}$ −117°±1.5° (*c*, 1.7% in water) (20)) was dissolved in dry pyridine (100 ml) and triphenylchloromethane (16.85 g) was added. After the solution had been kept at room temperature (25–26° C) for 41 hours isolation of the product (21) yielded ethyl 5-*O*-trityl-α-L-arabinoside as a syrup (yield 25.0 g or 98%), $[\alpha]_D^{22}$ −51.4° (*c*, 3.1% in methanol). Anal: Calc. for $C_{26}H_{28}O_5$: trityl, 59.8%; C, 74.26%; H, 6.71%. Found: trityl (22), 59.67%; C, 74.99%; H, 6.92%.

Ethyl 2,3-Di-O-benzyl-5-O-trityl-α-L-arabinoside

Ethyl 5-*O*-trityl-α-L-arabinoside (23 g) was benzylated according to the procedure of Dennison and McGilvray (23). The syrupy product showed very weak hydroxyl absorption in its infrared spectrum but the high yield (35.0 g, 106%) indicated incomplete removal of benzyl chloride or benzyl alcohol from the reaction mixture. $[\alpha]_D^{22}$ −40.5° ±1° (*c*, 1.7% in chloroform). Anal: Calc. for $C_{40}H_{40}O_5$: C, 80.0%; H, 6.71%. Found: C, 81.1%; H, 6.71%.

Ethyl 2,3-Di-O-benzyl-α-L-arabinofuranoside

Ethyl 2,3-di-*O*-benzyl-5-*O*-trityl-α-L-arabinoside (34.8 g) was dissolved in dry chloroform (100 ml) and the solution was saturated at 0° C with dry hydrogen chloride. After 1 hour, the solution was neutralized with silver carbonate, silver salts were filtered, and the filtrate was evaporated to a syrup. A methanolic solution of this syrup yielded crystals of triphenylcarbinol when kept at 0° C. The crystals (4.0 g) were removed and the filtrate was evaporated to dryness. The residue, slurried in petroleum ether (65–110° C), was added to a column of alumina (3.5×28 cm) which was then eluted with petroleum ether (700 ml) to remove triphenylmethane (10 g, m.p. 93° C). Elution of the column with methanol (500 ml) and evaporation of the eluate then yielded the syrupy product (14.66 g, 74.5%), $[\alpha]_D^{24}$ −28.5°±0.5° (*c*, 2.4% in chloroform). Anal: Calc. for $C_{21}H_{26}O_5$: C, 70.37%; H, 7.31%. Found: C, 70.53%; H, 7.10%.

Ethyl 2,3-Di-O-benzyl-5-O-methyl-α-L-arabinoside

Ethyl 2,3-di-*O*-benzyl-α-L-arabinofuranoside (14 g) was methylated with methyl iodide (125 ml) and silver oxide (19 g). Filtration and evaporation of the filtrate yielded a product which still had hydroxyl absorption in its infrared spectrum. One more methylation yielded a product (14.1 g, 96%) with no hydroxyl absorption in the infrared and having $[\alpha]_D^{24}$ −22.3°±0.5° (*c*, 2.0% in chloroform). Anal: Calc. for $C_{22}H_{28}O_5$: C, 70.94%; H, 7.58%. Found: C, 71.58%; H, 7.08%.

Ethyl 5-O-Methyl-α-L-arabinoside

Ethyl 2,3-di-*O*-benzyl-5-*O*-methyl-α-L-arabinoside (13.0 g) was debenzylated by reduction with sodium in alcohol (24) to yield a syrupy product (4.66 g, 78.5%). Analysis of this product by gas–liquid partition chromatography, column A, 170° C, 300 ml argon/min, showed the presence of one major component (68.9%) and four minor

components. The minor components were undoubtedly partially benzylated products that were soluble in water. Because of this evidence of a mixture, no analyses or measurement of specific rotation were done.

Hydrolysis of Ethyl 5-O-Methyl-α-L-arabinoside

The syrupy glycoside obtained above (2.5 g) was heated with 0.05 *N* sulphuric acid (50 ml) at 100° C and the hydrolysis was followed by gas–liquid partition chromatography using the conditions just described for examination of the products from debenzylation. The glycosides gradually disappeared and none could be detected after 2 hours of hydrolysis. The hydrolyzate was neutralized with barium carbonate, filtered, and the filtrate was evaporated to dryness yielding a syrup (1.9 g, 91.2%). Paper chromatography of this material showed the presence of a major component of R_G 0.39 and minor components of R_G values 1.1, 1.0, 0.77, 0.22, and 0.12 (R_G = movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose).

Isolation of 5-O-Methyl-L-arabinose

The syrupy mixture from the hydrolysis (1.9 g) was resolved by chromatography on 22 sheets of Whatman No. 3MM paper (46×57 cm) and elution of the appropriate areas yielded the major component (R_G 0.39), which was recovered as a syrup (0.90 g, 47.4%). The yield was corrected for material lost on the guide strips used to locate the sugars.

Dutton *et al.* (2) reported $[\alpha]_D^{25}$ −32.0° (*c*, 0.484% in water) for 5-*O*-methyl-L-arabinose. The sugar isolated in the present work had $[\alpha]_D^{25}$ −25.3°±1.5° (*c*, 1.0% in water) and a methoxyl content of 18.7%; a mono-*O*-methyl pentose requires a methoxyl content of 18.9%. On paper chromatography and paper electrophoresis this sugar gave R_G, R_F, and M_G values of 0.39, 0.32, and 0.74 respectively. Gas–liquid partition chromatography of a methanolyzate of this compound on column B showed only two peaks, which corresponded to the anomeric glycosides of 5-*O*-methyl-L-arabinose. These results show that 5-*O*-methyl-L-arabinose is readily purified by chromatography on cellulose despite the apparent difficulties, referred to in the discussion, in obtaining pure samples of arabinose methyl ethers by that procedure.

Methylation of 5-O-Methyl-L-arabinose

The mono-*O*-methyl pentose (ca. 2 mg) was heated at 100° C in a sealed tube with 2% methanolic hydrogen chloride (1.0 ml) for 12 hours. Acid was neutralized with silver carbonate, and the filtrate from removal of silver salts was evaporated to a syrup which was shaken overnight with silver oxide (20 mg), methyl iodide (0.2 ml), and dimethyl formamide (0.2 ml) (25). The reaction mixture was examined by gas–liquid partition chromatography using column B, 200° C, 85 ml argon/min. Two components were found, in the methylation reaction, with retention volumes (V_R), which were made relative to methyl 2,3,5-tri-*O*-methyl-α-L-arabinoside, of 1.00 and 1.28 corresponding to the α- and β-methyl glycosides respectively of 2,3,5-tri-*O*-methyl-L-arabinose. Under the conditions used, the α- and β-methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinose ran as one component with V_R = 1.75 and none was found in the methylated product.

5-O-Methyl-L-arabonolactone

A mixture of 5-*O*-methyl-L-arabinose (300 mg), water (3 ml), barium carbonate (300 mg), and bromine (30 drops) was kept in the dark at room temperature for 70 hours. Bromine was removed by aeration and the aqueous solution was extracted continuously with chloroform for 5 days. The lactone crystallized from the chloroform solution and

evaporation of the solution left more of the lactone as a crystalline residue. Recrystallization from ethanol – petroleum ether (30–60° C) yielded 5-*O*-methyl-L-arabonolactone (170 mg), m.p. 137–138° C; $[\alpha]_D^{25}$ $-79.1°\pm1° \rightarrow -71.3°\pm1°$, 6 days incomplete (*c*, 1.0% in water); ν_{max}^{Nujol} 1773 cm^{-1}. Reported (2) values are m.p. 135° C, $[\alpha]_D^{25}$ $-76.2°$ (*c*, 0.22% in water). Anal: Calc. for $C_6H_{10}O_5$: C, 44.44%; H, 6.22%; OCH_3, 19.3%. Found: C, 44.12%; H, 5.99%; OCH_3, 19.1%.

5-O-Methyl-L-arabonamide

5-*O*-Methyl-L-arabonolactone (50 mg) was dissolved in saturated methanolic ammonia and stored at 5° C for 20 hours. Evaporation of the solution and recrystallization from ethanol–ether yielded the amide, m.p. 141–143° C, $[\alpha]_D^{25}$ $+40.7°\pm2°$ (*c*, 0.8% in methanol). Anal: Calc. for $C_6H_{13}O_5N$: C, 40.22%; H, 7.31%; N, 7.82%. Found: C, 39.98%; H, 7.09%; N, 7.76%.

A portion of the amide (ca. 5 mg) was oxidized with sodium hypochlorite and on addition of semicarbazide hydrochloride (13) gave a white precipitate of hydrazodicarbonamide.

5-O-Methyl-L-arabonic Acid Phenylhydrazide

5-*O*-Methyl-L-arabonolactone (40 mg) was dissolved in methanol (3 ml), and freshly distilled phenylhydrazine (30 mg) was added. The solution was refluxed for 3 hours and then evaporated to a syrup which crystallized from ethanol–ether to give the phenylhydrazide, m.p. 176.5–178° C, $[\alpha]_D^{25}$ $+23.6°\pm3°$ (*c*, 0.38% in methanol). Anal: Calc. for $C_{12}H_{18}O_5N_2$: N, 10.37%. Found: N, 10.58%.

5-O-Methyl-L-arabinose Phenylosazone

A mixture of 5-*O*-methyl-L-arabinose (50 mg), 20% acetic acid (3 ml), sodium bisulphite (50 mg), and phenylhydrazine (0.5 ml) was heated for 1 hour at 80° C and was then left for 24 hours at room temperature. The yellow, crystalline precipitate (25 mg) was recrystallized from aqueous acetone to give 5-*O*-methyl-L-arabinose phenylosazone, m.p. 153–155° C, $[\alpha]_D^{25}$ $+3°\pm1°$ (*c*, 0.97% in methanol). Reported (2) values are m.p. 154.5° C, $[\alpha]_D^{25}$ $-16.6°$ (*c*, 0.4% in methanol). Anal: Calc. for $C_{18}H_{22}O_3N_4$: C, 63.14%; H, 6.48%; N, 16.4%; OCH_3, 9.05%. Found: C, 63.08%; H, 6.51%; N, 16.57%; OCH_3, 9.11%.

Periodate Oxidation of 5-O-Methyl-L-arabinose

5-*O*-Methyl-L-arabinose (90 mg) was dissolved in 0.10 *M* sodium metaperiodate (20 ml) and the solution was stored in the dark at room temperature for 24 hours. The solution was then distilled and to the distillate was added an aliquot (4 ml) of a solution of *p*-nitrophenylhydrazine (0.4 g) in water (25 ml) and concentrated hydrochloric acid (0.4 ml) (15). The dense yellow precipitate was filtered after 5 minutes and recrystallized from ethanol:water (2:3) to yield methoxyacetaldehyde-*p*-nitrophenylhydrazone, m.p. 114–116° C. Reported (15, 16) values are m.p. 116° C and 115–115.5° C. Anal: Calc. for $C_9H_{11}O_3N_3$: C, 51.67%; H, 5.30%; N, 20.09%. Found: C, 51.30%; H, 5.39%; N, 19.95%.

Periodate Oxidation of Methyl 5-O-Methyl-(α,β)-L-arabinoside

5-*O*-Methyl-L-arabinose (40 mg) was refluxed with 2% methanolic hydrogen chloride (3 ml) for 6 hours. The solution was neutralized with silver carbonate, filtered, and the filtrate was evaporated to a syrup. The syrup (33.7 mg) was oxidized in aqueous solution (25 ml) containing 0.3 *M* sodium metaperiodate (10 ml); a blank was run concurrently. The consumption of periodate, estimated by the arsenite method (26), was 0.96 mole

per mole of glycoside, constant after 24 hours. No formic acid or formaldehyde could be detected.

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