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ENZYMATIC REGIO- AND DIASTEREOSELECTIVE HYDROLYSIS OF PERACETYLATED GLYCEROL- AND ERYTHRITOL-β-GLUCOSIDES

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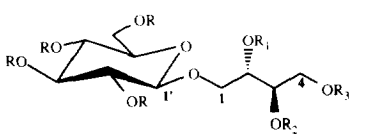
Abstract. Diastereomeric O-β-glucosides at the primary carbons of glycerol and erythritol have been subjected as their peracetates to enzymatic hydrolysis by PFL. Only acetyl groups of the aglycones are hydrolyzed, the diastereoselectivity being very high on the erythritol derivatives and fair on the glycerol derivatives. The chemical synthesis of (2*S*,3*R*)-1-(O-β-D-glucopyranosyl)-butane-2,3,4-triol heptaacetate is also reported.

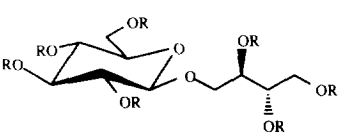
It is well known that β-glycosidases catalyze the transglycosidation reaction on polyols with a great selectivity towards primary hydroxy groups, the relative ratio of glycosidation at the secondary hydroxy groups depending upon the complexity of the substrates[1,2]. On the other hand, β-glycosidases show very poor stereoselectivity, generally leading to ca. 1:1 mixtures during transglycosidation of racemic alchools and polyols. We report here that the desired diastereoselectivity can be obtained by lipase catalyzed hydrolysis of the peracetylated diastereomeric polyol β–glucosides. The overall sequence involves the use of two enzymes, a β-glucosidase and a lipase, and results in the recovery of diastereomerically enriched or pure polyol glucosides. These two enzymes have been recently used in a reverse order (e.g. first the lipase and then the glucosidase) for the synthesis of aleppotrioloside, a naturally occurring glucoside[3].

As model substrates, glycerol- and erythritol-β-glucosides were selected. These compounds were recently obtained as mixtures of diastereomers by glucosidation using a crude homogenate of the thermophilic archeon *Sulfolobus solfataricus* containing a β-glycosidase activity[4,5].

Erythritol-β-glucoside was obtained as a 1:1 mixture of the two diastereomers **1** and **2** by glucosidation of *cis*-2-butene-1,4-diol followed by OsO_4 treatment[5] and acetylated to afford **3** and **4**. The mixture of **3** and **4** was hydrolyzed with *Pseudomonas fluorescens* lipase (phosphate buffer - acetone[6]) monitoring the reaction by TLC. After 24 h only ca. 20% of the product was hydrolyzed; chromatographic separation of the reaction

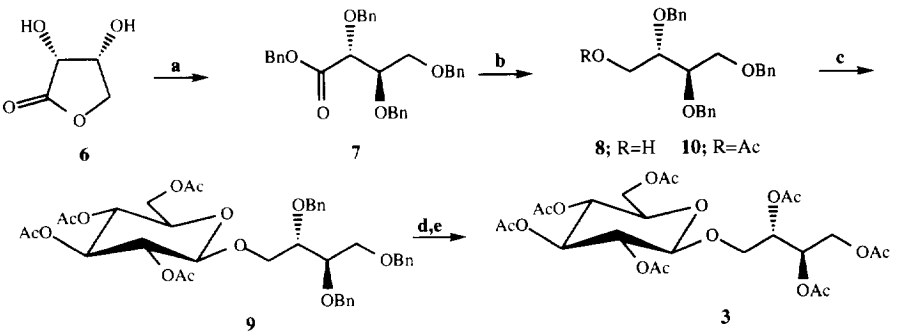


1: R=R_1=R_2=R_3=H
3: R=R_1=R_2=R_3=Ac
5; R=R_1=R_2=Ac R_3=H
11; R=R_1=R_3=Ac R_2=H
12; R=R_3=Ac R_1=R_2=H



2; R=H
4; R=Ac

Scheme 1



a) $C_6H_5CH_2Br$ (8 equiv.), Ag_2O (4 equiv.), Et_2O, 72 h r.t. (49%); b) $LiAlH_4$, Et_2O (85%); c) AgOTf, Ag_2CO_3, α-D-glucopyranosyl bromide tetracetate, CH_2Cl_2 (33%), d) H_2, Pd/C, EtOH (91%); e) Ac_2O, Py (quant.).

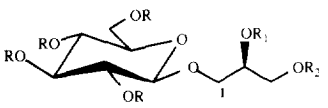
mixture yielded a compound deacetylated at the primary carbon of the aglycone (**5**), as the sole hydrolysis product, together with recovered starting material enriched in one of the two diastereomers. The diastereomeric purity of **5** was evident from its ^1H- and ^{13}C-NMR spectra[7] and was confirmed by acetylation which afforded diastereomerically pure **3**, whose stereochemistry was established by synthesis, as reported in Scheme 1.

D-Erythronolactone (**6**) was opened by reaction with benzyl bromide, using a slightly modified previously reported procedure[8], to afford the benzylester **7** which, in turn, was reduced with $LiAlH_4$ to the alcohol **8**. Reaction of **8** with acetobromoglucose using silver triflate as promoter[9] afforded the β-glucoside **9** in 33% yield, the major reaction product being the transesterification compound **10** (39.5%). Hydrogenolysis of **9** and acetylation afforded a compound which proved to be identical with **3**[10].

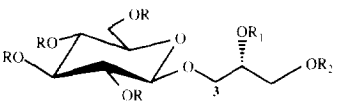
The unreacted product of the above enzymatic hydrolysis, enriched in the diastereomer **4**, was again reacted in the presence of the enzyme for 45 h. Chromatographic separation of the reaction mixture afforded unreacted **4**[11], slightly contaminated by ca. 10% of **3**, and a complex mixture of hydrolysis products among which again the compound **5** and, in addition, **11** and **12** were isolated by repeated chromatographic steps and identified by a combination of NMR and MS data[12]; also in this case, acetylation of **11** and **12** afforded pure **3**. Following the first removal of the acetyl group from the primary carbon of the polyol chain (**5**), which remains the most abundant product, ester hydrolysis apparently occurs at carbons 2 and 3 (**11**, **12**). It should be considered that the hydrolysis products at carbons 2 and 3 could arise in part by acetyl group migration towards the C-4, since it has been reported that this kind of migrations could occur in the enzymatic hydrolysis conditions[13]. It is noteworthy that PFL hydrolysis does not affect the ester groups on the glucose moiety, since on other glucosides having aglycones which do not display ester functions the hydrolysis of the acetyl groups on glucose does occur[14]. Finally, as it is evident from the structures of the hydrolysis products (**5**, **11**, **12**) the enzyme always hydrolyzes the diastereomer having the 2*S*, 3*R*-stereochemistry in the polyol chain, leaving unchanged the diastereomer with the 2*R*, 3*S*-stereochemistry.

Glucosidation of glycerol[4] afforded a 1:1 mixture of 1-O-*sn*- and 3-O-*sn*-β-glucosyl glycerol (**13** and **14**; 90%) together with a minor amount (10%) of the 2-O-β-glucosyl glycerol (**15**). The mixture was then acetylated and subjected to PFL hydrolysis[6], following the course of the reaction by HPLC and stopping the reaction when one of the two major diastereomers was reduced to a minimum in the HPLC trace (11h). Usual work-up and chromatographic separation afforded unreacted compounds **16** (hexaacetyllilioside D[15]) and **17**

(not separated) and a ca. 1:1 mixture of the product of hydrolysis at C-2 (**19**)[16] and at C-1 (**20**)[17]. The stereochemistry and the diastereoselectivity of the hydrolysis was ascertained by acetylation which afforded **18** (lilioside C hexaacetate[18]) from both compounds as the major product, contaminated by different amounts of **16** (d.e. >95% from **19**; d.e. ~70% from **20**).

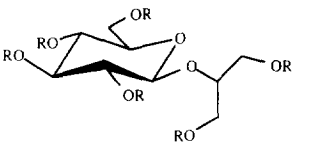


13: R=R$_1$=R$_2$=H
16: R=R$_1$=R$_2$=Ac



14: R=R$_1$=R$_2$=H
18: R=R$_1$=R$_2$=Ac

19: R=R$_2$=Ac R$_1$=H
20: R=R$_1$=Ac R$_2$=H



15: R=H
17: R=Ac

PFL hydrolyzes preferentially the 3-O-*sn*-β-glucosyl glycerol peracetate (**18**) both at C-1 and C-2 of the glycerol moiety. This result is not unexepected, since the enzyme has, *inter alia*, the 1,2-diacyl-3-glycosyl-*sn*-glycerides as natural substrates. It is noteworthy that also in this case no hydrolysis products of the acetyl groups in the sugar moiety were isolated, nor the 2-O-β-glucosyl glycerol peracetate (**17**) was affected by the enzyme. This latter has been previously found unaffected by *Pseudomonas cepacia* lipase, while it is hydrolyzed at the glycerol moiety by *Candida antarctica* lipase with a satisfactory diastereoselectivity[19].

The peracetylated derivatives of glycerol- and erythritol-β-glucosides examined in the present paper show the same behaviour as far as the regioselectivity of the hydrolysis with PFL is concerned, since in both cases the sugar moiety results unaffected, while the diastereoselectivity is very high in the case of the erythritol derivatives and fair in the glycerol derivatives.

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6. Naoshima, Y.; Kamezawa, M.; Tachibana, H.; Munakata, Y.; Fujita, T.; Kihara, K.; Raku, T. *J. Chem. Soc. Perkin Trans. I* **1993**, 557-561. 148 mg of **3** + **4** were hydrolyzed with 99 mg of PFL (Fluka cat. n° 62312; 42 U/mg) in 2.7 ml phosphate buffer (pH 7.02) and 2 ml of acetone. 227 mg of **16** + **18** were hydrolyzed with 86 mg of PFL in 2.4 ml phosphate buffer and 1.8 ml of acetone.
7. **5**: ^{1}H-NMR, δ (CDCl$_3$) 5.26 (q, J=10, 5.1, H-3), 5.18 (t, J=9.5, H-3'), 5.08 (t, J=9.6, H-4'), 5.05 (dt, J=9.7, 4.1, H-2), 4.98 (dd, J=9.4,7.9, H-2'), 4.55 (d, J=8, H-1'), 4.26 (dd, J=12.3, 4.8, H-6'a), 4.15 (dd, J=12.3, 2.4, H-6'b), 3.95 (dd, J=11.3, 5.3, H-4a), 3.8 (dd, J=11.3, 4.6, H-4b), 3.83 (m, H-1a), 3.72 (m, H-5'), 3.70 (m, H-1b); acetyl methyls: 2.091, 2.087, 2.081, 2.051, 2.021, 2.001. ^{13}C-NMR, δ 170.2, 169.4, 100.6, 72.8, 72.6 , 71.9, 71.1, 70.2, 68.2, 67.3, 61.8, 60.9, 20.9, 20.8, 20.7, 20.6. FABMS, m/z 537 $(M+H)^+$.

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9. Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C13.
10. **3**: M.p. 143-44°.$[\alpha]_D$-14.3 (c=1.15, $CHCl_3$). ^{1}H-NMR, δ ($CDCl_3$) 5.21 (m, H-2 + H-3),5.17 (t, J=9.4, H-3'), 5.04 (t, J=9.8, H-4'), 4.93 (dd, J=9.3, 7.9, H-2'), 4.53 (d, J=7.8, H-1'), 4.27 (dd, J=12.2, 3.0, H-4a), 4.21 (dd, J=12.3, 4.8, H-6'a), 4.15 (dd, J=12.2, 5.8, H-4b), 4.11 (dd, J=12.3, 2.3, H-6'b), 3.90 (dd, J=11.4, 5.2, H-1a), 3.73 (dd, J=11.4, 4.1, H-1b), 3.7 (m, H-5'); acetyl methyls: 2.06, 2.04 (x2), 2.02 (x2), 2.00, 1.97. ^{13}C NMR, δ 170.5, 170.4, 170.1, 169.6 (x2), 169.3, 169.1 , 100.3, 72.6, 71.9, 71.0, 69.9, 69.6, 68.3, 66.7, 61.8, 61.7, 20.7, 20.6, 20.5. FABMS, m/z 579 $(M+H)^+$.
11. **4**: ^{1}H-NMR, δ ($CDCl_3$) 5.23 (m, H-2 + H-3), 5.18 (t, J=9.6, H-3'), 5.07 (t, J=9.7, H-4'), 4.98 (dd, J=9.5, 8.1, H-2'), 4.51 (d, J=7.8, H-1'), 4.28 (dd, J=12.2, 2.7, H-4a), 4.25 (dd, J=12.3, 4.7, H-4b), 4.18 (dd, J=12.2, 5.6, H-6'a), 4.13 (dd, J=12.3, 2.2, H-6'b), 4.01 (dd, J=11.2, 3.8, H-1a), 3.69 (m, H-5'), 3.64 (dd, J=11.2, 5.4, H-1b); acetyl methyls: 2.09, 2.08, 2.07, 2.06 (x2), 2.02, 1.99. ^{13}C NMR, δ 170.6, 170.5, 170.2, 169.7, 169.3, 100.5, 72.7, 71.9, 70.9, 70.1, 69.6, 68.3, 66.8, 61.8 (x2), 20.8, 20.7, 20.5. FABMS, m/z 579 $(M+H)^+$.
12. **11**:^{1}H-NMR, δ 5.20 (t, J= 9.5, H-3'), 5.07 (t, J=9.7, H-4'), 5.00 (dd, J=9.5, 8.0, H-2'), 4.94 (dt, J=7.5, 3.7) 4.54 (d, J=8.0, H-1'), 4.20 (m, H-6'a + H-4a), 4.17 (m, H-6'b + H-4b), 4.08 (dd, J=11.4, 3.8 H-1a), 4.07 (H-3, overlapped with H-1a), 3.87 (dd, J=11.4, 3.6, H-1b), 3.72 (m, H-5'); acetyl methyls: 2.10, 2.09, 2.07, 2.04, 2.02, 2.01. FABMS, m/z 537 $(M+H)^+$. **12**: ^{1}H-NMR, δ ($CDCl_3$) 5.20 (t, J=9.5, H-3'), 5.05 (t, J=9.7, H-4'), 4.99 (dd, J=9.7, 8.0, H-2'), 4.55 (d, J=8.0, H-1'), 4.31 (dd, J=11.8, 3.1, H-4a), 4.22 (dd, J=11.8, 6.2, H-4b), 4.19 (m, H-6'), 3.97 (dd, J=10.9, 5.8, H-1a), 3.88 (dd, J=10.9, 3.0, H-1b), 3.80 (m, H-3), 3.73 (m, H-2 + H-5'); acetyl methyls: 2.11, 2.09, 2.06, 2.03, 2.01.^{13}C-NMR, δ 171.6, 170.7, 170.2, 169.5, 169.4, 101.3, 72.5, 72.2, 71.9, 71.2, 70.5, 70.4, 68.3, 65.8, 62.0, 20.9, 20.7, 20.6. FABMS, m/z 495 $(M+H)^+$. A product of hydrolysis of **3** at C-2 was also isolated in admixture with **11** and its structure was tentatively derived by interpretation of the ^{1}H-NMR spectrum.
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16. **19**: ^{1}H-NMR, δ ($CDCl_3$) 5.22 (t, J=9.5, H-3'), 5.07 (t, J=9.6, H-4'), 5.00 (dd, J=9.6, 8.0, H-2'), 4.55 (d, J=7.9, H-1'), 4.22 (dd, J=12.3, 5.1, H-6'a), 4.18 (dd, J=12.4, 2.7, H-6'b), 4.11 (d, J=5.1, H-1), 4.00 (q, J=5.0), 3.78 (d, J=4.9, H-3), 3.72 (m, H-5'); acetyl methyls: 2.09, 2.08, 2.06, 2.03, 2.00. ^{13}C-NMR, δ 170.6, 170.2, 169.3, 101.4, 72.6, 72.2, 72.0, 71.2, 68.9, 68.3, 65.2, 61.9, 20.8, 20.6. FABMS, m/z 465 $(M+H)^+$.
17. **20**: ^{1}H-NMR, δ ($CDCl_3$) 5.21 (t, J=9.5, H-3'), 5.06 (t, J=9.6, H-4'), 4.99 (dd, J=9.6, 8.0, H-2'), 4.98 (H-2, overlapped with H-2'), 4.54 (d, J=8.0, H-1'), 4.23 (dd, J=12.3, 4.9, H-6'a), 4.17 (dd, J=12.3, 2.4, H-6'b), 3.97 (dd, J=10.9, 4.5, H-3a), 3.78 (m, H-3b + H-1a), 3.72 (m, H-1b + H-5'); acetyl methyls: 2.09, 2.08, 2.05, 2.02, 2.00. ^{13}C-NMR, δ 170.5, 170.2, 169.4, 101.1, 72.8, 72.6, 72.0, 71.2, 68.3, 67.9, 61.6, 61.4, 21.0, 20.6. FABMS, m/z 465 $(M+H)^+$.
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