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Carbohydrate Research 339 (2004) 1215–1217
Carbohydrate RESEARCH

Note

A convenient preparation of 1,2,3-tri-*O*-acetyl-β-D-ribofuranose by enzymatic regioselective 5-O-deacetylation of the peracetylated ribofuranose ☆

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Received 26 October 2003; accepted 21 January 2004

This paper is dedicated to Professor Leroy B. Townsend on his 70th birthday

Abstract—The lipase from *Candida rugosa* (Sigma) was used to catalyze the regioselective deacetylation at the 5-position of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose on a preparative scale. Enzymatic deacetylation provides a convenient one-step preparation of 1,2,3-tri-*O*-acetyl-β-D-ribofuranose.

Keywords: Lipase; Regioselective; Deacetylation; Deprotection; Ribofuranose

Enzyme-catalyzed deacetylation reactions have been successfully utilized in selective deacetylation of acetylated sugars. Cleavage of the primary ester group is preferred in methyl glycosides.[2–11] However, a range of results were observed when deacetylation was carried out with peracetylated sugars.[3,9–12] Furthermore, most of the studies focused on hexopyranose derivatives; thus, pentofuranose derivatives received less attention.[3,4,13] In this investigation, a commercially available lipase (EC 3.1.1.3) from *Candida rugosa* (formerly *Candida cylindracea*) (CRL, type VII, Sigma L 1754) was selected for our study. This enzyme has previously been shown to regioselectively deacetylate the primary hydroxy group of peracetylated methyl hexopyranosides and methyl pentofuranosides.[2,3,5,7,9] Although several examples have demonstrated that CRL can also be used to deacetylate the acetyl ester on the primary hydroxy group of peracetylated hexopyranoses,[9,12] its action on the ribofuranose derivatives has been only rarely studied. A recent report from the Guisan's group shows that purified CRL immobilized on octyl agarose catalyzes the hydrolysis of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**1**) in sodium phosphate buffer to yield 1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**2**) in 47% yield. Although the conversion is fairly effective, the reaction was limited to the analytical scale and required purification and immobilization of the enzyme.[13] Herein, we would like to report an efficient enzyme-catalyzed regioselective deacetylation to prepare 1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**2**) on a preparative scale.

A variety of reaction conditions, including different enzyme/substrate ratios, solvent systems, reaction temperatures and reaction times were investigated. The reaction did not proceed when the reaction was performed in organic solvents such as methanol, ethanol, or hexane. However, the deacetylated product **2** was obtained in low yield with byproducts when the reaction was carried out in water at room temperature. By changing the solvent system to a mixture of 0.1 M pH 7.0 sodium phosphate buffer and DMF in a ratio of 9:1,[3] the desired product **2** was obtained in 75–80%

☆ Part X in a series. For the previous paper, see Ref. 1.

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doi:10.1016/j.carres.2004.01.010

Table 1. Enzymatic deacetylation of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**1**)

Enzyme/substrate (w/w)	Solvent(s)	Temperature	Time (h)	Yield[a] (%)
1:1 to 2:1	H_2O	rt	2–24	<25
1:1	Buffer[b]/DMF = 9:1	37 °C	2	50
2:1	Buffer[b]/DMF = 9:1	rt	24	75
1.5:1	Buffer[b]/DMF = 9:1	rt	24	80

[a] Isolated yields after flash column chromatography.
[b] 0.1 M sodium phosphate buffer, pH 7.0.

yield. It should be noted that the reaction was accelerated but accompanied with undesired byproducts when the reaction temperature was raised to 37 °C (Table 1).

The structural elucidation was carried out by intensive NMR studies. The ^{1}H and ^{13}C NMR spectra show that only one isomer was obtained. A singlet anomeric proton resonance suggests that the anomeric configuration is β-configuration based on Townsend's empirical rule.[14] The only D_2O-exchangeable proton (OH-5) shows correlations only with H-5 peaks from the COSY spectrum. In addition, the chemical shifts for the protons at the 5-position of the deacetylated product **2** appeared at 3.64 and 3.84 ppm, while the protons of the peracetylated ribofuranose **1** were at 4.15 and 4.38 ppm ($CDCl_3$). The tremendously upfield shifts indicated that the deacetylation was carried out at the 5-position. ^{1}H and ^{13}C NMR data are consistent with the data previously reported in the literature for the same compound obtained from chemical synthesis.[13,15,16]

This study has shown that 1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**2**) can be prepared effectively from the lipase-catalyzed deacetylation of the peracetylated ribofuranose (**1**) on a preparative scale. This partially acetylated sugar is an important building block for nucleoside or glycoside synthesis. Further studies of its applications are under investigation.

1. Experimental

1.1. General chemical procedures

NMR spectra were obtained with Varian Gemini-300 or Bruker DRX500 spectrometer. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter at 589 nm using a quartz cell with a 1 mL capacity and a 10 cm cell path length. Electrospray-ionization (ESI) mass spectra were recorded on a Micromass LCT mass spectrometer. Elemental analyses for C, H, and N were carried out on a Perkin–Elmer 240 elemental analyzer and were within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on E. Merck plates precoated with Silica Gel 60 containing fluorescent indicator. Compounds on thin-layer chromatography were visualized by dipping into 10% methanolic sulfuric acid, followed by charring on a hot plate. E. Merck Silica Gel 60 (230–400 mesh) was used for flash column chromatography as has been described.[17] Evaporations were carried out with a rotary evaporator under reduced pressure (water aspirator) with the bath temperature below 50 °C unless specified otherwise. Materials obtained from commercial suppliers were used without further purification.

1.2. 1,2,3-Tri-*O*-acetyl-β-D-ribofuranose (2)

A solution of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**1**, 3.82 g, 12 mmol) in *N*,*N*-dimethylformamide (30 mL) was diluted with 0.1 M pH 7.0 sodium phosphate buffer (270 mL), and then *C. rugosa* lipase [5.93 g, enzyme/substrate = 1.5:1 (w/w)] was added. The mixture was slowly stirred at room temperature and followed by TLC. After 24 h the reaction was completed, and the reaction mixture was extracted with EtOAc (3 × 500 mL). The organic layer was washed with satd NaCl solution, dried with anhyd $MgSO_4$, and evaporated to dryness. The resulting residue was purified by flash column chromatography (4:6 hexane–EtOAc) to give 1,2,3-tri-*O*-acetyl-β-D-ribofuranose [**2**, oil, 2.65 g, 9.59 mmol, 80%, R_f 0.32 (Et_2O), 0.25 (4:6 hexane–EtOAc)]. $[\alpha]_D^{20}$ −10.44° (*c* 2.14, EtOH) [lit.[18] $[\alpha]_D^{10}$ 3.3° (*c* 0.756, EtOH)]; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.13 (s, 1H, 1-H), 5.39 (dd, 1H, *J* 4.9, 7.0 Hz, 3-H), 5.33 (d, 1H, *J* 4.9 Hz, 2-H), 4.26–4.22 (m, 1H, 4-H), 3.87–3.80 (m, 1H, 5-H), 3.68–3.60 (m, 1H, 5-H), 2.26–2.22 (m, 1H, D_2O exchangeable, 5-OH), 2.12 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.06 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.6, 170.0, 169.8, 98.7 (1-CH), 82.9 (4-CH), 75.1 (2-CH), 70.4 (3-CH), 62.4 (5-CH_2), 21.6, 21.1 (2 × CH_3); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 6.01 (s, 1H, 1-H), 5.26–5.22 (m, 2H, 2-H, 3-H), 5.02 (t, 1H, D_2O exchangeable, *J* 5.7 Hz, 5-OH), 4.12 (dd, 1H, *J* 4.2, 10.0 Hz, 4-H), 3.57 (dt, 1H, *J* 4.8, 11.8 Hz, 5-H), 3.49–3.45 (m, 1H, 5-H), 2.09 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.04 (s, 3H, CH_3); ESIMS *m*/*z* 299 (100) (M+Na); HRMS calcd for $C_{11}H_{16}O_8$·Na (M+Na): 299.0743. Found: 299.0739. Anal. Calcd for $C_{11}H_{16}O_8$: C, 47.83; H, 5.84. Found: C, 47.80; H, 5.88.

Acknowledgements

This investigation was supported by a research grant (NSC90-2321-B002-002) from the National Science Council of Taiwan.

References

1. Chen, G. S.; Chen, C.-S.; Chien, T.-C.; Yeh, J.-Y.; Kuo, C.-C.; Talekar, R. S.; Chern, J.-W. *Nucleos. Nucleot. Nucl. Acids*, in press.
2. Sweers, H. M.; Wong, C. H. *J. Am. Chem. Soc.* **1986**, *108*, 6421–6422.
3. Hennen, W. J.; Sweers, H. M.; Wang, Y. F.; Wong, C. H. *J. Org. Chem.* **1988**, *53*, 4939–4945.
4. Moravcová, J.; Kefurt, K.; Hladuvková, R.; Staněk, J. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1619–1629.
5. Hsiao, K.-F.; Yang, F.-L.; Wu, S.-H.; Wang, K.-T. *Biotechnol. Lett.* **1995**, *17*, 963–968.
6. Kodera, Y.; Sakurai, K.; Satoh, Y.; Uemura, T.; Kaneda, Y.; Nishimura, H.; Hiroto, M.; Matsushima, A.; Inada, Y. *Biotechnol. Lett.* **1998**, *20*, 177–180.
7. Hsiao, K.-F.; Lin, H.-J.; Leu, D.-L.; Wu, S.-H.; Wang, K.-T. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1629–1632.
8. Chen, H.-P.; Hsiao, K.-F.; Wu, S.-H.; Wang, K.-T. *Biotechnol. Lett.* **1995**, *17*, 305–308.
9. Terreni, M.; Salvetti, R.; Linati, L.; Fernandez-Lafuente, R.; Fernandez-Lorente, G.; Bastida, A.; Guisan, J. M. *Carbohydr. Res.* **2002**, *337*, 1615–1621.
10. Horrobin, T.; Tran, C. H.; Crout, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1069–1080.
11. Copinet, A.; Couturier, Y.; Prudhomme, J. C. *Biotechnol. Lett.* **1998**, *20*, 447–450.
12. Bastida, A.; Fernandez-Lafuente, R.; Fernandez-Lorente, G.; Guisan, J. M.; Pagani, G.; Terreni, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 633–636.
13. Fernandez-Lorente, G.; Palomo, J. M.; Cocca, J.; Mateo, C.; Moro, P.; Terreni, M.; Fernandez-Lafuente, R.; Guisan, J. M. *Tetrahedron* **2003**, *59*, 5705–5711.
14. Townsend, L. B. Nuclear Magnetic Resonance Spectroscopy in the Study of Nucleic Acid Components and Certain Related Derivatives. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Wiley: New York, 1973; Vol. 2, pp 267–398, Chapter 7; See also: Stevens, J. D.; Fletcher, H. G., Jr. *J. Org. Chem.* **1968**, *33*, 1799–1805.
15. Beigelman, L. N.; Mikhailov, S. N. *Carbohydr. Res.* **1990**, *203*, 324–329.
16. Ebner, M.; Stütz, A. E. *Carbohydr. Res.* **1998**, *305*, 331–336.
17. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
18. Kanazawa, T.; Sato, T. *Nippon Kagaku Zasshi* **1959**, *80*, 200–203; Kanazawa, T.; Sato, T. *Chem. Abstr.* **1961**, 6385.