Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 145–149

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Chemical Synthesis of UDP-β-L-Arabinofuranose and its Turnover to UDP-β-L-Arabinopyranose by UDP-Galactopyranose Mutase

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Received 1 September 2000; accepted 26 October 2000

Abstract—Uridine-5′-diphospho-β-L-arabinofuranose, a possible donor of L-arabinofuranose residues in plants, was synthesized. This compound, in the presence of UDP-galactopyranose mutase, underwent interconversion with UDP-β-L-arabinopyranose that is a likely precursor of L-arabinofuranose in vivo. This result provided a working model for the biogenesis of arabinofuranose in plants.

Introduction

Glycoconjugates containing furanose residues are ubiquitous in nature.[1] Among the known naturally occurring furanose sugars, galactofuranose and arabinofuranose are most frequently found. They are especially abundant in the surface constituents of microorganisms, such as bacterial O-antigens, fungal exopolysaccharides, protozoal glycoproteins, and the cell walls of mycobacteria. For example, all of the galactose and arabinose units in two major mycobacterial cell wall polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM), have been shown to exist in the furanose form.[2] Arabinofuranose is also a common structural component in plant cell walls.[1,3] Its occurrence in plant arabinan, rhamnogalacturonan, arabinogalactan, and arabinogalactan proteins has been well documented, in which arabinofuranose is typically coupled to other sugar components via an α-L-(1→5), α-L-(1→2), or α-L-(1→3) linkage.[1]

The precursor of D-galactofuranose (D-Gal*f*) found in various glycoconjugates is UDP-D-Gal*f* (**1**), which is biosynthetically derived from the corresponding galactopyranose (**2**, UDP-D-Gal*p*) via an unusual ring contraction reaction catalyzed by UDP-galactopyranose mutase.[4–8] This enzyme mediated interconversion greatly favors the thermodynamically more stable pyranose form **2**.[5] The catalytic mechanism has recently been shown to involve cleavage of the anomeric C–O bond to form a bicyclo-acetal intermediate (**3**, Scheme 1).[9] While enzymes responsible for the biosynthesis and transfer of D-Gal*f* have attracted much attention due to their potential as drug targets for bacteria, fungi, and parasites, less is known about the biosynthesis of arabinofuranose (**4**, Ara*f*) at enzymatic level. Structurally, L-arabinofuranose differs from D-galactofuranose only by a shorter side chain at C-4. It is thus conceivable that biosynthesis of L-arabinofuranose follows a path similar to that of D-galactofuranose, involving an enzyme catalyzed ring contraction of L-arabinopyranose. Indeed, preliminary data have shown that the source of L-arabinofuranose (Ara*f*) residues of the plant polysaccharides is UDP-L-arabinopyranose (UDP-L-Ara*p*, **5**, see Scheme 3),[10,11] which may be the substrate of the putative mutase. In order to test the viability of this hypothesis, we have chemically synthesized UDP-L-Ara*f* (**4**) and examined its competence as a substrate for UDP-galactopyranose mutase, a homologue of the putative arabinopyranose mutase. Reported in this paper are the results of these experiments.

Results and Discussion

The synthesis of UDP-β-L-arabinofuranose (**4**) was accomplished by a sequence of reactions delineated in Scheme 2. L-Arabinose was converted, according to a

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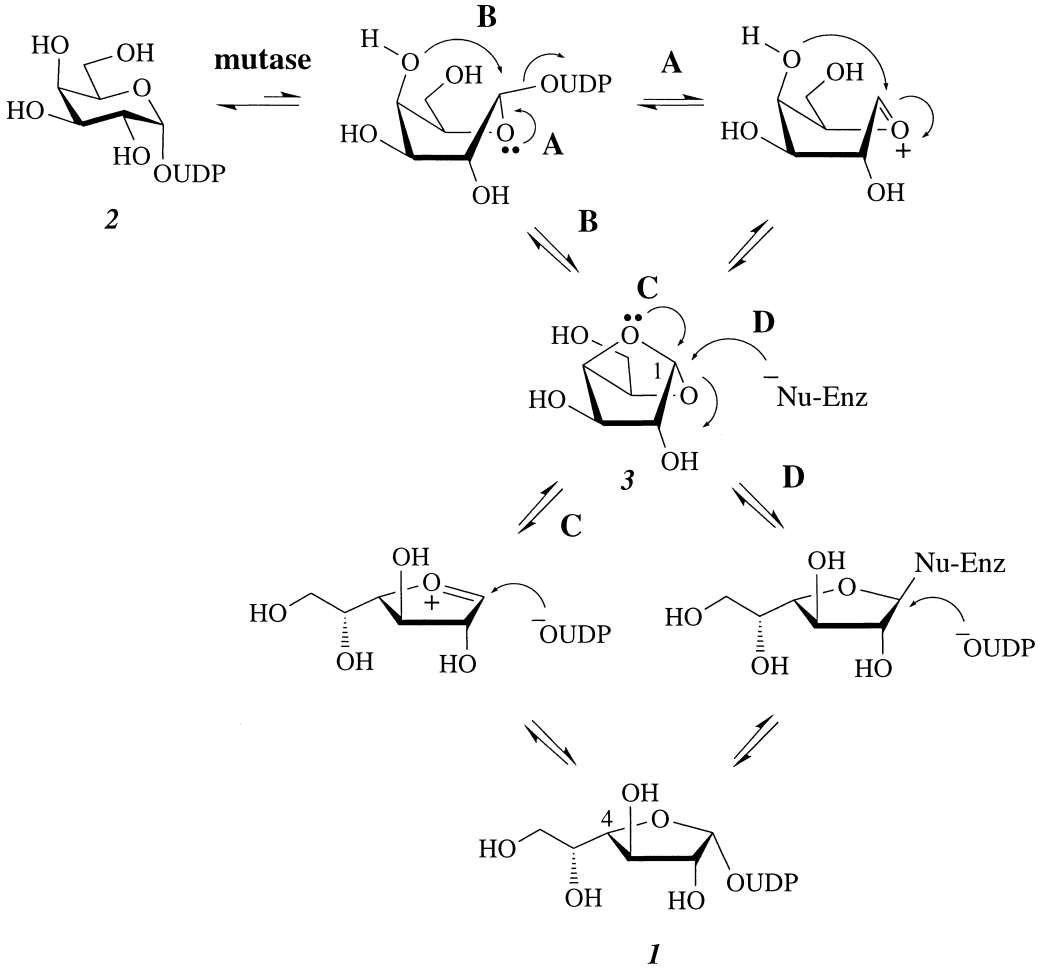
0960-894X/01/$ - see front matter
PII: S0960-894X(00)00616-8

one-pot procedure developed by Ichikawa et al.,[12] to tetra-*O*-acetyl-L-arabinofuranose (**6**) as a 1:1 mixture of α,β isomers. Subsequent preparation of β-L-arabinofuranosyl phosphate followed a strategy of de Lederkremer and co-workers.[13] As shown in Scheme 2, upon treatment with bromotrimethylsilane, tetra-acetate **6** was converted to the corresponding tri-*O*-acetyl-L-arabinofuranosyl bromide (**7**), which was chromatographically unstable on silica gel. Hence, without purification, compound **7** was reacted directly with excess triethylammonium dibenzyl phosphate in anhydrous toluene overnight. The resulting mixture was filtered and the filtrate was chromatographed on silica gel (ethyl acetate/hexanes, 3/5) to yield the desired product **8** as a single isomer. This product was isolated as a syrup. The 1-H signal in the ^{1}H NMR spectrum appeared as a doublet of doublets ($J_{1,2}=4.5$, $J_{H\text{-}1,P}=5.4$), and those of C-1 and C-2 in the ^{13}C NMR spectrum, due to coupling with ^{31}P, resonated as a doublet at δ 97.8 ($J=5$) and δ 75.9 ($J=5$), respectively. The observed coupling constant between 1-H and 2-H clearly indicates a *cis* relationship between the two hydroxyl groups at C-1 and C-2.[14] The product **8** was therefore characterized as dibenzyl (2,3,5-tri-*O*-acetyl-β-L-arabinofuranosyl) phosphate. The corresponding α isomer was not observed in this case.

Compound **8** was debenzylated by catalytic hydrogenation over 10% palladium on charcoal in the presence of triethylamine to afford the phosphate **9** as a monotriethylammonium salt in good yield (92%). Deacetylation of compound **9** was readily achieved by treatment with methanol/water (5/2) in the presence of triethylamine, providing the desired β-L-arabinofuranosyl phosphate (**10**) as its bistriethylammonium salt in quantitative yield. The final product UDP-L-Ara*f* (**4**) was synthesized from **10** and uridine phosphomorpholidate in anhydrous pyridine in the presence of 1*H*-tetrazole.[15,16] The reaction mixture was separated first by size exclusion chromatography (Sephadex LH-20, methanol), and then by HPLC on a semi-preparative C_{18} column (1.5% acetonitrile in 50 mM triethylammonium acetate) to give nearly homogeneous UDP-L-Ara*f* (**4**) as the bistriethylammonium salt in 42% yield. The structure of the purified product was confirmed spectroscopically by NMR (^{1}H, COSY, ^{13}C, and ^{31}P) and high-resolution ESI-MS. The presence of two doublets of similar intensity in the proton decoupled ^{31}P NMR confirmed the presence of a diphosphate linkage.[17] The availability of this furanose derivative is important for the bioassay. Since the equilibrium of the interconversion greatly favors the pyranose form, little

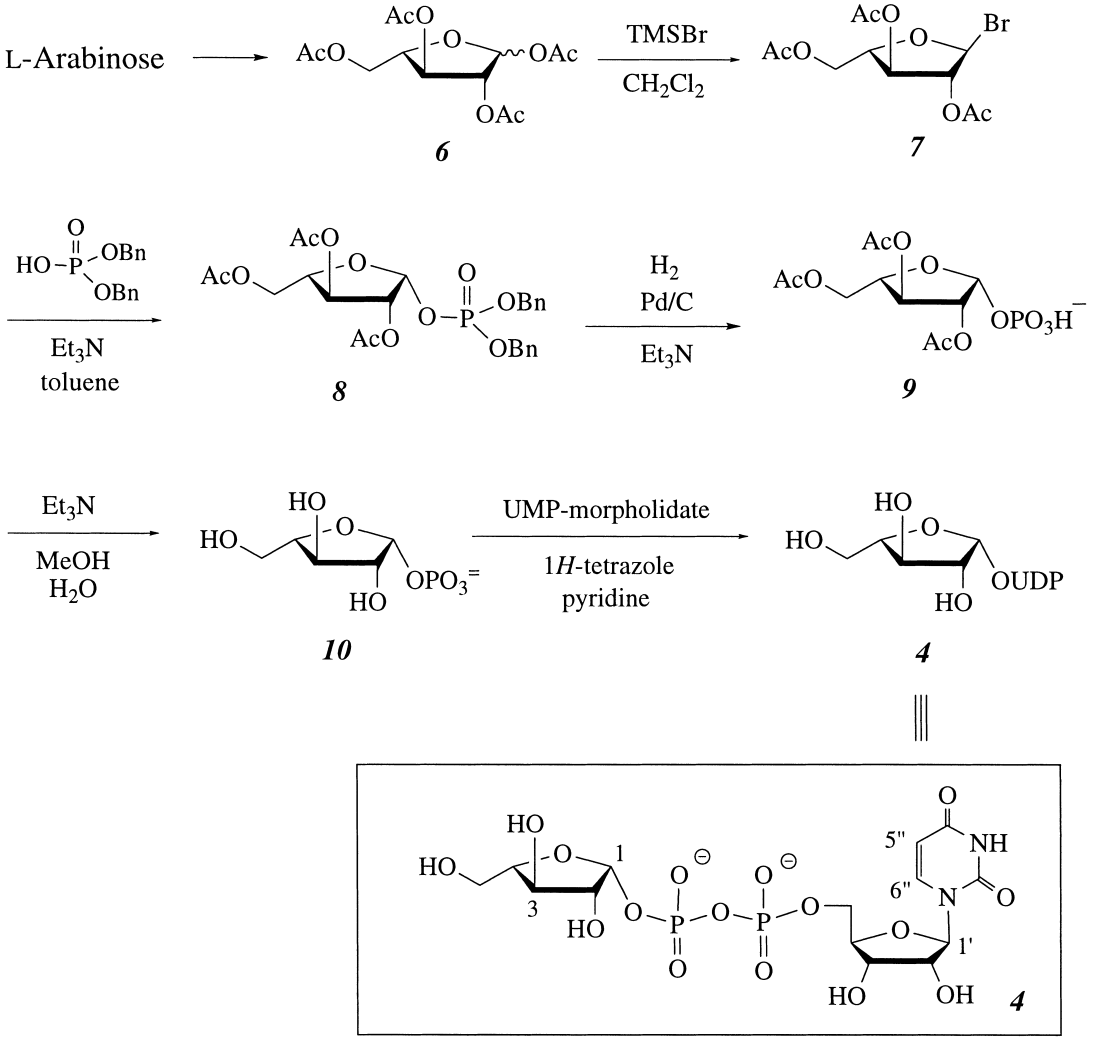


Scheme 1.

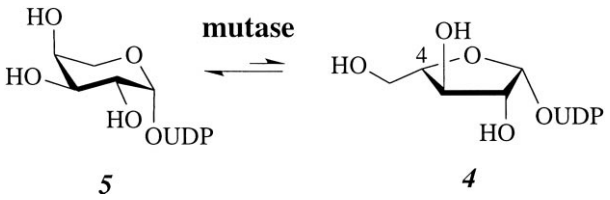
turnover would be seen if UDP-L-arabinopyranose was used as the substrate.

To demonstrate that UDP-L-Ara*f* (**4**) can undergo interconversion to generate UDP-L-Ara*p* (**5**) in the presence of a mutase, the synthetic product **4** was incubated with *Escherichia coli* UDP-galactopyranose mutase purified from an overproducing recombinant strain, *E. coli* BL-21 (DE3)/pQZ-1.[18] An assay solution consisting of 2 mM UDP-L-Ara*f* (**4**) and 72 μM mutase in 30 μL of 100 mM potassium phosphate buffer (pH 7.5) was incubated for 10 min at 37 °C. The resulting mixture was analyzed by reversed-phase HPLC (C_{18} column, 1.5% acetonitrile in 50 mM triethylammonium acetate). The detector was set at 262 nm and the flow rate was 1.0 mL/min. Under these conditions, a new peak with a retention time of 5.9 min was observed, whereas UDP-L-Ara*f* was eluted at 8.2 min (Fig. 1). More than 90% conversion of **4** to this new product could be achieved by prolonged incubation or by including 20 mM sodium dithionite in the reaction since the mutase is more reactive in its reduced form.[18] A preparative incubation allowed this product to be purified by semi-preparative HPLC under the aforementioned conditions. As expected, this compound was identified as UDP-L-Ara*p* (**5**) by NMR and MS analysis,[19] and by comparison to reported data.[20] The kinetic analysis was carried out by the same discontinuous HPLC assay. The product and substrate ratios were calculated from the integration of the corresponding peaks from the HPLC chromatogram. The K_m and k_{cat} for UDP-L-Ara*f* were determined to be 0.6 mM and 12 s^{-1}, respectively. The k_{cat} value is comparable to that ($k_{cat} = 27\ s^{-1}$) found for UDP-D-Gal*f*, but the K_m value is nearly 30-fold greater than that of UDP-D-Gal*f* ($K_m = 22\ \mu M$). These results suggest that significant interactions exist between the hydroxymethyl side chain of UDP-D-Gal*f* and the surrounding amino acid residues in the enzyme active site. Nevertheless, UDP-L-Ara*f* is clearly a reasonable substrate for UDP-galactopyranose mutase.

The work presented here is a proof of concept that UDP-L-Ara*f* can be converted from UDP-L-Ara*p* by the action of a mutase. On the basis of our data, this conversion can be catalyzed by a single enzyme, UDP-galactopyranose mutase, which works on both the D-Gal*p*/D-Gal*f* and L-Ara*p*/L-Ara*f* series. However, so



Scheme 2.



Scheme 3.

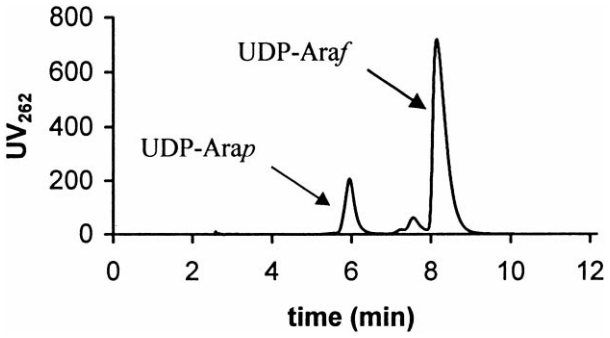


Figure 1. HPLC chromatogram of an incubation mixture of UDP-L-Ara*f* and UDP-galactopyranose mutase.

far all the arabinofuranose units found in the surface constituents of microorganisms are D-arabinose,[21,22] and the precursor of D-arabinofuranose in microorganisms has been established to be a β-D-arabinofuranosyl-1-monophosphoryldecaprenol. This notion is supported by many lines of evidence,[23–25] including the incorporation of chemically synthesized decaprenol phosphoarabinose into the oligosaccharide products in the presence of a membrane preparation from mycobacteria.[26] Therefore, in microorganisms it is likely that two distinct mutases are required to catalyze these two related ring contraction reactions, if D-arabinofuranose is indeed derived from D-arabinopyranose in its phosphoryldecaprenol form.[27] On the contrary, the arabinofuranose units found in plant polysaccharides are L-arabinose, where the scenario of one enzyme to catalyze the conversion of **2** to **1** and **5** to **4** is definitely a possibility. The ready availability of UDP-L-Ara*f* (**4**) through the chemical synthesis reported herein will certainly facilitate the detection and isolation of the desired mutase in plants. It would be interesting to compare whether the putative UDP-L-Ara*p* mutase and UDP-D-Gal*p* mutase are the same enzyme or are evolutionarily related, and whether the Ara*p* mutase can also accept Gal*p*/Gal*f* as substrates. Additionally, with large quantities of **4** available, one could also attempt to isolate the arabinofuranosyl transferase and study how nature incorporates arabinofuranose into biological molecules.

Acknowledgements

This work was supported in part by a grant from the National Institutes of Health (GM54346). H.-w. L. also thanks the National Institute of General Medical Sciences for a MERIT Award.

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15. Wittman, V.; Wong, C.-H. *J. Org. Chem.* **1997**, *62*, 2144. Specifically, the triethylammonium salt of β-L-arabinofuranosyl phosphate (**10**, 400 mg, 0.9 mmol) was dried by co-evaporating with anhydrous pyridine (4 mL) a few times. To this residue was added UMP-morpholidate (1.6 g, 2.1 mmol) in anhydrous pyridine (3 mL) and the solution was evaporated again to dryness under vacuum. This was followed by the addition of 1*H*-tetrazole (200 mg, 2.8 mmol) in anhydrous pyridine (5 mL) and the resulting solution was stirred at room temperature for 36 h. Removal of the solvent under vacuum gave a solid residue that was dissolved in methanol and chromatographed on a Sephadex LH-20 column (2.5×120 cm) using methanol as the eluent. Fractions containing the desired product, judging by TLC analysis ($EtOH/NH_4OH/H_2O$, 5/3/1), were pooled and evaporated to dryness under reduced pressure. Compound **4** (42% yield) was further purified by HPLC using a C_{18} column (Alltech, 10×250 mm). The eluent was 1.5% acetonitrile in 50 mM triethylammonium acetate buffer, pH 6.8, and the flow rate was 5 mL/min. The retention time of **4** under these conditions was 8.2 min.
16. See Tsvetkov, Y. E.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **2000**, 889 for an alternate method to make UDP-sugar.
17. All compounds afforded satisfactory NMR (^1H, ^{13}C, ^{31}P, and COSY) and high-resolution mass spectral characterization. Spectra data of **4** (bistriethylammonium salt): ^1H NMR (500 MHz, D_2O) δ 1.30 (18H, t, J=7.5 Hz, Et_3N-*Me*), 3.23 (12H, q, J=7.5, Et_3N-CH_2), 3.72 (1H, dd, J=13.0, 6.0, 5-H), 3.82 (1H, dd, J=12.5, 3.0, 5-H), 3.94 (1H, ddd, J=7.5, 6.0, 3.0, 4-H), 4.16 (2H, m, 2-H, 3-H), 4.22 (1H, ddd, J=11.5, 6.0, 3.0, 5′-H), 4.26 (1H, ddd, J=12.0, 5.0, 2.0, 5′-H), 4.30 (1H, m, 4′-H), 4.40 (2H, m, 2′-H, 3′-H), 5.66 (1H, dd, J=6.0, 3.5, 1-H), 6.00 (1H, d, J=8.5, 5″-H), 6.01 (1H, d, J=4.5, 1′-H), 7.99 (1H, d, J=8.5, 6″-H); ^{13}C NMR (125 MHz, D_2O) δ 9.8 (Et_3N-*Me*), 48.2 (Et_3N-CH_2), 63.8 (C-5), 66.5 (d, J=6, C-5′), 71.3 (C-2′), 74.8 (C-3), 75.4 (C-3′), 78.3 (d, J=8, C-2), 84.3 (C-4), 84.9 (d, J=9, C-4′), 89.9 (C-1′), 99.3 (d, J=6, C-1), 104.2 (C-5″), 143.2 (C-6″), 153.4 (C-2″), 167.8 (C-4″); ^{31}P NMR (121 MHz, D_2O) δ −12.3 (d, J=20.8), −10.9 (d,

$J=20.8$). Negative ion high-resolution ESI-MS calcd for $C_{14}H_{21}N_2O_{16}P_2$ $[M-H]^-$ 535.0372, found m/z 535.0370.
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19. Spectra data of **5** (bistriethylammonium salt): ^{1}H NMR (500 MHz, D_2O) δ 1.30 (18H, br, Et_3N-*Me*), 3.23 (12H, br, Et_3N-CH_2), 3.74 (1H, dd, $J=12.5$, 2.0 Hz, 5-H), 3.83 (1H, dt, $J=10.0$, 3.5, 2-H), 3.95 (1H, dd, $J=10.0$, 3.5, 3-H), 4.05 (1H, m, 4-H), 4.15 (1H, d, $J=12.5$, 5-H), 4.22 (1H, ddd, $J=12.0$, 5.5, 2.5, 5′-H), 4.27 (1H, ddd, $J=12.0$, 4.5, 2.5, 5′-H), 4.31 (1H, quint, $J=3.0$, 4′-H), 4.40 (2H, m, 2′-H, 3′-H), 5.63 (1H, dd, $J=7.5$, 3.5, 1-H), 6.00 (1H, d, $J=8.5$, 5″-H), 6.01 (1H, d, $J=5.0$, 1′-H), 7.99 (1H, d, $J=8.0$, 6″-H); ^{13}C NMR (75 MHz, D_2O) δ 9.8 (Et_3N-*Me*), 48.3 (Et_3N-CH_2), 65.6 (C-5), 66.5 (d, $J=6$, C-5′), 70.0 (d, $J=9$, C-2), 70.3 (C-3), 70.5 (C-4), 71.3 (C-2′), 75.4 (C-3′), 84.9 (d, $J=9$, C-4′), 89.9 (C-1′), 97.8 (d, $J=6$, C-1), 104.3 (C-5″), 143.2 (C-6″), 153.4 (C-2″), 167.9 (C-4″); ^{31}P NMR (121 MHz, D_2O) δ −12.3 (d, $J=21$), −10.7 (d, $J=21$). Negative ion high-resolution ESI-MS calcd for $C_{14}H_{21}N_2O_{16}P_2$ $[M-H]^-$ 535.0372, found m/z 535.0340.
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