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Lipase-catalysed deacetylation of botryodiplodin acetate

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Abstract—Enantioselective deacetylation of α-(±)-botryodiplodin acetate was successfully accomplished by means of lipase PS to afford (+)-botryodiplodin and α-(+)-botryodiplodin acetate with high enantiomeric excesses. Enzyme-mediated transesterification of the acetylated molecule with *n*-butanol, as well as its hydrolysis in several organic solvents, are also reported. The CD spectra of (+)-botryodiplodin and α-(+)-botryodiplodin acetate are also presented.

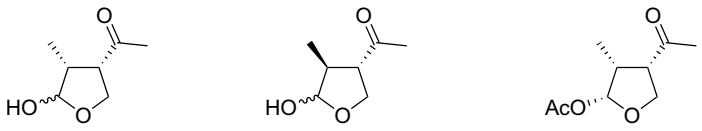
1. Introduction

Acylated hemiacetals are known to undergo enzymatic transformations by lipases at the anomeric position. For example, the α anomer of 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose was selectively hydrolysed at the acetyl group at C-1 by *Pseudomonas fluorescens* lipase (PFL),[1] while its β anomer was hydrolysed by *Candida antarctica* lipase.[2] Similarly, enantiomerically pure *cis*- and *trans*-hemiacetals of 2-bromo-5-hydroxypentanal were obtained by *C. antarctica* lipase B (CALB)-catalysed kinetic resolution of the corresponding acetates,[3] and acetylated hemiacetals of substituted cyclopropanone were also kinetically resolved by lipase.[4] On the other hand, enzymatic kinetic resolution of 6-acetyloxy-2*H*-pyran-3(6*H*)-one was achieved by lipase PS transesterification in hexane/*n*-butanol.[5]

Botryodiplodin (−)-**1** (Fig. 1) is a natural mycotoxin[6] with wide biological activities.[7] Its absolute configuration is (2*R*/2*S*,3*R*,4*S*), as it is a 2:3 mixture of α-(2*R*) and β-(2*S*) anomers. Its nonnatural diastereomer *epi*-botryodiplodin (−)-**2**,[6] also present as a 1:2 mixture of α and β anomers, has the (2*R*/2*S*,3*S*,4*S*)-configuration. We have recently proposed a new stereoselective synthesis of the acetate **3** of the α anomer of botryodiplodin in racemic form,[8,9] and we report here on its lipase-catalysed kinetic resolution.

2. Results and discussion

Initially, in order to verify the stability of the botryodiplodin acetate (±)-**3** in water, it was set aside in phosphate buffer at pH 7.4. After 2.5 h, it was transformed into botryodiplodin (±)-**1** for 40% (HRGC) and after 23 h, botryodiplodin (±)-**1** thus formed was partially (20%) converted into its diastereomer (±)-**2** (Fig. 1). The easy hydrolysis of β-botryodiplodin acetate in water at room temperature had already been observed.[10]



botryodiplodin (–)-**1** *epi*botryodiplodin (–)-**2** α-botryodiplodin acetate (±)-**3**

Figure 1. Botryodiplodin (−)-**1**, *epi*-botryodiplodin (−)-**2** and α-botryodiplodin acetate (±)-**3**.

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In spite of these results, we attempted a lipase PS catalysed hydrolysis[11] of compound **3**, to verify whether the enzymatic hydrolysis could be faster than the chemical hydrolysis. After 1 h 17 min, the corresponding α-botryodiplodin acetate (+)-**3**, $[\alpha]_D^{25} = +22.2$ (*c* 0.18, $CHCl_3$), 33% ee, and botryodiplodin (+)-**1**, $[\alpha]_D^{25} = +19.5$ (*c* 0.22, $CHCl_3$), lit.[6b] $[\alpha]_D^{25} = -69$ (*c* 0.85, $CHCl_3$), lit.[6c] $[\alpha]_D^{25} = -68$ (*c* 0.35, MeOH), 18% ee (60% conversion), were recovered. The enantiomeric excesses were determined by chiral HRGC.[6c,12]

The α-botryodiplodin acetate (±)-**3** was then subjected to lipase PS-catalysed deacetylation reaction with *n*-butanol[5] and MeOH[3] in organic solvent. The main results are shown in Table 1.

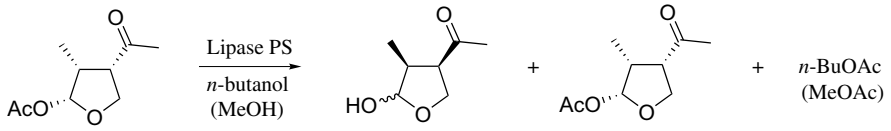
The conditions proposed by Parve et al.[3] for the enzymatic deacetylation of hemiacetals of α-bromo-ω-hydroxyaldehydes with *C. antarctica* lipase B, namely, MeOH in $CHCl_3$, gave the lowest selectivity ($E = 7$), when used with lipase PS, while the highest activity ($E > 200$) was observed using *n*-butanol as the alcohol and toluene as the organic solvent. However, although under these conditions the enantiomeric ratio was high, the reaction rates were low, so we decided to carry out the hydrolysis of α-(±)-**3** in both non-anhydrous organic solvents and in a biphasic system.[15] The best results are reported in Table 2.

In toluene, the presence of *n*-butanol had no effect, while in DME the selectivity was higher without adding *n*-butanol ($E = 175$ vs 86; Table 2, entry 1 and Table 1, entry 1) but with a longer reaction time, while in the biphasic system DME/buffer, the reaction was faster but the selectivity was lower. The best conditions were found to be diisopropyl ether, as the reaction proceeded faster (20 h) and with high enantioselectivity ($E > 200$; Table 2, entry 4).

In $CDCl_3$ the ^1H NMR spectrum[6b,c] of botryodiplodin (+)-**1** (ratio of the α and β anomers, 2:3; the attributions will be given later) showed both anomeric protons at 5.18, as a singlet for the β anomer and a doublet of doublets for the α anomer (0.4H, $J_1 = 12.1$ Hz, $J_2 = 4.8$ Hz) (Fig. 2a). Addition of a few drops of deuteriated benzene allowed the separation of the anomeric protons, the dd of the α anomer being shifted to 5.10 ppm and the singlet of the β anomer to 4.97 (Fig. 2b). In $CDCl_3$, their respective OH signals resonated at 4.87 ppm as a doublet (0.4H, $J = 12.1$ Hz) and at 2.77 ppm (0.6H) as a broad singlet (Fig. 2a). When benzene was added, their resonance positions moved to 4.76 and 2.21 ppm, respectively, this latter signal being overlapped with H-3 of the α anomer (Fig. 2b).

By the addition of D_2O to the sample containing C_6D_6, the OH signal of the β-isomer at 2.21 ppm rapidly disappeared,

Table 1. Lipase PS catalysed transesterification of α-botryodiplodin acetate (±)-**3**



α-(±)-**3** (+)-**1** α-(+)-**3**

Entry	Solvent	Time (d)	Conv.[b] (%)	ee[c] (%) (+)-**1**	ee[c] (%) (+)-**3**	*E*[13]
1	DME[a]	7	47	94	84	86
2	Toluene[a]	7	51	95	99	>200
3	CH_2Cl_2[a]	8	51	85	88	35
4	MeOH/$CHCl_3$[3]	8	17	73	15	7

[a] Conditions: botryodiplodin acetate **3** (0.06 g, 0.32 mmol), 2 mL of organic solvent, *n*-butanol (0.15 mL, 1.6 mmol), lipase PS (0.05 g).
[b] Calculated values, Ref. 14.
[c] Determined by chiral HRGC.

Table 2. Lipase PS catalysed deacetylation of botryodiplodin acetate α-(±)-**3**

Entry	Solvent	Time	Conv.[b] (%)	ee[c] (+)-**1** (%)	ee[c] (+)-**3** (%)	*E*[13]
1	DME[a]	13 d	46	97	84	175
2	DME/buffer[16]	15 h	52	90	99	99
3	Light petroleum[a]	5 d	51	93[d]	97	115
4	*i*-Pr_2O[a]	20 h	50	98	99	>200
5	THF[a]	4 d	53	87	97	59
6	Toluene[a]	5 d	49	97	93	>200

[a] Conditions: botryodiplodin acetate **1** (0.06 g, 0.32 mmol), 2 mL of organic solvent, lipase PS (0.05 g). At the end of the reaction lipase PS was filtered and washed with ethyl acetate.
[b] Calculated values, Ref. 14.
[c] Determined by chiral HRGC.
[d] Botryodiplodin was extracted with ethyl acetate because of its insolubility in light petroleum.

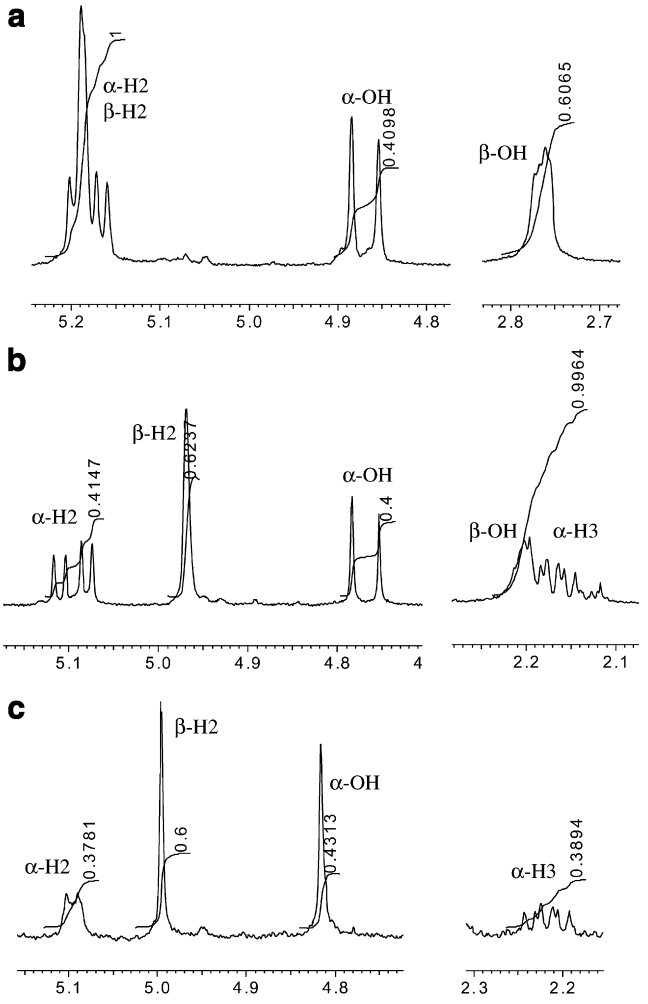


Figure 2. Part of ^{1}H NMR spectrum of botryodiplodin (+)-**1**: (a) in $CDCl_3$, (b) in $CDCl_3/C_6D_6$, (c) the same as (b) with a few drops of D_2O added.

whereas that of the α-isomer did not exchange with deuterium (Fig. 2c). Strangely enough, it became a singlet and as a consequence the double doublet at 5.10 ppm became a doublet. This result was interpreted in terms of the existence of an intramolecular hydrogen bond involving the OH group of the α anomer and the carbonyl group at C-4, which would lower its mobility. The presence of a small amount of D_2O would simply eliminate coupling but would not be sufficient enough to exchange with OH with the same velocity as for the β anomer.

Finally, the attribution of the descriptors α and β to the anomers of botryodiplodin **1** should be noted. This was made by means of NOE different measurements. Irradiation of the signal relative to H-3 at 2.45 ppm of (0.4H) caused an enhancement of the H-2 signal (dd, 4%), thus demonstrating that these two signals belonged to the α anomer (Fig. 3). Figure 3 also shows the possible orientation of the OH group which would account for both the high value observed for the $^3J_{H2-OH}$ coupling constant (12.1 Hz) and the scarce mobility of the hydroxy proton itself.

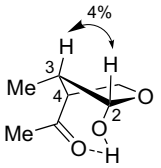


Figure 3. α-Anomer of botryodiplodin **1**.

The enantiomerically pure α anomer of botryodiplodin acetate **3** showed a positive specific rotation $\{[\alpha]_D^{25} = +87.9$ (c 1.65, $CHCl_3$)}, while the β anomer of botryodiplodin acetate **3** is reported[17] to have negative optical rotation power $\{[\alpha]_D = -104$ (c 0.09, $CHCl_3$)}, thus suggesting that the sign of the specific rotation does depend solely on the configuration at C-2. As to the CD spectra run in MeOH, the α anomer of botryodiplodin acetate (+)-**3** showed two negative Cotton effects at 218 and 283 nm ($\Delta\varepsilon_{218}$ −0.2, $\Delta\varepsilon_{283}$ −0.4), which can be attributed to the n→π* transition bands of the acetate group and the acetyl group, respectively. In the same solvent, botryodiplodin (+)-**1**, which has the carbonyl group as a single chromophore, showed a positive Cotton effect at 284 nm ($\Delta\varepsilon_{284}$ +0.9).

Notwithstanding botryodiplodin acetate **3** is more stable than botryodiplodin **1**, it is sensitive to acidic conditions and should be manipulated very carefully, and stored preferably in CH_2Cl_2 or $CHCl_3$ solution at −20 °C. Interestingly, at 4 °C the pure α anomer of botryodiplodin acetate **3** slowly converted into the more stable β anomer. This is rather surprising as acetylated hemiacetals are usually considered stable structures, at least in sugar chemistry. This unusual behaviour could be attributed to the easy removal of the acetyl group, owing to its leaving group ability, with generation of an ion pair, which would eventually lead to the more stable β anomer.

3. Conclusion

In conclusion we have successfully accomplished the enantioselective deacetylation of α-(±)-botryodiplodin acetate by means of lipase PS to afford (+)-botryodiplodin and α-(+)-botryodiplodin acetate with high enantiomeric excesses. This method, we have developed, demonstrates that an easy to hydrolyse acetal function can be resolved by lipase-catalysed hydrolysis in organic solvent or in a biphasic system and not only by a transesterification process. Studies on the enzymatic resolution of botryodiplodin acetate analogues (with alternative alkyl substituents to methyl at C-3) have been scheduled and will appear in due course.

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9. Compound α-**3**: Colourless oil, found: C, 58.0; H, 7.5. $C_9H_{14}O_4$ requires C, 58.05; H, 7.58; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (3H, d, J 7.3 Hz, CH_3C(3)), 2.08 (3H, s, CH_3COO), 2.28 (3H, s, CH_3C=O), 2.83 (1H, m, J 5.0, 7.2, 9.9 Hz, C(3)H), 3.33 (1H, ddd, J 5.9, 7.5, 9.9 Hz, C(4)H), 4.04 (1H, dd, J 7.5, 9.1 Hz, C(5)H), 4.42 (1H, dd, J 5.9, 9.1 Hz, C(5)H), 6.22 (1H, d, J 5.0 Hz, C(2)H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.6 (CH$_3$C(3)), 20.6 (CH$_3$COO), 30.0 (CH$_3$C=O), 40.3 (C(3)), 52.4 (C(4)), 68.7 (C(5)), 98.6 (C(2)), 169.8 (COO), 206.7 (C=O); IR (film): 1743 (C=O), 1713 (C=O) cm^{-1}, m/z (EI): 143 (1%, M$^+$−43), 127 (17), 126 (18), 98 (10), 83 (57), 55 (29), 43 (100).
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11. To a suspension of botryodiplodin acetate **3** (0.120 g, 0.5 mmol) in 15 mL of phosphate buffer (pH 7.4, 0.1 M), lipase PS (0.100 g, 3000 U) was added under vigorous stirring. The course of the reaction was monitored with a pH-STAT, with continuous addition of 1.0 M NaOH. At about 50% conversion, the reaction mixture was filtered, extracted with ether and ethyl acetate and the organic phase dried with anhydrous Na_2SO_4. After evaporation of the solvent, the crude was purified by flash chromatography (light petroleum/ethyl acetate 7/3).
12. Chiral HRGC analyses were run on a Shimadzu GC-14B instrument, the capillary column being a DiMePe β-cyclodextrin (25 m × 0.25 mm, carrier gas He, 110 KPa, split 1:50, 100 °C for 10 min, 3 °C/min until 150 °C) retention time: (+)-(2*R*,3*R*,4*S*)-**3**, 29.01 min and (−)-(2*S*,3*S*,4*R*)-**3**, 29.70 min; (−)-(2*R*/*S*,3*R*,4*S*)-**1**, 25.91 min and (+)-(2*R*/*S*,3*S*,4*R*)-**1**, 26.28 min, the two anomers having the same retention time.
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16. To a suspension of botryodiplodin acetate **3** (0.300 g, 1.6 mmol) in 10 mL of DME and 5 mL of phosphate buffer (pH 7.4, 0.1 M), lipase PS (0.250 g) was added under vigorous stirring. After 15 h, water was added and the reaction mixture was extracted with ether and ethyl acetate, and the organic phase dried with anhydrous Na_2SO_4. After evaporation of the solvent, the crude mixture was purified by flash chromatography to afford botryodiplodin acetate **3** (0.08 g, 27% yield, $[\alpha]_D^{25} = +87.9$ (c 1.65, $CHCl_3$), $[\alpha]_D^{25} = +100$ (c 0.35, MeOH), $\Delta\varepsilon_{218} = -0.2$ (MeOH), $\Delta\varepsilon_{283} = -0.4$ (MeOH), ee 99%) and botryodiplodin **1** (0.06 g, 26% yield, $[\alpha]_D^{25} = +65$ (c 0.85, $CHCl_3$), $[\alpha]_D^{25} = +56.5$ (c 0.55, MeOH), $\Delta\varepsilon_{284} = +0.9$ (MeOH), ee 90%).
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