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Note

Facile synthesis of 6-deoxy-L-*arabino*-hexulose (L-rhamnulose) 1-phosphate via regioselective phosphorylation of the unprotected sugar

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There is currently an intense interest in the utilization of enzymic approaches for the synthesis of carbohydrates and their analogues [1,2]. The aldolases comprise one class of enzymes that have been successfully used in this regard, particularly in the synthesis of iminoalditols [3,4], carbohydrate analogues having potent inhibitory activities against various glycohydrolytic enzymes. In the course of our work on the exploitation of L-rhamnulose-1-phosphate aldolase (EC 4.1.2.19) in carbohydrate synthesis, it was necessary to assay the activity of this aldolase from bacterial sources and therefore its substrate, 6-deoxy-L-*arabino*-hexulose (L-rhamnulose) 1-phosphate (**4**) was required.

Phosphate **4** has previously been prepared by enzymic phosphorylation of L-rhamnulose [5,6] (**2**). However, to use this enzymic method, L-rhamnulokinase is required. Furthermore, a tedious purification of the product from the enzymic incubation mixture is necessary, including the separation of L-rhamnulose 1-phosphate from various nucleotides. We have found it difficult to prepare sufficient quantities of L-rhamnulose 1-phosphate by this method. Very recently Wong and coworkers [7] reported the synthesis of phosphate **4** by aldol condensation of L-lactaldehyde and dihydroxyacetone phosphate, catalyzed by crude L-rhamnulose-1-phosphate aldolase from *E. coli* K-40. However, the crude enzyme we obtained from *E. coli* (ATCC 8739) contained L-rhamnulose-1-phosphate aldolase together with fructose-1,6-diphosphate aldolase, and so cannot be directly used as a catalyst in the aldol addition without further purification. This has led us to investigate the chemical synthesis of **4**.

To minimize the number of synthetic steps involved, an approach utilizing selective phosphorylation of the primary hydroxyl group in L-rhamnulose (**2**)

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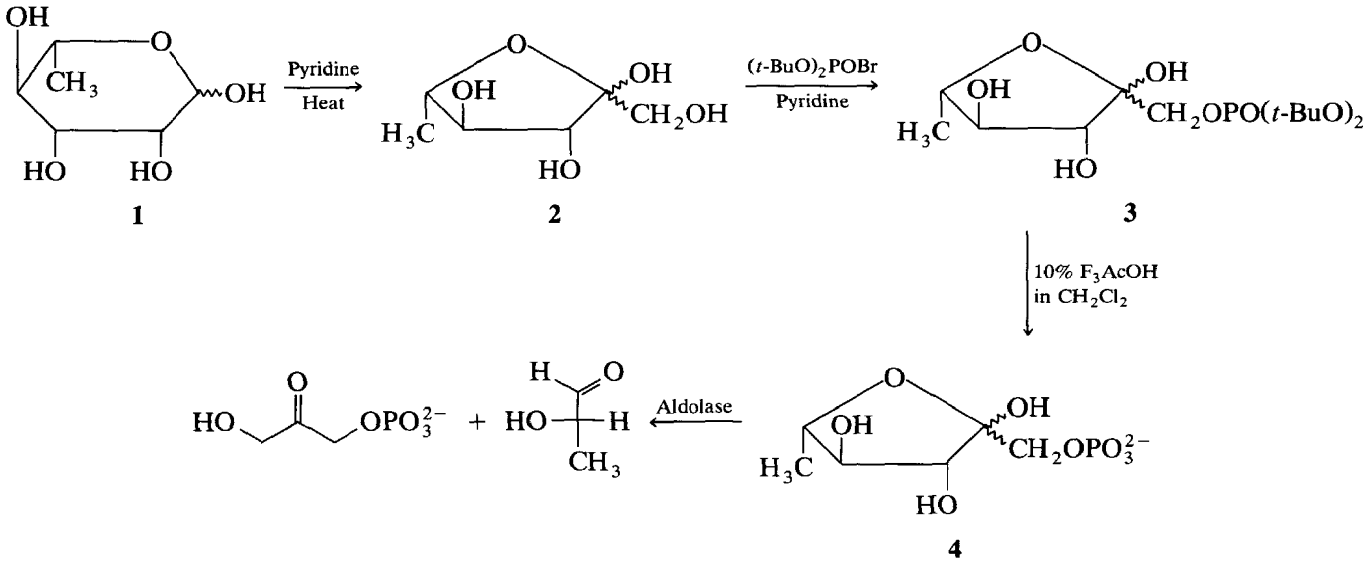
OH
O
CH3
OH
HO
HO
1
Pyridine
Heat
OH
OH
CH2OH
H3C
HO
2
(t-BuO)2POBr
Pyridine
OH
OH
CH2OPO(t-BuO)2
H3C
HO
3
10% F3AcOH
in CH2Cl2
HO
OPO3 2−
+
HO
H
CH3
Aldolase
OH
OH
CH2OPO3 2−
H3C
HO
4

Scheme 1.

instead of routes involving various protection and deprotection sequences was investigated. Commercial diphenyl phosphorochloridate [8] and readily accessible di(2-*tert*-butylphenyl) phosphorochloridate [9] have been used in the regiospecific phosphorylation of primary hydroxyl groups in selected carbohydrates and nucleosides, but, in our hands, phosphorylation of L-rhamnulose with these two reagents under various conditions (−40 to 20°; various ratios of reagent to pyridine) afforded only complex mixtures of products.

Phosphorylation using di-*tert*-butyl phosphorobromidate [10] was examined next. It has been reported that this reagent exhibits preferential reactivity with primary alcohols in pyridine solution and, in addition, the *tert*-butyl protecting groups may be effectively cleaved under mild acidic conditions [10]. The highest yield (20–30%) of **3** was obtained by the slow addition of this reagent to a solution of L-rhamnulose in dry pyridine at −40° under nitrogen, followed by warming to room temperature over 2 h. Surprisingly, increasing the ratio of di-*tert*-butyl phosphorobromidate to L-rhamnulose did not improve the yield, but interfered with the purification of product **3**. The *tert*-butyl protecting groups in **3** were removed using 10% trifluoroacetic acid in dichloromethane at room temperature to give the desired phosphate **4**.

The position of the phosphate ester group at the primary position on L-rhamnulose was confirmed spectroscopically as well as by the fact that the optical rotation of the barium salt of **4** is consistent with the reported value for the enzymic product [5], and its R_F value on paper chromatography [6] is identical with that of a sample prepared enzymically. Furthermore, synthetically prepared L-rhamnulose 1-phosphate was used successfully as a substrate for the measurement of L-rhamnulose-1-phosphate aldolase activity [11].

The facile preparation of L-rhamnulose 1-phosphate by selective phosphorylation with di-*tert*-butyl phosphorobromidate followed by deprotection should facilitate studies on the isolation and biochemical properties of L-rhamnulose-1-phosphate aldolase and encourage the enzyme's use in the synthesis of novel L-rhamnulose analogues.

EXPERIMENTAL

General methods.—Optical rotations were measured with a JASCO DIP-360 digital polarimeter. NMR spectra were recorded with a Bruker AC-200 or a Bruker AM-250 spectrometer. Chemical shifts are reported as δ values, referenced to internal Me_4Si for ^1H and ^{13}C shifts in $CDCl_3$, to external trimethyl phosphate for ^{31}P shifts in $CDCl_3$, to external sodium 3-(trimethylsilyl)-1-propanesulfonate for ^1H and ^{13}C shifts in D_2O, and to external 85% phosphoric acid for ^{31}P shifts in D_2O. Pyridine was distilled from CaH_2 and stored over 4A molecular sieves. L-Rhamnulose (**2**) was readily prepared by the isomerization of L-rhamnose (**1**) in boiling pyridine [12].

6-Deoxy-L-arabino-hexulose (L-rhamnulose) 1-phosphate bis(cyclohexylammonium) salt.—Di-*tert*-butyl phosphorobromidate * (0.74 g, 2.7 mmol) was slowly added to a solution of L-rhamnulose (0.37 g, 2.3 mmol) in anhyd pyridine (20 mL) with stirring at −40° under N_2. The mixture was then slowly warmed to room temperature. After 2 h, MeOH (1 mL) was added to decompose the unreacted reagent. The solvent was evaporated at room temperature under vacuum. The residue was chromatographed on silica gel using 10:1 (v/v) EtOAc–MeOH as eluent. Compound **3** was obtained as an oil (0.19 g, 23%). NMR revealed it to be a mixture of two anomers in a ratio of 1:4; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 1.45 (s, 18 H, *t*-Bu), 1.46 (d, 3 H, *J* 6.0 Hz, Me), 4.25–3.65 (m, 5 H), 4.65 (br, O*H*); ^{13}C-NMR ($CDCl_3$, 62.9 MHz): minor anomer, δ 19.4 (C-6), 29.9 (d, $J_{C,P}$ 3.4 Hz, CH_3 of *t*-Bu), 67.3 (C-1), 81.3, 81.5, 82.3, 84.0 (d, $J_{C,P}$ 7.4 Hz, CCH_3 of *t*-Bu), 105.1 (d, $J_{C,P}$ 6.5 Hz, C-2); major anomer, δ 19.9 (C-6), 29.9 (d, $J_{C,P}$ 3.4 Hz, CH_3 of *t*-Bu), 68.6 (d, $J_{C,P}$ 6.8 Hz, C-1), 77.0, 77.5, 81.0, 83.8 (d, $J_{C,P}$ 7.4 Hz, CCH_3 of *t*-Bu), 99.9 (d, $J_{C,P}$ 9.4 Hz, C-2); ^{31}P-NMR ($CDCl_3$, 81.0 MHz): minor anomer, δ −8.5; major anomer, δ −9.9.

The oily triester (**3**) was dissolved in 10% trifluoroacetic acid in CH_2Cl_2 (5 mL). After 0.5 h at room temperature, toluene (10 mL) was added to the mixture and the solvent was evaporated at room temperature under vacuum. The residue was dissolved in water (1 mL) and the solution was adjusted to pH 7.5 with satd aq $Ba(OH)_2$. Anhydrous EtOH (4 mL) was added and the mixture was kept at 0° for 1 h. The white precipitate was collected by centrifugation, washed with EtOH, and dried under vacuum to give the barium salt of **4** (0.072 g, 36%); $[\alpha]_D^{22}$ + 8.7° (*c* 0.56, HOAc); lit. [5] $[\alpha]_D^{30}$ + 8.9°; ^{1}H-NMR (D_2O, 250 MHz): δ 1.2 (d, 3 H, *J* 4.0 Hz, H-6), 3.4–4.3 (m, 5 H); ^{13}C-NMR (D_2O, 62.9 MHz): minor anomer, δ 20.5 (C-6), 67.9 (C-1), 80.5, 83.7, 85.0, 106.7 (C-2); major anomer, δ 21.6 (C-6), 69.3 (C-1), 78.9, 79.3, 82.0, 103.3 (C-2); ^{31}P-NMR (D_2O, 101.3 MHz): minor anomer, δ 7.0; major anomer, δ 6.4. This barium salt was passed through a Dowex 50W-X8 (H^+) column using water as eluent. The fractions containing compound **4** were lyophilized to dryness. The residue was dissolved in EtOH (1 mL) and cyclohexylamine (90 μL, 0.79 mmol) was added. The mixture was kept at 0° for 1 h and the precipitate was then collected by filtration, washed with diethyl ether, and dried under vacuum to give the bis(cyclohexylammonium) salt (0.08 g).

Anal. Calcd for $C_{18}H_{39}N_2O_8P$: C, 48.86; H, 8.88; N, 6.33; P, 7.00; Found: C, 48.82; H, 8.90; N, 6.46; P, 6.95.

Enzymic assay was performed by the measurement of dihydroxyacetone phosphate formation, as reported by Chiu [11].

* Di-*tert*-butyl phosphorobromidate is stable for three months when stored at −70° in a desiccator over KOH.

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