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Enzymic Hydrolysis of Acetylated D-Ribose Derivatives*

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Tetra-*O*-acetyl-β-D-ribopyranose (**1**) and tetra-*O*-acetyl-β-D-ribofuranose (**2**) were submitted to hydrolysis catalyzed by several hydrolytic enzymes. The best results were achieved with the esterase from rabbit serum (RS), porcine pancreatic lipase (PPL) and porcine liver esterase (PLE). Regioselective enzymic deacetylation of the ester group on the anomeric centre of **1** produced in all three cases 2,3,4-tri-*O*-acetyl-β-D-ribopyranose (**3**) as the main product. Tetraacetate **2** underwent enzymic hydrolysis followed most probably by acetyl migration and the mixture of two triacetates 1,2,5-tri-*O*-acetyl-β-D-ribofuranose (**4**) and 1,3,5-tri-*O*-acetyl-β-D-ribofuranose (**5**) was isolated. Structures of prepared compounds were established by ^{1}H and ^{13}C NMR spectroscopies.

Keywords
• Acetylated ribopyranose and ribofuranose
• hydrolases
• enzymic hydrolysis

INTRODUCTION

Various applications of esterases, lipases and proteases as catalysts have shown promising results in the synthesis of acylated carbohydrates.[1,2] In comparison to chemical methods[3–7] enzymic reactions have some significant advantages, one of them being the regioselectivity and/or chemoselectivity of such transformations.

The regioselective access to a specific hydroxyl function is an important target in carbohydrate chemistry. Enzyme catalyzed deacetylations have been effective in removing one or more acetyl groups from peracetylated monosaccharides.[1,2,8] The rate of hydrolysis of the primary esters is often comparable to that of the anomeric position. Selective enzymic hydrolysis of other secondary esters in peracetylated derivatives has shown only limited success due to the comparable chemical reactivity of secondary hydroxyl groups.[1]

Enzyme catalyzed reactions leading to regioselective and/or chemoselective protection and deprotection of various acylated pyranoses can also be successfully extended to acylated furanoses. Thus, acylated substrates with five membered sugar rings can also undergo hydrolysis with a pronounced degree of regioselectivity.[1,2]

In continuation of our work on enzymic regioselective and/or chemoselective deacylations of acylated monosaccharide[8–16] and nucleoside[17] derivatives with esterases isolated from mammalian sera and commercially available enzymes we wish to report on the enzymic regioselective hydrolysis of peracetylated derivatives of D-ribose.

EXPERIMENTAL

General

Tetra-*O*-acetyl-β-D-ribopyranose (**1**) was purchased from Aldrich and tetra-*O*-acetyl-β-D-ribofuranose (**2**) from Ega-Chemie. Lipase from porcine pancreas (PPL, 27 U/mg), butyrylcholinesterase from equine serum (BChE, 17.6 U/mg),

* Dedicated to Professor Nikola Kallay on the occasion of his 65th birthday.

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TABLE I. Enzyme catalyzed hydrolysis of tetraacetate **1**.

1 mg	enzyme mg or μL	phosphate buffer mL	reaction time h	yield of **3** %	recovery of **1** %
100	RS / 500 μL	7.5	8	45	53
50	PPL / 6 mg	5	3	37	46
50	PLE / 0.5 mg	5	1.5	40	58
50	BChE / 9 mg	5	3	– [(a)]	–
10	α-chymotrypsin / 1 mg	1	24	– [(b)]	–
10	subtilisin / 2.5 mg	1	48	– [(b)]	–

[(a)] unselective hydrolysis; products were not isolated
[(b)] to slow and unselective hydrolysis; products were not isolated

proteinase from *Bacillus subtilis* (subtilisin, 13.1 U/mg) and α-chymotrypsin from bovine pancreas (61 U/mg) were obtained from Fluka/BioChemika. Esterase from porcine liver (PLE, 41 U/mg) was purchased from Sigma. Esterase fraction from rabbit serum (RS) was partially purified as previously described.[18]

Phosphate buffered (0.1 mol dm^{-3}, pH = 7) solutions of substrates were incubated with enzymes at 37 °C. Control reactions were performed parallel to every enzymic hydrolysis and contained all reactants except the enzyme. pH values of reaction mixtures were monitored periodically by pH-indicator paper Neutralit (pH = 5.5–9.0) from Merck and adjusted with 0.1 mol dm^{-3} NaOH. Reactions were stopped by adding EtOH, the solvents evaporated under reduced pressure and the products subjected to column chromatography. In the case of RS the precipitated proteins were removed by centrifugation before evaporation.

All solvents were reagent grade and distilled before use. Column chromatography was performed on silica gel (Merck) and TLC monitoring on Merck silica gel plates (60 F 254; 0.25 mm) with solvent A, EtOAc-C_6H_6 (2:1). Visualisation was effected by charring with H_2SO_4. Optical rotations were measured at ≈20 °C using the Schmidt + Haensch Polartronic NH8.

^{1}H and ^{13}C NMR spectra (300 MHz, $CDCl_3$) were recorded with a Bruker AV300 spectrometer. Chemical shifts (δ/ppm) are referred to TMS. Elemental analyses were performed in the Microanalytical laboratory at the Ruđer Bošković Institute, Zagreb, Croatia.

*Enzymic Deacetylation of Tetra-O-acetyl-β-D-ribopyranose (**1**)*

Reaction conditions and yields for enzymic hydrolyses of tetraacetate **1** are listed in Table I. Column chromatography of the residues after hydrolysis with RS, PPL and PLE gave 2,3,4-tri-*O*-acetyl-β-D-ribopyranose (**3**) as an oil; $[\alpha]_D$ –7.9° (c 0.53, $CHCl_3$); $R_f \approx 0.37$ (solvent A); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.08 (s, 3H, *O*Ac), 2.11 (s, 3H, *O*Ac), 2.12 (s, 3H, *O*Ac), 3.81 (dd, 1H, J = 6.86 Hz, J = 12.01 Hz, H-5b), 4.09 (dd, 1H, J = 3.83 Hz, J = 12.06 Hz, H-5a), 4.95 (dd, J = 3.25 Hz, J = 5.24 Hz, 1H, H-2), 5.02–5.14 (m, 2H, H-1, H-4), 5.53 (app t, 1H, J = 3.14 Hz, J = 3.23 Hz, H-3). ^{13}C NMR ($CDCl_3$) δ/ppm: 20.66, 20.74, 20.78 (***C***H_3CO, 2-*O*Ac, 3-*O*Ac, 4-*O*Ac), 61.54 (***C***-5), 66.71, 66.91, 69.79 (***C***-2, ***C***-3, ***C***-4), 93.21 (***C***-1), 169.76, 169.86, 170.29 (***C***=O, 2-*O*Ac, 3-*O*Ac, 4-*O*Ac). *Anal.* Calcd. for $C_{11}H_{16}O_8$ (M_r = 276.08): C 47.83, H 5.84 %; found: C 47.69, H 5.56 %. In all cases starting unreacted compound **1** was recovered as well (Table I).

Conventional pivaloylation of **3** (31 mg, 0.112 mmol) with pivaloyl chloride/pyridine, followed by column chromatography (solvent A) gave 2,3,4-tri-*O*-acetyl-1-*O*-pivaloyl--β-D-ribopyranose (**6**) as an oil (31 mg, 78 %); $[\alpha]_D$ –31.3° (c 0.16, $CHCl_3$); $R_f \approx 0.66$ (solvent A); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.23 (s, 9H, 1-*O*Piv), 2.09 (s, 3H, *O*Ac), 2.10 (s, 3H, *O*Ac), 2.11 (s, 3H, *O*Ac), 3.92 (dd, 1H, J = 6.27 Hz, J = 12.25 Hz, H-5b), 4.01 (dd, 1H, J = 3.47 Hz, J = 12.32 Hz, H-5a), 5.09 (dd, J = 3.47 Hz, J = 5.08 Hz, 1H, H-2), 5.16 (m, 1H, H-4), 5.49 (app t, 1H, J = 3.24 Hz, J = 3.29 Hz, H-3), 6.00 (d, 1H, J = 5.13 Hz, H-1). ^{13}C NMR ($CDCl_3$) δ/ppm: 20.62, 20.70, 20.75 (***C***H_3CO, 2-*O*Ac, 3-*O*Ac, 4-*O*Ac), 26.86 ((***C***H_3)$_3$CCO, 1-*O*Piv), 38.95 ((CH$_3$)$_3$***C***CO, 1-*O*Piv), 62.66 (***C***-5), 66.21, 66.60, 67.32 (***C***-2, ***C***-3, ***C***-4), 90.87 (***C***-1), 169.35, 169.52, 169.82 (***C***=O, 2-*O*Ac, 3-*O*Ac, 4-*O*Ac), 176.25 (***C***=O, 1-*O*Piv). *Anal.* Calcd. for $C_{11}H_{16}O_8$ (M_r = 360.31): C 53.33, H 6.71 %; found: C 52.95, H 6.47 %.

*Enzymic Deacetylation of Tetra-O-acetyl-β-D-ribofuranose (**2**)*

Reaction conditions and yields for enzymic hydrolyses of tetraacetate **2** are listed in Table II. Column chromatography of the residues after hydrolysis with RS, PPL (**4** : **5** = 1 : 1.2) and PLE gave in all three cases a mixture of 1,2,5-tri-*O*-acetyl-β-D-ribofuranose (**4**) and 1,3,5-tri-*O*-acetyl-β-D-ribofuranose (**5**) as an oil; $R_f \approx 0.35$ (solvent A); ^{1}H NMR ($CDCl_3$) δ/ppm: 2.09 (s, 3H, *O*Ac), 2.11 (s, 3H, *O*Ac), 2.18 (s, 3H, *O*Ac), 4.13–4.21 (m, 2H, H-4, H-5b), 4.33–4.44 (m, 2H, H-3, H-5a), 5.11–5.14 (m, 1H, H-2), 6.13 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ/ppm: 20.87, 20.96, 20.96 (***C***H_3CO, 1-*O*Ac, 2-*O*Ac, 5-*O*Ac), 64.04 (***C***-5), 73.65 (***C***-3), 76.06 (***C***-2), 81.42 (***C***-4), 98.06 (***C***-1), 168.85, 170.23, 170.71 (***C***=O, 1-*O*Ac, 2-*O*Ac, 5-*O*Ac) for the 1,2,5-triacetate **4** and ^{1}H NMR ($CDCl_3$) δ/ppm: 2.10 (s, 3H, *O*Ac), 2.10 (s, 3H, *O*Ac), 2.17 (s, 3H, *O*Ac), 4.13–4.21 (m, 1H, H-5b), 4.33–4.44 (m, 3H, H-2, H-4, H-5a), 5.11–5.14 (m, 1H,

TABLE II. Enzyme catalyzed hydrolysis of tetraacetate **2**

2 mg	enzyme mg or μL	phosphate buffer mL	reaction time h	yield of **4** + **5** %	recovery of **2** %
50	RS / 1000 μL	2.5	1.75	33	65
50	PPL / 6 mg	5	24	30	68
100	PLE / 1 mg	7.5	1.5	45	52
50	BChE / 9 mg	5	2.5	– [a]	–
10	α-chymotrypsin / 1 mg	1	48	– [b]	–
10	subtilisin / 2.5 mg	1	48	– [b]	–

[a] unselective hydrolysis; products were not isolated
[b] to slow and unselective hydrolysis; products were not isolated

H-3), 6.10 (s, 1H, H-1); ^{13}C NMR ($CDCl_3$) δ/ppm: 20.52, 20.58, 20.61 (***C***H_3CO, 1-*O*Ac, 3-*O*Ac, 5-*O*Ac), 63.94 (***C***-5), 70.44 (***C***-2), 72.86 (***C***-3), 79.38 (***C***-4), 100.87 (***C***-1), 169.43, 170.05, 170.42 (***C***=O, 1-*O*Ac, 3-*O*Ac, 5-*O*Ac) for the 1,3,5-triacetate **5**. In all cases starting unreacted compound **2** was recovered as well (Table II).

Conventional pivaloylation of the mixture of triacetates **4** and **5** obtained after hydrolysis with PPL (30 mg, 0.11 mmol) with pivaloyl chloride/pyridine, followed by column chromatography (solvent A) produced a mixture of 1,2,5-tri-*O*-acetyl-3-*O*-pivaloyl-β-D-ribofuranose (**7**) and 1,3,5-tri-*O*-acetyl-2-*O*-pivaloyl-β-D-ribofuranose (**8**) in the ratio of 1 : 1.4 as an oil (36 mg, 91 %); $R_f \approx 0.66$ (solvent A); ^{1}H NMR ($CDCl_3$) δ/ppm: 1.20 (s, 9H', *O*Piv'), 1.24 (s, 9H, *O*Piv), 2.055 (s, 3H', *O*Ac'), 2.097 (s, 3H', *O*Ac'), 2.114 (s, 3H', *O*Ac'), 2.099 (s, 3H, *O*Ac), 2.104 (s, 3H, *O*Ac), 2.108 (s, 3H, *O*Ac), 4.12–4.19 (m, 1H+1H', H-5b+H-5b'), 4.22–4.39 (m, 2H+2H', H-4+H-5a, H-4'+H-5a'), 5.29–5.38 (m, 2H+2H', H-3+H-2, H-3'+H-2',), 6.15 (s, 1H, H-1), 6.17 (s, 1H', H-1'); ^{13}C NMR ($CDCl_3$) δ/ppm: 20.41–21.01 (6 ***C***H_3CO, *O*Ac, *O*Ac'); 26.94, 27.04 ((***C***H_3)$_3$CCO, *O*Piv, *O*Piv'); 38.81, 38.96 ((CH$_3$)$_3$***C***CO, *O*Piv, *O*Piv'); 63.71, 63.88 (***C***-5, ***C***-5'); 70.66, 70.71 (***C***-3, ***C***-3'); 73.79, 74.13 (***C***-2, ***C***-2'); 79.26, 79.76 (***C***-4, ***C***-4'); 98.22, 98.27 (***C***-1, ***C***-1'); 168.98–170.41 (6 ***C***=O, *O*Ac, *O*Ac'); 176.83 (2 ***C***=O, *O*Piv, *O*Piv').

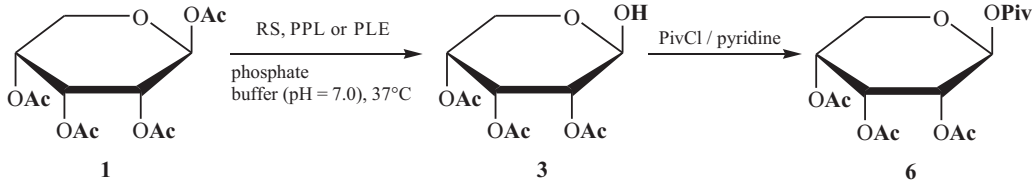
RESULTS AND DISCUSSION

Tetraacetates **1** and **2** were submitted to hydrolysis catalyzed by various commercially available enzymes (PPL, PLE, BChE, α-chymotrypsin and subtilisin) and the esterase fraction which was partially purified from rabbit serum (RS).[18] Reactions were performed in phosphate buffered (pH = 7) solutions at 37 °C without the addition of organic cosolvents since satisfactory solubility of starting materials in these conditions was achieved. To exclude the possibility of spontaneous hydrolysis and migrations effected by the reaction medium, control reactions were carried out parallel to every enzymic hydrolysis.

The reactions were monitored by TLC and stopped when optimal conversions were observed. The products were further purified by column chromatography on silica gel.

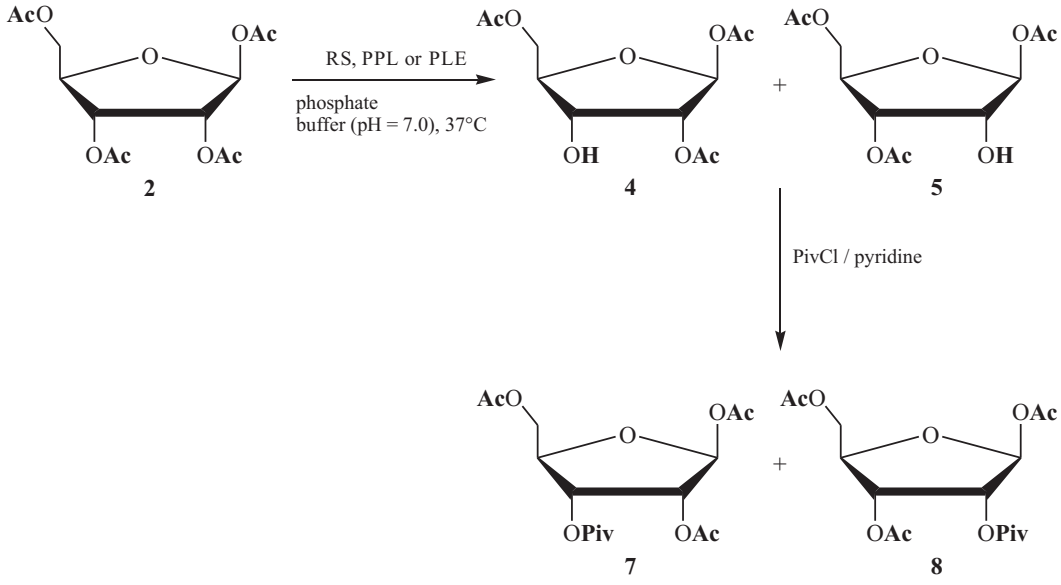
Regioselective deacetylations of both tetraacetates were observed when RS, PLE and PPL were used as catalysts. Even partially purified esterase fraction from rabbit serum (RS) proved to be adequately efficient and above all cheap source of enzyme activity when compared to commercial catalysts used in the hydrolysis of tetraacetates **1** and **2**. Reactions in which BChE, α-chymotrypsin and subtilisin were used as possible catalysts were to slow and/or unselective to allow successful determination of structures and yields.

Acylated pyranose derivative **1** was regioselectively hydrolyzed at the anomeric position and 2,3,4-tri-*O*-acetyl-β-D-ribopyranose (**3**) was isolated (Table I, Scheme 1). This was to be expected since there is no primary ester group in **1** which in sugar rings is of comparable reactivity with the ester on the anomeric centre. This result is in accord with enzyme catalyzed deprotection of other peracetylated pyranoses where anomeric position is the one preferentially deprotected.[1,2] Hydrolysis product **3** was pivaloylated in the next step with the purpose of addi-



1 **3** **6**

Scheme 1



Scheme 2.

tional identification, to give the 2,3,4-tri-*O*-acetyl-1-*O*-pivaloyl-β-D-ribopyranose **6** (Scheme 1).

The furanose tetraacetate **2** was hydrolyzed in all three reactions with RS, PPL, and PLE as catalysts (Table II, Scheme 2). The result of these reactions was the mixture of triacetates **4** and **5**. Such a mixture may be the result of regioselective hydrolysis of 2- and 3-acetates or it may be the result of the hydrolysis of more reactive 1- or 5-acetyls, followed by intramolecular migrations of acetates from positions 2 and 3 in the sugar molecule toward the liberated hydroxyl (1-OH or 5-OH). The disadvantage of the acetyls as a common protecting group in carbohydrate chemistry is that they are prone to intramolecular migrations even under mild reaction conditions.[4,19,20] It was previously reported that acetyl group at the anomeric centre in the furanose tetraacetate **2** was removed with complete selectivity in a reaction catalyzed by the lipase from *Aspergillus niger*.[21] Our previous experiments with radiolabelled peracetylated glucopyranose derivative showed that the preferential hydrolysis of the 1-acetate occurs catalyzed by RS, and no subsequent migration was observed.[8] When the position 1 was blocked by an ether group (*O*Me) hydrolysis of the acetate on the most reactive primary hydroxyl group (6-OH) occurred as expected, followed by intramolecular transesterifications leading to products with deprotected secondary 3- and 4-OH groups. The ratio of these products was dependent on reaction times. These results indicated that migrations of acetyl groups in the sugar ring occur with acetyls migrating from secondary groups towards the primary hydroxyl (6-OH) liberated in the enzyme catalyzed reaction but no migration occurs towards the hemiacetal hydroxyl (1-OH) liberated as well in the enzyme catalyzed reaction. Therefore, it is possible that in the furanose acetate **2** 2-OH and 3-OH products are formed as a result of direct hydrolytic reactions. Based on previous results it is more probable that under the reaction conditions used, and with chosen enzymes, 5-acetate is hydrolyzed enzymically followed by the migration of secondary acetyls towards the liberated primary 5-OH. This could also explain the lack of acetyl migration in the peracetylated ribopyranose derivative which has no primary acetyls to be possibly hydrolyzed in the first step leading to further migrations of acetyls.

Pivaloylation of the resulting mixture produced a mixture of 1,2,5-tri-*O*-acetyl-3-*O*-pivaloyl-β-D-ribofuranose (**7**) and 1,3,5-tri-*O*-acetyl-2-*O*-pivaloyl-β-D-ribofuranose (**8**) respectively (Scheme 2). It might be possible that acetyl migrations occur during pivaloylation procedures. In that case the ratio of **7** : **8** would not reflect the ratio of the acetylated compounds **4** : **5** which were the result of the hydrolysis reaction. We, therefore, calculated the ratio of **4** : **5** from the ^{1}H NMR spectrum of the mixture which was formed in the PPL catalyzed hydrolysis of **2**. The ratio of **7** : **8** which was the result of the pivaloylation of the mixture of hydrolyzed products, **4** and **5**, was calculated as well using ^{1}H NMR spectral data. No significant difference in the ratios of **4** : **5** and **7** : **8** was found indicating that possible migrations of acetyls may primarily occur during enzyme catalyzed hydrolysis reactions.

All compounds were identified by NMR spectroscopies. Chemical shifts and splitting pattern of ring protons were in satisfactory accordance with previously reported data for similar structures.[22,23]

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SAŽETAK

Enzimska hidroliza acetiliranih derivata D-riboze

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Tetra-*O*-acetil-β-D-ribopiranoza (**1**) i tetra-*O*-acetil-β-D-ribofuranoza (**2**) podvrgnuti su hidrolizama kataliziranima hidrolitičkim enzimima. Najbolji su rezultati postignuti uporabom esteraze iz seruma kunića (RS), esteraze iz svinjske jetre (PLE) i lipaze iz svinjske gušterače (PPL). Sva tri navedena enzima kataliziraju regioselektivnu deacetilaciju estera na anomernom centru u molekuli spoja **1** te je dobivena 2,3,4-tri-*O*-acetil-β-D-ribopiranoza (**3**). Tetraacetat **2** podliježe regioselektivnoj hidrolizi popraćenoj migracijom acetila te je kao konačan produkt izolirana smjesa dvaju triacetata 1,2,5-tri-*O*-acetil-β-D-ribofuranoze (**4**) i 1,3,5-tri-*O*-acetil-β-D-ribofuranoze (**5**). Strukture svih pripravljenih spojeva utvrđene su ^{1}H i ^{13}C NMR spektroskopijama.