Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 2915–2924

TETRAHEDRON: *ASYMMETRY*

Lipase-catalysed chemoselective monoacetylation of hydroxyalkylphenols and chemoselective removal of a single acetyl group from their diacetates

Pietro Allevi, Pierangela Ciuffreda, Alessandra Longo and Mario Anastasia *

Department of Medical Chemistry and Biochemistry, University of Milan, Via Saldini 50, 20133 Milan, Italy

Received 10 July 1998; accepted 17 July 1998

Abstract

It was demonstrated that *Pseudomonas cepacia* PS lipase adsorbed on Celite, has the ability to catalyse the chemoselective monoacetylation of various hydroxyalkylphenols or the chemoselective removal of a single acetyl group from the corresponding acetate.

1. Introduction

An important topic in organic synthesis is the development of chemical procedures to obtain chemoselectively monoacetylated compounds from molecules containing phenolic and aliphatic hydroxyl groups, as reflected by the number of methods developed recently to effect such transformations.[1–3]

The literature shows this goal can be achieved by two routes, one involving the selective monoacylation[3] of the dihydroxy compounds, the other the selective removal[4–6] of a single acyl group from the diacetylated compounds. The usual methods (using acyl chlorides or anhydrides) are generally orientated towards the acylation of phenols,[3] while the selective acylation of the alcoholic hydroxyls is better achieved by relatively recent methods based on transesterification processes that couple heterogeneous catalysts such as Al_2O_3[7,8] or $NaHSO_4$ supported on silica gel ($NaHSO_4 \cdot SiO_2$).[1] In the latter case, the acetylation yield is high and the conditions required are milder (6–18 h at 67–80°C) than those reported for selective acylation using tightly twisted active amides (72–120 h at 80°C).[3] The acetyl group is selectively removed from phenols in excellent yield by treating the phenols with guanidine[5] and, in the case of the 3-hydroxybenzylalcohols, by treatment with $NaBH_4$ in dimethoxyethane (for 18 h at 40°C).[6]

* Corresponding author. E-mail: skinski@imiucca.csi.unimi.it

0957-4166/98/$ - see front matter
PII: S0957-4166(98)00285-7

Less explored is the possibility of using enzymes in organic solvents to catalyse the chemoselective acylation of hydroxyalkylphenols or the chemoselective deacetylation of the diacetates, though enzymes have been used in the enantioselective acylation of unsymmetrical alcohols, diols or polyols,[9] in the regioselective acylation of hydroxyls in polydric phenols[10,11a,b] and in the regioselective deacylation of polyacetylated phenols.[12–15] In fact, to the best of our knowledge, only three papers have dealt with this topic. One gave consideration only to the ability of some lipases, from *Candida cylindracea* (CCL),[16] to mediate the selective acetylation of the alcoholic groups of 2-, 3- and 4-hydroxybenzyl alcohols, together with the ability of the same enzymes to mediate the chemoselective transesterification of the diacetate. The acetylation reaction always occurs quantitatively and with complete and exclusive esterification of the benzylic hydroxy group. Similarly, deacetylation on the phenolic ester takes place with satisfactory results except for the 2-acetoxybenzyl alcohol acetate, which shows poor yield and poor selectivity.[16] Another of these papers[17] discusses the ability of lipase from *Aspergillus niger* to mediate the acetylation of the said hydroxybenzylacohols. The reaction was difficult and a considerable amount of the starting diols was recovered (7–23%; after 48 h). The third paper[18] studied the selective enzymatic hydrolysis of phenolic acetates in the presence of alkyl acetates. Pig liver acetone powder (PLAP) that contains lipases was used for competitive experiments. Only the hydroxyalkylphenol diacetate was tested, 3-(2-acetoxypropyl)phenyl acetate, resulting in a partial hydrolysis (12%) of both esters.

From these results it was concluded that the tested enzymes could catalyse, by chemoselective acetylation, the preparation of acetoxyphenols, but were ineffective when preparing the complementary isomeric compound (i.e. of the benzylic alcohols with an acetylated phenolic group) by deacetylation. Thus, there appeared to be a gap in the enzyme-catalysed reactions, compared to reactions mediated by chemical reagents.

In the course of research concerning the transformation of 4′-demethylepipodophyllotoxin (**1a**) into the etoposide, a phenolic anticancer O-glycoside,[19a,b] we searched for an enzymatic route suitable for the facile acetylation of the alcoholic hydroxyl of **1a**. This led us to an extensive study of monoacylation in organic solvents; we considered various hydroxyalkylphenols and used lipases from *Pseudomonas cepacia* PS or PLAP as catalysts in the acetylations.[20,21] The next step was the study of the deacetylation reactions of the corresponding diacetylated compounds in aqueous neutral buffer.

Our results show that Lipase PS can be used to selectively prepare phenolic or alcoholic acetates of hydroxyalkylphenols containing one or two methoxy groups adjacent to the phenolic hydroxyl. This selectivity occurs as the alcoholic hydroxyl prevails in enzymatic acetylation in organic solvents, while in the case of the hydrolysis of the corresponding diacetates what prevails is the ester of the alcoholic hydroxyl. Moreover, in unsubstituted hydroxyalkylphenols, Lipase PS causes the alcohols (in acetylation) and the phenol esters (in deacetylation) to be more reactive, always leading to acetylated alcohols with a free phenol, with complete chemoselectivity. Other lipases, PLAP and lipase from *Candida cylindracea*,[16] do not show this reversal of chemoselectivity and are, in some cases, ineffectual, while in others they catalyse both the acetylation and the deacetylation in noticeably longer times than those required by Lipase PS.

2. Results and discussion

First we evaluated the possibility of obtaining the two monoacetyl esters of the hydroxyalkylphenols **2a–5a**, by enzyme-catalysed acetylation and/or deacetylation of the corresponding diacetates. Compound **2a**, which contains a phenolic moiety similar to that present in 4′-demethylepipodophyllotoxin (**1a**),[19a] is part of a group of well known synthons used for various synthetic purposes in flavonoid or lignan

Table 1
Lipase-catalysed chemoselective acetylation (**Ac**) of diols (**2a–5a**) and deacetylation (**D**) of the corresponding diacetates (**2d–5d**)

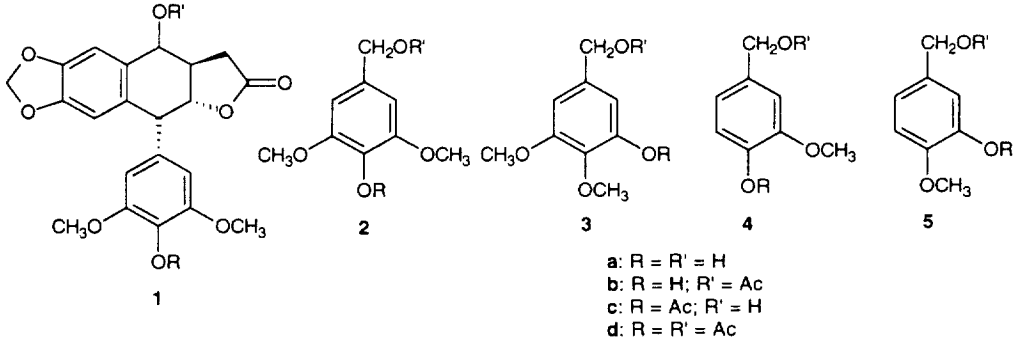
Substrate	Reaction-Enzyme	Time (h)	Compound(s)[a]
2a	**Ac**-PS	0.25	**2b**
	Ac-PLAP	70	**2b** (Y=66%)[b]
2d	**D**-PS	0.83	**2c**
	D-PLAP	1.0	**2a**
3a	**Ac**-PS	0.25	**3b**
	Ac-PLAP	40	**3b**
3d	**D**-PS	0.83	**3c**
	D-PLAP	1.0	**3b**
4a	**Ac**-PS	0.25	**4b**
	Ac-PLAP	18	**4b**
4d	**D**-PS	1.0	**4a** (Y=55%) and **4b** (Y=45%)
	D-PLAP	1.2	**4a** (Y=15%) and **4b** (Y=85%)
5a	**Ac**-PS	0.25	**5b**
	Ac-PLAP	70	**5b** (Y=81%)[b]
5d	**D**-PS	1.0	**5c**
	D-PLAP	3	**5b**

[a] The conversion and yield of the crude products was quantitative (Y = 96-98%) unless otherwise indicated. [b] In this case the reaction was interrupted at the conversion indicated, before the disappearance of the starting material.

chemistry.[22–24] In addition, some of these congeries/synthons are used as model compounds to evaluate chemoselective acylations performed by chemical methods.[3]

In separate experiments, the mono and dimethoxylated hydroxyalkylphenols **2a–5a** were subjected to acetylation with vinyl acetate in diisopropyl ether, catalysed by Lipase PS or PLAP. In all cases, the alcohol acetylation prevailed, resulting in excellent chemoselectivity regardless of the enzyme used (Table 1).

Lipase PS showed very high activity, instead PLAP was lower and required noticeably longer reaction times.[25]

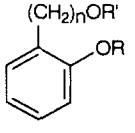


When the corresponding diacetates **2d–5d** were subjected to deacetylation in acetone–aqueous phosphate buffer (pH=7), in the presence of free Lipase PS or PLAP (Table 1), the chemoselectivity for the enzymes was, in two cases, opposing. In fact, Lipase PS catalyses the chemoselective hydrolysis of the alcoholic acetate esters of **3d** and **5d**, while PLAP catalyses the hydrolysis of the phenolic esters or, in the case of **2d** and **4d**, of both esters.[25]

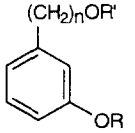
On the basis of these results, it can be seen that the same compounds **2b–5b**, with acetylated

alcohols and a free phenol group, can be obtained in good yield by acetylation of the corresponding diols under Lipase PS or PLAP catalysis, or by the hydrolysis of the corresponding diacetates in the presence of PLAP. Conversely, the isomeric monoacetylated phenols **2c**, **3c** and **5c** are obtained in good yield in deacetylation reactions under Lipase PS catalysis. Only in the case of the 4-acetoxy-3-methoxybenzylalcohol acetate **4d** did the deacetylation mediated by Lipase PS afford 4-hydroxy-3-methoxybenzylalcohol acetate **4b** (43% yield), accompanied by the 4-hydroxy-3-methoxybenzyl alcohol **4a** (53% yield). The same deacetylation under PLAP catalysis afforded the phenolic benzylalcohol acetate **4b** (in 82% yield), accompanied by a minor amount of the dihydroxycompound **4a** (14% yield).

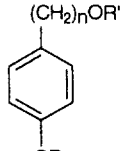
These encouraging results prompted us to evaluate Lipase PS and PLAP behaviour in similar reactions but involving simpler hydroxyalkylphenols. Thus, a study was made of three classes of compounds, corresponding to the possible reciprocal positions in a benzene ring (*ortho*, *meta* and *para*) of a phenolic hydroxy group and an alkyl chain bearing the alcoholic function. The alkyl chains chosen were of three different lengths (from one to three carbon atoms) in order to also assess the influence of the distance between the molecules' hydroxyls on the reaction course.



6



7



8

a: n = 1; R = R' = H
b: n = 1; R = H; R' = Ac
c: n = 1; R = Ac; R' = H
d: n = 1; R = R' = Ac
e: n = 2; R = R' = H
f: n = 2; R = H; R' = Ac
g: n = 2; R = Ac; R' = H
h: n = 2; R = R' = Ac
i: n = 3; R = R' = H
j: n = 3; R = H; R' = Ac
k: n = 3; R = Ac; R' = H
l: n = 3; R = R' = Ac

The results, summarised in Table 2, show that all the tested hydroxy compounds (*ortho*: **6a**, **6e** and **6i**; *meta*: **7a**, **7e** and **7i** and *para*: **8a**, **8e** and **8i**) are chemoselectively acetylated at the alcoholic hydroxy group affording, exclusively, phenols with an esterified alcoholic group (**6b**, **6f** and **6j**; **7b**, **7f** and **7j**; **8b**, **8f** and **8j**); this occurs irrespective of the reciprocal position and distance between the two hydroxyls in the starting compound. In no case was acetylation of the phenol observed, even on prolonging the reaction time to several hours after the disappearance of the starting substrate.

PLAP also catalysed the acetylation of the alcoholic hydroxyl, but very long times were needed (the reactions were incomplete even after 3 days) and are not reported in Table 2.

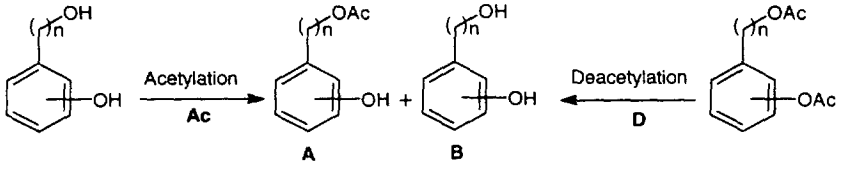
In the deacetylation of the diacetates (*ortho*: **6d**, **6h** and **6l**; *meta*: **7d**, **7h** and **7l** and *para*: **8d**, **8h** and **8l**), regardless of the enzyme catalyst used, the regeneration of the phenolic group always prevailed, the only exception being the case of the hydroxymethylphenol diacetates **6d**, **7d** and **8d** where some dihydroxy compound (5–30%) accompanied the benzylic alcohol ester. In these cases, prolonging the reaction time after the disappearance of the starting diacetate results in dihydroxy compounds as exclusive reaction products.

Thus, with non-hindered phenols, Lipase PS and PLAP show similar behaviour and selectivity, the selectivity increasing with the length of the alkyl chain, regardless of its position with respect to the phenol hydroxyl.

The reported results show that the ability of enzymes to discriminate between alcoholic and phenolic groups in acetylation reactions, and between acetates in deacetylation reactions, has marked potential in organic syntheses. Indeed enzymatic methods could be used as a good alternative to current chemical methods for obtaining the same results.

In our laboratory, we used enzymes to prepare 4′-demethylepipodophyllotoxin 4-acetate **1b** and α- and β-estradiol 17-acetates. The acetate **1b** was obtained in quantitative yield by hydrolysis of its diacetate

Table 2
Lipase-catalysed chemoselective acetylation (**Ac**) of hydroxyalkylphenols of type (**6–8**) and deacetylation (**D**) of the corresponding diacetates



Substrate	Reaction-Enzyme[a]	Ratio (A : B)[b]	Time (h)
ortho			
6a; n = 1	**Ac**-PS	100 : 0	0.25
6d; n = 1	**D**-PS	72 : 28	0.25
	D-PLAP	68 : 32	0.25
6e; n = 2	**Ac**-PS	100 : 0	2.0
6h; n = 2	**D**-PS	98 : 2	0.17
	D-PLAP	96 : 4	0.33
6i; n = 3	**Ac**-PS	100 : 0	1.5
6l; n = 3	**D**-PS	96 : 4	0.83
	D-PLAP	94 : 6	0.75
meta			
7a; n = 1	**Ac**-PS	100 : 0	0.50
7d; n = 1	**D**-PS	87 : 7[c]	0.08
	D-PLAP	95 : 5	0.25
7e; n = 2	**Ac**-PS	100 : 0	2.0
7h; n = 2	**D**-PS	100 : 0	0.08
	D-PLAP	100 : 0	0.08
7i; n = 3	**Ac**-PS	100 : 0	1.5
7l; n = 3	**D**-PS	100 : 0	0.75
	D-PLAP	100 : 0	0.75
para			
8a; n = 1	**Ac**-PS	100 : 0	0.50
8d; n = 1	**D**-PS	84 : 16	0.42
	D-PLAP	80 : 20	0.33
8e; n = 2	**Ac**-PS	100 : 0	1.8
8h; n = 2	**D**-PS	100 : 0	0.08
	D-PLAP	100 : 0	0.08
8i; n = 3	**Ac**-PS	100 : 0	1.5
8l; n = 3	**D**-PS	100 : 0	0.75
	D-PLAP	100 : 0	0.67

[a] PLAP catalyses the acetylation of the alcoholic hydroxyl but the reactions are incomplete after 3 days.
[b]Conversion and yield of crude product(s) were quantitative (Y= 96-98 %); [c]In this case some alcohol (6%) acetylated at the phenolic group was obtained.

1d mediated by PLAP, however lipase PS did not catalyse the deacetylation of compound **1d**. The two epimeric estradiol 17-acetates were obtained by chemoselective deacetylation of the corresponding diacetates mediated by either PLAP or Lipase PS. These compounds are of interest for synthetic studies in our laboratory,[19a,b,26] and have already been obtained by more tedious chemical procedures involving the selective protection of the phenolic group, acetylation of the alcohol and deprotection of the phenol.[19a,27,28]

In the pioneering work done by Caspi and Njar[29] in this area the α-estradiol 17-acetate yield was poor (25%), while that of the β-estradiol 17 acetate was good (90%). These pioneers obtained the two isomers

by the enzymatic transesterification of the corresponding diester, mediated by *Candida cylindracea* in organic solvents.

In conclusion, though it is still not possible to predict the general course of the enzyme-catalysed reactions of hydroxyalkylphenols, Lipase PS merits some reflection. In the case of the hydroxyalkylphenols with unhindered alcoholic and phenolic hydroxyls, Lipase PS preferentially catalyses the acetylation of the alcohol, but in the hydrolysis of the corresponding diacetates it shows chemoselectivity for the phenolic ester. With an unhindered alcoholic group and a hindered phenolic hydroxyl, Lipase PS still catalyses the acetylation of the alcoholic group but in the hydrolysis of the corresponding diacetate the chemoselectivity reverts and hydrolysis of the alcoholic ester is catalysed. Thus, Lipase PS leads to complementary isomeric products in acetylation–deacetylation steps. On the contrary, when both the alcoholic and the phenolic hydroxyls are equally hindered (as in **1d**), Lipase PS is unable to catalyse the acetylation or the hydrolysis of the corresponding esters. Both PLAP and CCL are less active than Lipase PS, however when they are effective, they catalyse the alcoholic group acetylation and phenol ester hydrolysis or the hydrolysis of both esters.

Also of interest is the behaviour of Lipase PS. It changes its chemopreference not only on the basis of the steric hindrance but also on the basis of the position of the phenol group. Such knowledge leads to a better understanding of the characteristics of this enzyme.

3. Experimental section

^{1}H NMR spectra (500.13 MHz) were recorded in $CDCl_3$ at 303 K, the reference being $CHCl_3$ at 7.24 ppm. Mass spectra (MS) were recorded on a Hewlett Packard 5988A mass spectrometer using a direct inlet at an ionising voltage of 70 eV by electron impact. Mass spectra were reported as *m/z* (relative abundance). HPLC analyses were carried out on a Merck superspher 100 RP-18 column (4 mm×15 cm; eluent H_2O:MeOH, 50:50; v/v). The flow rate was 1 mL/min and detection was performed at 270 nm. GLC analyses were carried out on a SPB-5 Supelco column (30 m×0.32 mm; 0.25 μm film thickness): column temperature, initially set at 110°C for 1 min, increased by 20°C/min until 280°C where it was kept for 2 min. TLC was carried out on silica gel 60 F_{254} microplates eluting with hexane:ethyl acetate (60:40; v/v). Column chromatography refers to flash chromatography.[30] The progress of all the reactions, column chromatography and compound purity were monitored by TLC, GLC and/or HPLC.

Particular attention was given to the course of the reaction as the starting diacetates and acetoxyphenols used show very similar R_f in the best eluent used (hexane:ethyl acetate, 60:40; v/v), as do the diols and alcohols with an acetylated phenol. Experiments with lipase from CCL were performed according to the general conditions described by Servi and Pedrocchi.[16] All organic solvents were dried before use. Starting substrates were obtained as follows and showed satisfactory elemental analyses and significant spectroscopic data (IR, ^{1}H NMR and mass spectra).

The starting diols **4a–8a**, **6e–8e**, **8i**, 17α- and 17β-estradiol were purchased (Aldrich). Diols **2a** and **3a** were obtained by $NaBH_4$ reduction of the corresponding aldehydes (Aldrich), compound **6i** was obtained by $LiAlH_4$ reduction of dihydrocoumarin (Aldrich) at 25°C, and the diol **7i** was prepared from 3-(3-methoxyphenyl)propionic acid (Aldrich), described below; 4′-demethylepipodophillotoxin was obtained from commercial podophyllotoxin.[19a] The acetates **1d**[31]–**8d**, **6h–8h**, **6l–8l** and those of 17α- and 17β-estradiol were obtained by usual acetylation with acetic anhydride in pyridine and were identified by their physical and/or spectroscopic data. All compounds showed correct elemental analyses.

3.1. Preparation of 3-(3-hydroxypropyl)phenol (7i)

3-(3-Methoxyphenyl)propionic acid (600 mg; 3.33 mmol) was dissolved in CH_3CN (5 mL) and added to NaI (599 mg; 3.99 mmol) and $(CH_3)_3SiCl$ (0.5 mL; 3.99 mmol). The mixture was heated at 60°C for 4 h, quenched with a saturated solution of NaCl and extracted with $CHCl_3$. The organic layers were washed, dried and evaporated to afford a crude product (560 mg) which, without purification, was dissolved in methanol (5 mL) and treated with H_2SO_4 (0.5 mL; 96%), for 12 h at 25°C. The solution was then concentrated under reduced pressure, poured into an ice cold aqueous solution of NaOH (pH=12) and washed with ethyl acetate. The aqueous layer was then acidified with HCl (2 M) and extracted with ethyl acetate. The obtained organic layers were dried and the solvent evaporated to afford the crude methyl ester (310 mg). This was dissolved in anhydrous diethyl ether (10 mL) and treated with $LiAlH_4$ (150 mg) at 25°C for 6 h to give, after usual work-up, the diol **7i** (200 mg): a viscous oil, IR (neat) 3380, 1580, 780 cm^{-1}; ^1H NMR δ 7.12 (1H, dd, J=8.0, 8.0 Hz), 6.72 (1H, d, J=8.0 Hz), 6.67–6.64 (2H, m), 3.62 (2H, t, J=7.5 Hz, CH_2OH), 2.61 (2H, t, J=7.5 Hz, $ArCH_2$), 1.92 (2H, tt, J=7.5, 7.5 Hz, CH_2); MS *m/z* 152 (M^+, 26), 133 (15), 121 (9), 108 (100), 107 (55), 91 (23), 77 (49).

3.2. Enzyme-mediated acetylation of diols. General procedure

Vinyl acetate (0.18 mL; 2 mmol) and enzyme (Lipase PS: 125 mg or PLAP: 125 mg) were added to a solution of the diol (1 mmol) in diisopropyl ether (20 mL). The resulting suspension was shaken at 25°C and monitored by TLC, GLC and/or HPLC. After the consumption of the starting diol the reaction was quenched by filtering off the enzyme and the solvent was then removed in vacuo to give the identified compound(s).

3.3. Enzyme-mediated deacetylation of diacetates. General procedure

The diacetate (1 mmol) was dissolved in acetone (4 mL) and added to phosphate buffer (20 mL; pH=7) and the enzyme (free Lipase PS: 65 mg, or PLAP: 125 mg). The resulting suspension was shaken at 25°C and monitored by TLC, GLC and/or HPLC. After consumption of the starting diacetate the reaction was quenched by adding ethyl acetate and the resulting mixture was filtered through a pad of Celite. The organic layer was then separated and dried and the solvent removed *in vacuo* to give a crude residue which, if necessary, was chromatographed to afford the identified compound(s).

3.3.1. 2,6-Dimethoxy-4-(acetoxymethyl)phenol (2b)

IR (neat) 3420, 1730 cm^{-1}; ^1H NMR δ 6.58 (2H, s, H-3 and H-5), 5.56 (1H, s, O*H*), 4.98 (2H, s, CH_2), 3.86 (6H, s, 2×OCH_3), 2.06 (3H, s, $OCOCH_3$); MS *m/z* 226 (M^+, 23), 182 (50), 167 (100), 148 (44), 136 (29), 123 (31), 107 (19), 95 (24), 79 (27), 65 (32).

3.3.2. 2,6-Dimethoxy-4-(hydroxymethyl)phenyl acetate (2c)

IR (neat) 3430, 1770 cm^{-1}; ^1H NMR δ 6.60 (2H, s, H-3 and H-5), 4.61 (2H, s, CH_2), 3.79 (6H, s, 2×OCH_3), 2.30 (3H, s, $OCOCH_3$); MS *m/z* 226 (M^+, 3), 184 (100), 167 (24), 155 (15), 141 (8), 123 (25), 109 (21), 95 (17), 81 (15), 53 (17).

*3.3.3. 2,3-Dimethoxy-5-(acetoxymethyl)phenol (**3b**)*

IR (neat) 3420, 1725 cm^{-1}; ^1H NMR δ 6.59 (1H, d, *J*=2.1 Hz), 6.45 (1H, d, *J*=2.1 Hz), 5.82 (1H, s, O*H*), 4.97 (2H, s, CH_2), 3.87 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 2.08 (3H, s, OCOCH_3); MS *m/z* 226 (M^+,4), 184 (83), 169 (9), 167 (20), 141 (15), 109 (100), 95 (37), 81 (48), 53 (39).

*3.3.4. 2,3-Dimethoxy-5-(hydroxymethyl)phenyl acetate (**3c**)*

IR (neat) 3435, 1770 cm^{-1}; ^1H NMR δ 6.80 (1H, d, *J*=2.1 Hz), 6.63 (1H, d, *J*=2.1 Hz), 4.57 (2H, s, CH_2), 3.84 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 2.29 (3H, s, OCOCH_3); MS *m/z* 226 (M^+, 15), 184 (100), 169 (9), 167 (11), 141 (19), 109 (74), 95 (21), 81 (21), 53 (20).

*3.3.5. 2-Methoxy-4-(acetoxymethyl)phenol (**4b**)*

IR (neat) 3430, 1735 cm^{-1}; ^1H NMR δ 6.88–6.85 (3H, m, H-2, H-3 and H-5), 5.70 (1H, s, O*H*), 5.00 (2H, s, CH_2), 3.87 (3H, s, OCH_3), 2.06 (3H, s, OCOCH_3); MS *m/z* 196 (M^+, 32), 154 (33), 137 (100), 122 (32), 107 (22), 94 (21), 93 (26), 65 (51).

*3.3.6. 2-Methoxy-5-(acetoxymethyl)phenol (**5b**)*[3,6]

IR (neat) 3440, 1730 cm^{-1}; ^1H NMR δ 6.92 (1H, d, *J*=2.1 Hz, H-6), 6.83 (1H, dd, *J*=2.1, 7.7 Hz, H-4), 6.80 (1H, d, *J*=7.7 Hz, H-3), 5.67 (1H, s, O*H*), 4.98 (2H, s, CH_2), 3.86 (3H, s, OCH_3), 2.06 (3H, s, OCOCH_3); MS *m/z* 196 (M^+, 33), 154 (54), 137 (100), 125 (8), 122 (38), 93 (30), 65 (13).

*3.3.7. 2-Methoxy-5-(hydroxymethyl)phenyl acetate (**5c**)*[3]

IR (neat) 3400, 1770 cm^{-1}; ^1H NMR δ 7.16 (1H, dd, *J*=2.1, 7.7 Hz, H-4), 7.06 (1H, d, *J*=2.1 Hz, H-6), 6.92 (1H, d, *J*=7.7 Hz, H-3), 4.57 (2H, s, CH_2), 3.80 (3H, s, OCH_3), 2.29 (3H, s, OCOCH_3); MS *m/z* 196 (M^+, 7), 154 (100), 137 (27), 125 (19), 122 (18), 93 (36), 65 (25).

*3.3.8. 2-(Acetoxymethyl)phenol (**6b**)*[16]

IR (neat) 3380, 1740 cm^{-1}; ^1H NMR δ 7.28–7.22 (2H, m), 6.92 (1H, d, *J*=7.7 Hz), 6.89 (1H, dd, *J*=7.7, 7.7 Hz), 5.10 (2H, s, CH_2), 2.09 (3H, s, OCOCH_3); MS *m/z* 166 (M^+, 33), 137 (11), 107 (100), 106 (96), 78 (96), 77 (56).

*3.3.9. 2-(2-Acetoxyethyl)phenol (**6f**)*[1]

IR (neat) 3250, 1700 cm^{-1}; ^1H NMR δ 7.14–7.06 (2H, m), 6.84 (1H, dd, *J*=7.7, 7.7 Hz), 6.80 (1H, d, *J*=7.7 Hz), 4.27 (2H, t, *J*=7.0 Hz, CH_2OAc), 2.94 (2H, t, *J*=7.0 Hz, ArCH_2), 2.05 (3H, s, OCOCH_3); MS *m/z* 180 (M^+, 2), 137 (3), 120 (100), 107 (42), 91 (30), 77 (25).

*3.3.10. 2-(3-Acetoxypropyl)phenol (**6j**)*

IR (neat) 3400, 1720 cm^{-1}; ^1H NMR δ 7.08 (1H, dd, *J*=2.1, 7.7 Hz), 7.05 (1H, ddd, *J*=2.1, 7.7, 7.7 Hz), 6.84 (1H, dd, *J*=7.7, 7.7 Hz), 6.75 (1H, d, *J*=7.7 Hz), 5.08 (1H, s, O*H*), 4.10 (2H, t, *J*=7.0 Hz, CH_2OAc), 2.69 (2H, t, *J*=7.0 Hz, ArCH_2), 2.05 (3H, s, OCOCH_3), 1.97 (2H, tt, *J*=7.0, 7.0 Hz, CH_2); MS *m/z* 194 (M^+, 4), 134 (100), 133 (39), 119 (38), 117 (8), 107 (43), 91 (35), 77 (42).

*3.3.11. 3-(Acetoxymethyl)phenol (**7b**)*[16]

IR (neat) 3380, 1730 cm^{-1}; ^1H NMR δ 7.19 (1H, dd, *J*=7.7, 7.7 Hz, H-5), 6.89 (1H, d, *J*=7.7 Hz, H-4), 6.82 (1H, d, *J*=2.1 Hz, H-2), 6.78 (1H, dd, *J*=2.1, 7.7 Hz, H-6), 5.99 (1H, s, O*H*), 5.04 (2H, s, CH_2), 2.09 (3H, s, OCOCH_3); MS *m/z* 166 (M^+, 42), 137 (3), 107 (90), 106 (29), 78 (74), 77 (100).

3.3.12. 3-(2-Acetoxyethyl)phenol (7f)

IR (neat) 3380, 1700 cm^{-1}; ^1H NMR δ 7.14 (1H, dd, *J*=7.7, 7.7 Hz, H-5), 6.75 (1H, d, *J*=7.7 Hz, H-4), 6.69 (1H, dd, *J*=2.1, 7.7 Hz, H-6), 6.67 (1H, d, *J*=2.1 Hz, H-2), 5.52 (1H, s, O*H*), 4.26 (2H, t, *J*=7.0 Hz, CH_2OAc), 2.87 (2H, t, *J*=7.0 Hz, ArCH_2), 2.03 (3H, s, OCOCH_3); MS *m/z* 180 (M^+, 1), 137 (2), 120 (100), 107 (16), 91 (14), 77 (18).

3.3.13. 3-(3-Acetoxypropyl)phenol (7j)

IR (neat) 3380, 1710 cm^{-1}; ^1H NMR δ 7.12 (1H, dd, *J*=7.7, 7.7 Hz, H-5), 6.72 (1H, d, *J*=7.7 Hz, H-4), 6.66 (1H, dd, *J*=2.1, 7.7 Hz, H-6), 6.64 (1H, d, *J*=2.1 Hz, H-2), 5.60 (1H, s, O*H*), 4.07 (2H, t, *J*=7.0 Hz, CH_2OAc), 2.61 (2H, t, *J*=7.0 Hz, ArCH_2), 2.04 (3H, s, OCOCH_3), 1.92 (2H, tt, *J*=7.0, 7.0 Hz, CH_2); MS *m/z* 194 (M^+, 6), 134 (100), 133 (97), 119 (16), 117 (77), 107 (71), 91 (40), 77 (44).

3.3.14. 4-(Acetoxymethyl)phenol (8b)[16]

IR (neat) 3380, 1725 cm^{-1}; ^1H NMR δ 7.22 (2H, d, *J*=7.7 Hz), 6.80 (2H, d, *J*=7.7 Hz), 5.71 (1H, s, O*H*), 5.02 (2H, s, CH_2), 2.07 (3H, s, OCOCH_3); MS *m/z* 166 (M^+, 15), 137 (2), 124 (17), 107 (100), 78 (30), 77 (26).

3.3.15. 4-(2-Acetoxyethyl)phenol (8f)[1,16]

IR (neat) 3380, 1700 cm^{-1}; ^1H NMR δ 7.04 (2H, d, *J*=8.4 Hz), 6.75 (2H, d, *J*=8.4 Hz), 5.72 (1H, s, O*H*), 4.22 (2H, t, *J*=7.0, CH_2OAc), 2.84 (2H, t, *J*=7.0 Hz, ArCH_2), 2.02 (3H, s, OCOCH_3); MS *m/z* 180 (M^+, 0.2), 137 (4), 120 (100), 107 (63), 91 (10), 77 (21).

3.3.16. 4-(3-Acetoxypropyl)phenol (8j)

IR (neat) 3380, 1710 cm^{-1}; ^1H NMR δ 7.00 (2H, dd, *J*=7.7 Hz), 6.74 (2H, d, *J*=7.7 Hz), 5.70 (1H, s, O*H*), 4.06 (2H, t, *J*=7.0 Hz, CH_2OAc), 2.59 (2H, t, *J*=7.0 Hz, ArCH_2), 2.04 (3H, s, OCOCH_3), 1.89 (2H, tt, *J*=7.0, 7.0 Hz, CH_2); MS *m/z* 194 (M^+, 6), 134 (80), 133 (76), 119 (8), 117 (6), 107 (100), 91 (14), 77 (33).

3.3.17. 4′-Demethylepipodophyllotoxin 4-acetate (1b)

M.p. 172–174°C; was identical with that reported.[28] ^1H NMR δ 6.84 (1H, s, H-5), 6.53 (1H, s, H-8), 6.26 (2H, s, H-2′ and H-6′), 6.12 (1H, d, *J*=3.5 Hz, H-4), 5.97, 5.94 (2H, 2×d, *J*=1.0 Hz, OCH_2O), 4.63 (1H, d, *J*=5.0 Hz, H-1), 4.31 (1H, dd, J 9.5 and *J*=7.0 Hz, H-11a), 3.87 (1H, dd, *J*=11.0, 9.5 Hz, H-11b), 3.75 (6H, s, 3′-OCH_3 and 5′-OCH_3), 3.19 (1H, dd, *J*=14.0, 5.0 Hz, H-2), 2.95 (1H, dddd, *J*=14.0, 11.0, 7.0, 3.5 Hz, H-3), 2.08 (3H, s, OCOCH_3).

3.3.18. 17β-Acetoxy-1,3,5(10)-estratrien-3-ol

M.p. 217–220°C; all chemicophysical properties were identical with an authentic sample.[1,3b]

3.3.19. 17α-Acetoxy-1,3,5(10)-estratrien-3-ol

M.p. 178–180°C; all chemicophysical properties were identical with an authentic sample.[32]

Acknowledgements

We would like to thank G. Gritti for the technical assistance and A. Lorenzi for the mass spectra, and gratefully acknowledge the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) for the financial support of this work.

References

1. Breton, G. W. *J. Org. Chem.* **1997**, *62*, 8952–8954 and references cited therein.
2. Li, A.-X.; Li, T.-S.; Ding, T.-H. *J. Chem. Soc., Chem. Commun.* **1997**, *67*, 1389–1390.
3. (a) Yamada, S.; Sugaki, T.; Matsuzaki, K. *J. Org. Chem.* **1996**, *61*, 5932–5938. (b) Nagao, Y.; Fujita, E.; Kohno, T.; Yagi, M. *Chem. Pharm. Bull.* **1981**, *29*, 3202–3207.
4. *Protective Groups in Organic Synthesis*; Greene, T. W., Wuts, P. G. M., Eds; Wiley: New York, 1991; p. 90.
5. Kunesch, N.; Miet, C.; Poisson, J. *Tetrahedron Lett.* **1987**, *28*, 3569–3572.
6. Quick, J.; Crelling, J. K. *J. Org. Chem.* **1978**, *43*, 155–156.
7. Rana, S. S.; Barlow, J. J.; Matta, K. L. *Tetrahedron Lett.* **1981**, *22*, 5007–5010.
8. Posner, G. H.; Oda, M. *Tetrahedron Lett.* **1981**, *22*, 5003–5006.
9. *Enzymatic Reactions in Organic Media*; Koskinen, A. M. P., Klibanov, A. M., Eds; Chapman & Hall: Glasgow, 1996.
10. Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, M. *J. Agric. Biol. Chem.* **1989**, *53*, 1879–1884.
11. (a) Nicolosi, G.; Piattelli, M.; Sanfilippo, C. *Tetrahedron* **1992**, *48*, 2477–2482. (b) *ibid.* **1993**, *49*, 3143–3148.
12. Natoli, M.; Nicolosi, G.; Piattelli, M. *Tetrahedron Lett.* **1990**, *31*, 7371–7374.
13. Natoli, M.; Nicolosi, G.; Piattelli, M. *J. Org. Chem.* **1992**, *57*, 5776–5778.
14. Parmar, V. S.; Pati, H. N.; Sharma, S. K.; Singh, A.; Malhotra, S.; Kumar, A.; Bisht, K. S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2269–2274.
15. Parmar, V. S.; Kumar, A.; Bisht, K. S.; Mukherjee, S.; Prasad, A. K.; Sharma, S. K.; Wengel, J.; Olsen, C. E. *Tetrahedron* **1997**, *53*, 2163–2176.
16. Pedrocchi-Fantoni, G.; Servi, S. *J. Chem Soc., Perkin Trans. 1* **1992**, 1029–1033.
17. Hsiao, K.-F.; Yang, F.-L.; Wu, S.-H.; Wang, K.-T. *Biotechnol. Lett.* **1996**, *18*, 1277–1282.
18. Basavaiah, D.; Raju, S. B. *Synth. Commun.* **1994**, *24*, 467–473.
19. (a) Allevi, P.; Anastasia, M.; Cajone, F.; Ciuffreda, P.; Sanvito, A. M. *J. Org. Chem.* **1993**, *58*, 5000–5002. (b) Allevi, P.; Anastasia, M.; Ciuffreda, P. *Tetrahedron Lett.* **1993**, *34*, 7313–7316.
20. Tested lipases were as follows: Lipase from *Pseudomonas cepacia* (Lipase PS, Amano). Lipase PS used in acetylations was immobilised on Hyflo Super Cell according to Ref. 21. Pig liver acetonic powder type II (PLAP) was from Aldrich. Lipase from *Candida cylindracea* type VII (CCL) was from Sigma.
21. Bovara, R.; Carrera, G.; Ferrara, L.; Riva, S. *Tetrahedron: Asymmetry* **1991**, *2*, 931–936.
22. Iinuma, M.; Tanaka, T.; Ito, K.; Mizuno, M. *Chem. Pharm. Bull.* **1987**, *35*, 660–667.
23. Planchenault, D.; Dhal, R. *Tetrahedron* **1995**, *51*, 1395–1404.
24. Karhunen, P.; Rummakko, P.; Pajunen, A.; Brunow, G. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2303–2308.
25. Preliminary experiments, using CCL in the reported conditions,[16] performed on compounds **2a–5a** showed that their acetylation occurred at the alcoholic hydroxyl but the reaction rate was relatively lower (2–3 days). The deacetylation of **4d** and **5d** was comparably low and involved the phenolic ester. The deacetylation of **2d** and **3d** did not occur.
26. Allevi, P.; Anastasia, M.; Ciuffreda, P.; Sanvito, A. M.; Scala, A. *Chem. Phys. Lipids* **1992**, *63*, 179–189.
27. Thurston, L. S.; Imakura, Y.; Haruna, M.; Li, D. H.; Liu, Z. C.; Liu, S. Y.; Cheng, Y. C; Lee, K. H. *J. Med. Chem.* **1989**, *32*, 604–608.
28. Gupta, R. S.; Chenchaiah, P. C. *Anti-Cancer Drug Design* **1987**, *2*, 13–23.
29. Njar, V. C. O.; Caspi, E. *Tetrahedron Lett.* **1987**, *28*, 6549–6552.
30. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
31. Kuh, M.; Keller-Juslen, C.; von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 944–947.
32. Nambara, T.; Numazawa, M.; Takahashi, H. *Chem. Pharm. Bull.* **1968**, *16*, 1148–1151.