Regioselective Enzymatic Acylation and Deacetylation of Secoiridoid Glucosides

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Candida antarctica **lipase (CAL) catalyses the regioselective cinnamoylation and benzoylation of the aglycone moiety of 10-hydroxyoleoside dimethyl ester a secoiridoid glucoside. This enzyme catalyses as well regioselective deacetylation of the aglycone moiety of 10,2′,3′,4′,6′-pentaacetoxyoleoside dimethyl ester. Further action of the enzyme results in deacetylation at C-6′ and C-4′ of the glucoside moiety.**

Key words lipase; secoiridoid; regioselectivity; acylation; deacetylation

Iridoids and secoiridoids are compounds widely distributed in the vegetal kingdom, whose varied biological activities are extensively reported.[1,2] These activities could be enhanced by the presence or absence of certain functional groups in the molecule. Hydrolases regioselectivity regarding the modification of natural glycosides has been amply used.[3]

In a previous work,[4] we reported about the regioselective acetylation catalyzed by lipase from *Candida antarctica* in the aglycone moiety of 10-hydroxyoleoside dimethyl ester as well as the obtainment of two new secoiridoids glucosides by means of this enzymatic route. In this work we describe the cinnamoylation and the benzoylation of the aglycone moiety of 10-hydroxyoleoside dimethyl ester (**1a**), catalyzed by *Candida antarctica* lipase (CAL) in tetrahydrofuran (THF) where the acyl group is provided by vinyl cinnamate or vinyl benzoate. Cinnamoylation yielded the already described natural product **2**.[5] Benzoylation produced the derivative **3**, whose structure unknown as of yet, was established by spectroscopic methods.

Furthermore, we give herein, an account of the regioselective enzymatic deacetylation of 10-hydroxyoleoside dimethyl ester pentaacetate (**1b**) by the action of the same enzyme. The deacetylation occurred at the C-10 position, that is in the aglycone moiety, to give the tetraacetate derivative **4**. Further deacetylation of the tetraacetate in the same conditions yielded the diacetate **5**. Compounds **4** and **5** have not been previously described and their structures were determined by spectroscopic studies.

Acylation of 1a Cinnamoylation of **1a** was carried out with 80% yield after 7 d of reaction and a great excess of vinyl cinnamate. The product of reaction **2** was identified by comparison with an authentic sample.[5] On the other hand, benzoylation took place in 3 d with 90% yield and not so great excess of vinyl benzoate. ^{1}H-NMR spectrum (Table 1) of **3** shows signals at δ 4.98 and 4.92 of the protons H-10a and H-10b, which means paramagnetic shifts with respect to the corresponding values in the starting compound **1a**, those being at δ 4.15 and 4.28, respectively. Signals for the aromatic protons of the benzoyl group are also observed at δ 7.97, 7.38 and 7.50 corresponding to H-2″+H-6″, H-3″+H-5″ and H-4″, respectively. The rest of the signals in this spectrum were in the same range from those for the starting compound. ^{13}C-NMR (Table 1), correlation spectroscopy (COSY), heteronuclear multiple bond coherence (HMBC) and heteronuclear single quantum coherence (HSQC) experiments provided additional information which supported the proposed structure. If these two times of reaction between themselves and with those already published[3] for acetylation of **1a** are compared, it seems evident that the size of the acyl group has an influence on the rate of reaction, though the amount of enzyme used in each process is the same.

Nevertheless, it is important to observe that these three processes of acylation were produced in the aglycone moiety, which means not a very common regioselectivity[4,6,7] in enzymatic reactions of this type in natural glucosides. Figure 1 shows both processes of acylation.

Deacetylation of 1b and 4 Deacetylation of **1b** to render **4** (Fig. 1) took place in 48 h and 90% yield. ^{1}H-NMR spectrum of **4** (Table 1) showed signal for the protons H-10a and H-10b at δ 4.37 and 4.18, which correspond to the diamagnetic shifted signals at δ 4.81 and 4.74, respectively, for the starting pentaacetate (**1b**), which indicates that the deacetylation occurred at the site C-10. The chemical shifts for the rest of the protons, along with ^{13}C-NMR (Table 1), COSY, HMBC and HSQC studies, strongly supported the structure given for this compound. The aglycone moiety is again the chosen site for the enzyme to react. The preference of this deacetylation and acylations for the site C-10, may be due to the rotation of the double bond 8—9 is restricted. Deacetylation of **4** to **5** is slower; it needed 96 h and was carried out only with 50% yield. ^{1}H-NMR spectrum (Table 1) showed diamagnetic shifts for the protons H-4′, H-6′a and H-6′b from values of δ 5.09—5.15, 4.29 and 4.14, respectively, for the original tetraacetate to the corresponding values 3.80, 3.92 and 3.84, which allowed us to conclude that deacetylations were produced at the C-4′ and C-6′ positions. This conclusion was supported by ^{13}C-NMR (Table 1), COSY, HMBC and HSQC. In this case, the enzyme did not discriminate the sites C-6′ and C-4′ of the glucoside ring. Comparison of these results with those of acetylation of **1a**, already published, allowed us to observe that the site C-2′ of the glucoside ring remained unchanged, both in acetylation and deacetylation processes, suggesting that this site is far from the active centre of the enzyme.

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Table 1. ^{1}H- and ^{13}C-NMR Data in $CDCl_3$ of Compounds **3**, **4** and **5**

	3		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.81 (s)	93	5.68 (s)	92.9	5.73 (s)	93.2
3	7.47 (s)	153.5	7.46 (s)	153.1	7.46 (s)	153.4
4		108.0		108.6		108.3
5	4.04 (dd, 8.7, 4.1)	31.3	4.10 (dd, 10.2, 3.8)	30.3	4.05 (dd, 10.4, 3.8)	30.6
6a	2.83 (dd, 14.8, 4.1)	40.1	2.88 (dd, 15.9, 3.8)	39.9	2.91 (dd, 15.4, 3.8)	40.1
6b	2.42 (dd, 14.8, 9.8)		2.39 (dd, 15.9, 10.1)		2.41 (dd, 15.4, 10.4)	
7		171.7		172.1		172.5
8	6.25 (t, 6.6)	123.6	6.15 (t, 6.5)	128.7	6.15 (t, 7.0)	129.5
9		132.4		129.1		128.3
10a	4.98 (dd, 13.2, 7.6)	61.1	4.37 (dd, 13.5, 7.7)	58.8	4.36 (dd, 13.1, 7.8)	58.7
10b	4.92 (dd, 13.2, 6.1)		4.18 (dd, 13.5, 6.2)		4.17 (dd, 13.1, 6.1)	
11		166.4		166.4		166.5
1′	4.82 (d, 7.9)	99.9	5.0 (d, 8.0)	97.1	5.0—5.13 (H-1′+H-2′+H-3′)	97.5
2′	3.46—3.65 (H-2′+H-3′+H-4′)	73.1	5.09—5.15 (H-2′+H-4′)	70.7	5.0—5.13 (H-1′+H-2′+H-3′)	70.8
3′	3.46—3.65 (H-2′+H-3′+H-4′)	78.1	5.28 (t, 9.5)	72.5	5.0—5.13 (H-1′+H-2′+H-3′)	75.7
4′	3.46—3.65 (H-2′+H-3′+H-4′)	69.7	5.09—5.15 (H-2′+H-4′)	68.2	3.80 (m)	68.9
5′	3.38 (m)	76.2	3.75 (m)	72.3	3.50 (m)	76.3
6′a	3.82 (d, 10.5)	61.6	4.29 (dd, 12.4, 4.7)	61.7	3.92 (dd, 12.0, 3.0)	61.7
6′b	3.74 (dd, 10.5, 4.42)		4.14 (dd, 12.4, 2.45)		3.84 (dd, 11.9, 4.8)	
2′-CH_3CO			2.02 (s)	20.6	2.05 (s)	20.8
				169.2		171.7
3′-CH_3CO			2.03 (s)	20.6	2.13 (s)	20.8
				170.1		169.6
4′-CH_3CO			2.03 (s)	20.6		
				169.2		
6′-CH_3CO			2.09 (s)	20.6		
				170.6		
7-OCH_3	3.61 (s)	51.6	3.63 (s)	51.5	3.68 (s)	51.6
11-OCH_3	3.70 (s)	51.9	3.72 (s)	51.9	3.74 (s)	52.2
1″		129.8				
2″	7.97 (d, 7.4)	129.7				
3″	7.38 (t, 7.4)	128.4				
4″	7.50 (t, 7.6)	133.1				
5″	7.38 (t, 7.4)	128.4				
6″	7.97 (d, 7.4)	129.7				
7″		166.5				

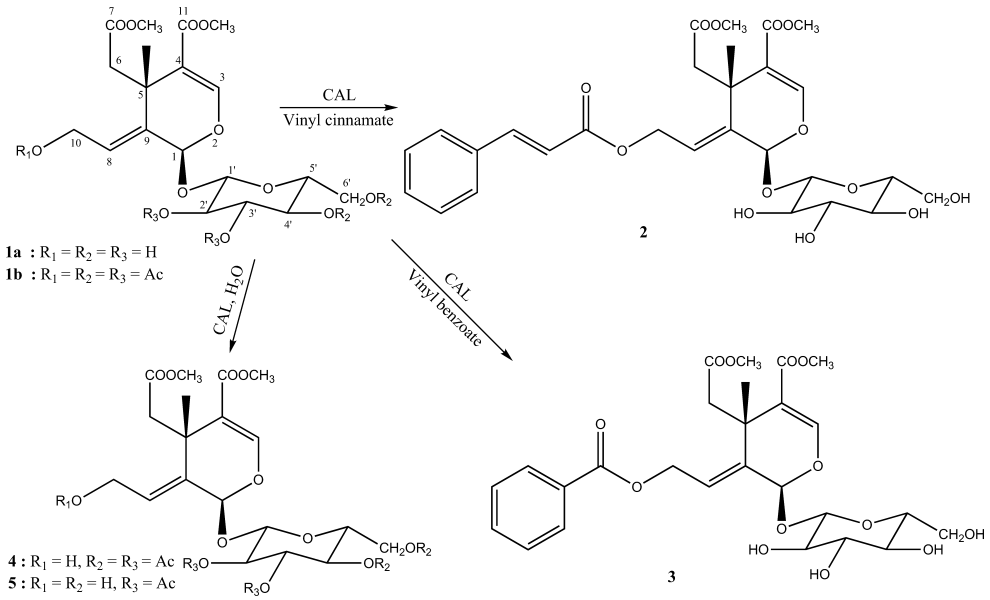

Fig. 1. Reactions of Acylation and Deacetylation Catalysed by CAL

Experimental

General Experimental Procedures One- and two-dimensions NMR studies were measured in a Bruker AMX-400 spectrometer, using TMS as an internal reference. HR-ESI-MS spectra were obtained in a Micromass LCT Premier XE (Waters) and the IR spectra were obtained on a Bruker IFS 55 (FTIR) spectrometer. HPLC works were carried out in a JASCO PU-1580, equipped with a JASCO-UV-5755 detector and a Kromasil Si (Teknokroma) (250×10 mm i.d., 5 μm) column. Different mixtures of $CHCl_3$ and MeOH were used as eluent. Conversion percentages expressed above have been estimated from the area of the chromatogram peaks.

Enzymatic Reactions Enzymatic acylation reactions were carried out on substrate obtained from *Jasminum odoratissimum*, after the already described method in a previous article.[8] The enzyme used was lipase de *C. antarctica* (Novozyme Corp.) from Sigma. In a typical experiment, 100 mg of lipase were added to a mixture of 50 mg of substrate and 30 μl of vinyl cinnamate or vinyl benzoate in 15 ml of THF. The incubation is carried out at 20 °C with stirring, being monitored by HPLC. The products of each reaction were purified by HPLC. Compound **2** was identified by comparison with authentic samples isolated from *Jasminum odoratissimum*. The starting compound **1b** was obtained by acetylation of **1a** in Ac_2O/Py.[9] One hundred milligrams of lipase were added to a solution of 50 mg of substrate **1b** in MeOH/H_2O/acetone (2 : 50 : 48) with stirring at 20 °C The reaction was monitored by HPLC and the products were purified by semipreparative HPLC.

10-Benzoyloxyoleoside Dimethyl Ester (3) Obtained as a yellow oil. $[\alpha]_D^{25}$ −142 (c=0.1, $CDCl_3$). ^{1}H- and ^{13}C-NMR see Table 1. IR (Film) ν_{max} cm^{-1}: 3402, 1710, 1636, 1442, 1273, 1703 1046, 757, 717. HR-ESI-MS (positive ions) m/z: 561.1589 $[M+Na]^+$ (Calcd for $C_{25}H_{30}O_{13}Na$: 561.1584).

10-Hydroxy-2′,3′,4′,6′-tetraacetoxyoleoside Dimethyl Ester (4) This compound was isolated as a yellow oil. $[\alpha]_D^{25}$ −135 (c=0.1, $CDCl_3$). ^{1}H- and ^{13}C-NMR see Table 1. IR (Film) ν_{max} cm^{-1}: 3512, 1738, 1707, 1634, 1437, 1214, 1034, 909, 757. HR-ESI-MS (positive ions) m/z: 625.1738 $[M+Na]^+$ (Calcd for $C_{26}H_{34}O_{16}Na$: 625.1745).

10,6′,4′-Trihydroxy-2′,3′-diacetoxyoleoside Dimethyl Ester (5) Colourless compound with $[\alpha]_D^{25}$ −128 (c=0.1, $CDCl_3$). ^{1}H- and ^{13}C-NMR see Table 1. IR (Film) ν_{max} cm^{-1}: 3432, 1729, 1633, 1438, 1370, 1242, 1074, 906. HR-ESI-MS (positive ions) m/z: 541.1526 $[M+Na]^+$ (Calcd for $C_{22}H_{30}O_{14}Na$: 541.1533).

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