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Regioselective enzymatic acylation of polyhydroxylated sesquiterpenoids

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Abstract

Regioselective acetylation of some protoilludane sesquiterpenes has been performed using a set of commercially available lipases. While esterification of the illudane "Illudine S" (**1**) gave the expected derivative mono-acetylated at the primary C(15)-OH, acylation of the protoilludane "Tsugicoline A" (**2**) and of its derivatives **3** and **4** gave different products depending on the lipase used. Preferential regioselective esterification of the less chemically reactive secondary C(6)-OH in **4** and of the tertiary C(5)-OH in **3** was obtained by action of *Candida rugosa* lipase and lipase A from *Aspergillus niger*, respectively.

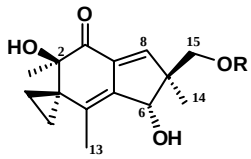
Keywords: Lipases; Regioselectivity; Enzymatic acylation; Basidiomycetes; Sesquiterpenes

1. Introduction

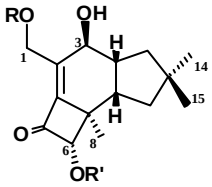
Basidiomycetes are a particular class of highly specialized and advanced fungi, their name deriving from "basidium", the club-shaped cell in which meiosis occurs. Sesquiterpenes are the major constituents of the secondary metabolites produced by these fungi and, specifically, a number of Basidiomycetes are known to produce sesquiterpenoids with an illudane (**1**), a protoilludane (**2**) or a sterpurane (**4**) skeleton [1]. The compounds belonging to these classes usually show antifungal and antibacterial activity, and are produced as chemical defences agents against predators.

As a part of our continuing search for new biologically active metabolites, we have recently described the isolation of the illudane sesquiterpene Illudine S (**1**), a potent cytotoxic compound, from a strain of *Omphalotus olearius* [2]. The protoilludane sesquiterpene Tsugicoline A (**2**) was also isolated by us from a strain of the fungus *Laurilia tsugicola* [3]. The anomalous presence of a carbonyl function in the four membered ring of **2** is the key to understand the reactivity of this interesting metabolite, and leads, for instance, to a Michael addition of NH_3 to the α,β unsaturated double bond, followed by intramolecular acetalization, to form compound **3** (2-amino-Tsugicoline E) or to an easy opening of the C(6)–C(7) bond to give **4** (a sterpurane derivative) [4].

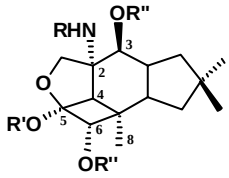
In order to extend the number of the available derivatives of these compounds, we undertook a comparative study of their enzymatic acylation. In this report the results obtained using a set of different lipases to catalyze the regioselective esterification of **1**–**4** are described.



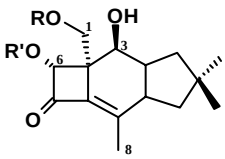
1 : R = H
1a : R = Ac



2 : R = R' = H
2a : R = Ac ; R' = H
2b : R = R' = Ac



3 : R = R' = R'' = H
3a : R = R' = Ac ; R'' = H
3b : R = R' = R'' = Ac



4 : R = R' = H
4a : R = Ac ; R' = H
4b : R = H ; R' = Ac
4c : R = R' = Ac

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2. Experimental

2.1. Materials and methods

Metabolites **1** and **2** were isolated from MPGA (malt–peptone–glucose–agar) cultures of *O. olearius* (ATCC 164.51) and *L. tsugicola* (CBS 248.51), respectively, as previously described [2,3], while compounds **3** and **4** were obtained from **2** [4]. Lipases from *Candida rugosa* and from porcine pancreas were purchased from Sigma. Lipase PS (from *Burkholderia cepacia*), lipase CE-5 (from *Humicola lanuginosa*) and lipase A (from *Aspergillus niger*) were obtained from Amano. *Chromabacterium viscosum* lipase was from Finnsugar. Novozym 435 (immobilized lipase B from *Candida antarctica*) and Lipozyme (immobilized lipase from *Mucor miehei*) were a gift from Novo-Nordisk. Lipase PS was adsorbed on celite following an optimized protocol [5]. All other chemicals were of analytical grade.

Thin-layer chromatography (TLC) was carried out on Merck precoated 60 F_{254} plates and detection was performed with the molybdate reagent ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 42 g; $Ce(SO_4)_2$, 2 g; conc. H_2SO_4, 62 ml made up to 1 l of deionized water). Flash column chromatography was performed on silica gel 60 (0.040–0.063 mm, Merck).

HPLC analyses were performed using a LiChroCART 125-4 Lichrospher Si60 5 μm (Merck)and a Jasco 880/PU instrument equipped with a Jasco 875 UV/VIS detector (reading was done at 254 nm). Compound **2**: eluent AcOEt:petroleum ether, 1:1; flow rate 2.0 ml/min; ret. time (min): **2b**, 1.4; **2a**, 2.5; **2**, 11.0. Compound **4**: eluent AcOEt:petroleum ether, 6:4; flow rate 2.0 ml/min; ret. time (min): **4c**, 1.7; **4b**, 3.4; **4a**, 7.1; **4**, 12.0.

NMR spectra were measured on a Bruker AC250L sprectometer operating at 250.1 MHz for ^{1}H NMR, using Me_4Si as internal standard. Mass spectra were obtained with a Finnigan-MATT TSQ70 spectrometer.

2.2. Acetylation of Illudine S (*1*)

Illudine S (1, 30 mg, 0.114 mmol) was dissolved in methyl *tert*-butyl ether (1 ml). Vinyl acetate (5 ml) was added together with 100 mg of lipase PS on celite. The reaction was conducted under shaking (250 rpm) at 45 °C and monitored by TLC (AcOEt:hexane 45:55). After 7 h, conversion was almost quantitative. The enzyme was filtered off, the solvent was evaporated and the residue purified by flash chromatography (eluent: AcOEt:hexane 3:7) to give 15-*O*-acetyl illudine S (**1a**, 23 mg, 0.075 mmol, 66% yield). Oil. EI-MS: *m*/*z* 306 (M^+), 246 ($M^+ - 60$), 231. Selected ^{1}H NMR data, δ ($CDCl_3$): 6.47 (1H, br s, H-8), 4.61 (^{1}H, s, H-6), 3.96 and 3.92 (1H each, d each, $J = 15.9$ Hz, CH_2-15), 2.05 (3H, s, CH_3CO), 1.68 (3H, s, CH_3-13), 1.38 (3H, s, CH_3-10), 1.22 (3H, s, CH_3-14).

Table 1
Regioselective acetylation of **2** catalyzed by different lipases[a]

Lipase	**2a**:**2b**[b]
Porcine pancreatic lipase	92:8
Lipase PS	16:84
Chromobacterium viscosum lipase	94:6
Lipase CE-5	96:4
Novozym 435	79:21
Lypozime	92:8
C. rugosa lipase	96:4
Lipase A	81:19

[a] Determined after 24 h, at 100% conversion.
[b] Evaluated by HPLC (see Section 2).

2.3. Lipases screening for the acetylation of Tsugicoline A (*2*)

Tsugicoline A (0.5 mg) was dissolved in 400 μl methyl *tert*-butyl ether containing 100 μl of vinyl acetate. A sample of 1 of the 8 different lipases was added (pancreatic lipase, 50 mg; lipase PS on celite, 25 mg; *C. viscosum* lipase, 50 mg; lipase CE-5, 50 mg; Novozym 435, 5 mg; Lipozyme, 25 mg; *C. rugosa* lipase, 50 mg; lipase A, 50 mg) and the eight reaction mixtures were shaken (250 rpm) at 45 °C. At defined times samples were taken and analyzed by TLC and HPLC. Conversions were quantitative in 24 h. The products ratio (**2a** and **2b**) are reported in Table 1.

2.4. Acetylation of Tsugicoline A (*2*)

(a) Tsugicoline A (**2**, 25 mg, 0.094 mmol) was dissolved in methyl *tert*-butyl ether (1 ml). Vinyl acetate (5 ml) was added together with 50 mg of Lipozyme. The reaction was shaken (250 rpm) at 45 °C and monitored by TLC (AcOEt:hexane 1:1). After 16 h, conversion was almost quantitative (**2a** to **2b** ratio, 99:1). The enzyme was filtered off, the solvent was evaporated and the residue purified by flash chromatography (eluent: AcOEt:hexane 3:7) to give 1-*O*-acetyl tsugicoline A (**2a**, 20 mg, 0.065 mmol, 69% yield). Oil. CI-MS (isobutene): *m*/*z* 309 ($M + H^+$). Selected ^{1}H NMR data, δ ($CDCl_3$): 4.98 and 4.89 (1 H each, d each, $J = 15$ Hz, CH_2-1), 4.37 (1H, s, H-6), 4.17 (1H, d, $J = 8.5$ Hz, H-3), 2.17 (3H, s, CH_3CO), 1.13 (3H, s, CH_3-8), 0.99 (6H, s, CH_3-14 and CH_3-15).

(b) Tsugicoline A (**2**, 25 mg, 0.094 mmol) was dissolved in methyl *tert*-butyl ether (1 ml). Vinyl acetate (5 ml) was added together with 100 mg of lipase PS on celite. The reaction mixture was shaken (250 rpm) at 45 °C and monitored by TLC (AcOEt:hexane 1:1) for 6 days (**2a** to **2b** ratio, 3:97). The enzyme was filtered off, the solvent was evaporated and the residue purified by flash chromatography (eluent: AcOEt:hexane 25:75) to give 1,6-di-*O*-acetyl tsugicoline A (**2b**, 16 mg, 0.046 mmol, 49% yield). Oil. CI-MS (isobutene): *m*/*z* 351 ($M + H^+$). Selected ^{1}H NMR data, δ ($CDCl_3$): 5.10 (1H, s, H-6),

5.02 and 4.90 (1H each, d, $J = 15$ Hz, CH_2-1), 4.18 (1H, d, $J = 8.5$ Hz, H-3), 2.19 and 2.14 (3H each, s, CH_3CO), 1.10, 1.00 and 0.92 (3H each, s, CH_3-8, CH_3-14 and CH_3-15).

2.5. Acetylation of 2-amino-tsugicoline E (3)

2-amino-tsugicoline E (**3**, 20 mg, 0.071 mmol) was dissolved in *tert*-amyl alcohol (1 ml). Vinyl acetate (5 ml) was added together with 200 mg of Lipase A. The reaction mixture was shaken (250 rpm) at 45 °C and monitored by TLC (AcOEt:MeOH:H_2O:AcOH 8:2:1:0.1) for 5 days. The enzyme was filtered off, the solvent was evaporated and the residue purified by flash chromatography (eluent: AcOEt:MeOH 8:1) to give 2-*N*-acetyl-5-*O*-acetyl amino-tsugicoline E (**3a**, 8 mg, 0.022 mmol, 31% yield). Oil. FAB-MS (thioglycerol): *m/z* 368 ($M + H^+$), 326. Selected ^{1}H NMR data, δ (acetone-d_6): 7.40 (1H, br s, NH), 4.14 (1H, s, H-6), 4.03 and 3.95 (1H each, d, $J = 15$ Hz, CH_2-1), 4.01 (1H, d, $J = 7.5$ Hz, H-3), 2.24 (1H, s, H-4), 1.90 and 1.86 (3H each, s, CH_3CO), 1.0.9, 1.03 and 1.00 (3H each, s, CH_3-8, CH_3-14 and CH_3-15).

2.6. Lipases screening for the acetylation of the sterpurane derivative (4)

Sterpurane (0.5 mg) was dissolved in 400 μl methyl *tert*-butyl ether containing 100 μl of vinyl acetate. A sample of the different lipases was added (pancreatic lipase, 50 mg; lipase PS on celite, 25 mg; *C. viscosum* lipase, 50 mg; lipase CE-5, 50 mg; Novozym 435, 5 mg; Lipozyme, 25 mg; *C. rugosa* lipase, 50 mg; lipase A, 50 mg) and the eight reaction mixtures were shaken (250 rpm) at 45 °C. At defined times samples were taken and analyzed by TLC and HPLC. The products ratio (**4a** to **4b**) after 24 h are reported in Table 2.

2.7. Acetylation of the sterpurane derivative (4)

(a) Compound **4** (14 mg, 0.053 mmol) was dissolved in methyl *tert*-butyl ether (0.56 ml). Vinyl acetate (2.8 ml) was added together with 300 mg of Lipase A. The reaction mixture was shaken (250 rpm) at 45 °C and monitored by TLC (AcOEt:hexane 6:4) for 4 days (62% conversion; **4a** to **4b** ratio, 81:19). The enzyme was filtered off, the solvent was evaporated and the residue purified by flash chromatography (eluent: AcOEt:hexane 6:4) to give the 1-*O*-acetyl derivative **4a** (2 mg, 0.006 mmol, 12% yield). Oil. CI-MS (isobutene): *m/z* 309 ($M + H^+$). Selected ^{1}H NMR data, δ ($CDCl_3$): 4.99 (1H, s, H-6), 4.75 and 4.10 (1H each, d each, $J = 14$ Hz, CH_2-1), 4.16 (1H, br s, H-3), 2.05 (3H, s, CH_3CO), 2.01 (3H, s, CH_3-8), 1.10 (3H, s, CH_3-14), 1.00 (3H, s, CH_3-15).

(b) Compound **4** (13 mg, 0.049 mmol) was dissolved in methyl *tert*-butyl ether (1 ml). Vinyl acetate (5 ml) was added together with 300 mg of *C. rugosa* lipase. The reaction mixture was shaken (250 rpm) at 45 °C and monitored by TLC (AcOEt:hexane 1:1) for 2 days (64% conversion; **4a** to **4b** ratio 25:75). The enzyme was filtered off, the solvent was evaporated and the residue purified by flash chromatography (eluent:AcOEt:hexane 55:45) to give the 6-*O*-acetyl derivative **4b** (2 mg, 0.006 mmol, 12% yield). Oil. CI-MS (isobutene): *m/z* 309 ($M + H^+$). Selected ^{1}H NMR data, δ ($CDCl_3$): 5.44 (1H, s, H-6), 4.24 (1H, br s, H-3), 3.82 and 3.70 (1H each, d each, $J = 14$ Hz, CH_2-1), 2.19 (3H, s, CH_3CO), 2.04 (3H, s, CH_3-8), 1.06 (3H, s, CH_3-14), 1.01 (3H, s, CH_3-15).

If the same mixture was let to react for 15 days, flash chromatography allowed the isolation of the 1,6-di-*O*-acetyl derivative **4c** (2.2 mg, 0.006 mmol, 13% yield). Oil. CI-MS (isobutene): *m/z* 351 ($M + H^+$). Selected ^{1}H NMR data, δ ($CDCl_3$): 5.38 (1H, s, H-6), 4.28 and 4.12 (1H each, d each, $J = 12.4$ Hz, CH_2-1), 4.13 (1H, br s, H-3), 2.17 and 2.05 (3H each, s, CH_3CO), 2.05 (3H, s, CH_3-8), 1.08 (3H, s, CH_3-14), 1.01 (3H, s, CH_3-15).

Table 2
Regioselective acetylation of **4** catalyzed by different lipases[a]

Lipase	Conversion (%)[b]	**4a**:**4b**[b]
Porcine pancreatic lipase	66	38:62
Lipase PS	100	82:18
Chromobacterium viscosum lipase	41	0:100
Lipase CE-5	82	22:78
Novozym 435	77	57:43
Lypozime	93	71:29
C. rugosa lipase	100	0:100
Lipase A	100	100:0

[a] Determined after 24 h.
[b] Evaluated by HPLC (see Section 2).

3. Results and discussion

A set of eight commercially available lipases, known to be able to catalyze the regioselective acylation of polyhydroxylated compounds [6], has been screened with the sesquiterpene Illudine S (**1**). Esterification reactions were performed in methyl *tert*-butyl ether containing vinyl acetate. Acetylation was, in all cases, quantitative within 24 h and selectively directed to the primary C(15)–OH to give the expected product **1a**. The structure of **1a** (and of the other compounds that will be described later) was easily determined by comparing the ^{1}H NMR spectra of the starting materials and of the acetate derivatives: a significant downfield shift of the signals due to the protons linked to the acylated carbon C(15) was clearly observed.

In contrast, our second target compound, Tsugicoline A (**2**), gave different reaction products depending on the lipase used. As shown in Table 1, the expected marked preference for the primary C(1)–OH to give the monoacetate **2a** was the reaction outcome with most of the lipases tested (quantitative conversion was again observed). However, lipase PS

catalyzed the formation of the diacetate **2b**, with a complete regio-preference for the secondary C(6)–OH over the C(3)–OH.

Even more interesting results were obtained with the Tsugicoline derivatives **3** and **4**. Only lipase A and lipase CE-5 were able to acetylate 2-amino-tsugicoline E (**3**) to give the same product, as judged by TLC. The compound was isolated and proved to be the unexpected diacetate **3a** in which, in addition to the acylation of the C(2)–NH_2, the tertiary C(5)–OH resulted to be esterified. To demonstrate the unusual acetylation pattern in C(5)–OH, compound **3a** was successively reacted with pyridine and Ac_2O to give the tetraacetate derivative **3b**, whose NMR spectrum was identical to the published data [4]. In comparison to the spectrum of **3a**, the peracetylated compound **3b** showed a marked downfield shift of the signals due to C(3)–H (at 5.71 from 4.01 ppm) and C(6)–H (at 5.08 from 4.14), therefore demonstrating that the corresponding C(3)–OH and C(6)–OH were not acetylated in **3a**.

Finally, as shown in Table 2, using the sterpurane derivative **4** as a substrate, the lipases directed their catalytic action either on the primary C(1)–OH and/or on the secondary C(6)–OH. In this way the two regioisomers **4a** and **4b** could be isolated independently. Quite peculiar is the marked preference of *C. rugosa* lipase for the chemically less reactive secondary C(6)–OH.

The results obtained are not easy to rationalize in terms of the interaction of the substrates with the enzymatic active sites. Nevertheless, they do confirm the versatility of action of lipases and the peculiar capacity of these enzymes to discriminate functional groups with equivalent chemical reactivity. Additionally, the present report further supports the importance of developing libraries of enzymes [7] in order to exploit the complementary performances of different biocatalysts belonging to the same class.

References

[1] W.A. Ayer, L.M. Browne, Tetrahedron 37 (1981) 2199–2248.
[2] A. Arnone, L. Merlini, G. Nasini, O. Vajna de Pava, F. Zunino, J. Chem. Soc., Perkin Trans. I (2001) 610–616.
[3] A. Arnone, U. Brambilla, G. Nasini, O. Vajna de Pava, Tetrahedron 51 (1995) 13357–13364.
[4] A. Arnone, C. De Gregorio, A. Mele, G. Nasini, O. Vajna de Pava, J. Chem. Soc., Perkin Trans. I (2000) 745–751 and literature therein.
[5] R. Bovara, G. Carrea, L. Ferrara, S. Riva, Tetrahedron: Asymmetry 2 (1991) 831–838.
[6] (a) S. Riva, G. Roda, in: M.N. Gupta (Ed.), Methods and Tools in Biosciences and Medicine, Birkhäuser Verlag, Basel, 2000, Chapter 9, pp. 146–159;
(b) G. Carrea, S. Riva, Angew. Chem. Int. Ed. 39 (2000) 2227–2254;
(c) B. Danieli, M. Luisetti, G. Sampognaro, G. Carrea, S. Riva, J. Mol. Catal. B: Enzym. 3 (1997) 193–201;
(d) C.-H. Wong, R.L. Halcomb, Y. Ichikawa, T. Kajimoto, Angew. Chem. Int. Ed. 34 (1995) 412–432.
[7] (a) M. Casali, L. Tarantini, S. Riva, Z. Huňková, L. Weignerová, V. Křen, Biotechnol. Bioeng. 77 (2002) 105–110;
(b) F. Secundo, G. Carrea, M. De Amici, S. Jappolo di Ventimiglia, J.S. Dordick, Biotechnol. Bioeng. 81 (2003) 391–396;
(c) J.O. Rich, P.C. Michels, Y.L. Khmelnitsky, Curr. Opin. Chem. Biol. 6 (2002) 161–167, and references therein.