Regioselective Enzymatic Acetylation of the Aglycone Moiety of a Secoiridoid Glucoside. Two New Secoiridoid Glucoside Acetates

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Candida antarctica lipase (CAL) catalyses the regioselective acetylation of the 10-hydroxyl group of 10-hydroxyoleoside dimethyl ester, a secoiridoid glucoside, using THF as a solvent and ethyl acetate or vinyl acetate as acetyl group suppliers. Two acetyl derivatives at 3'- and 6'-sites of the glucosidic ring of 10-acetoxyoleoside dimethylester, not previously described, were obtained by acetylation in the same conditions.

Key words lipase; secoiridoid; regioselective; enzymatic; acetylation

10-Hydroxyoleoside dimethyl ester (**1**) is a secoiridoid glucoside isolated from *Jasminum odoratissimum* L. (Oleaceae) along with 10-acetoxyoleoside dimethyl ester (**2**).¹⁾ Compounds of this type show a variety of biological and pharmacological activities.²⁾ Specific functionalisation, such as those at hydroxyl groups, could be of interest to improve their initial activities. The regioselective enzymatic acylation of natural glycosides in organic solvents has been extensively used,³⁾ but is quite unusual in the aglycone moiety.⁴⁾ This methodology, to the best of our knowledge, has not been used with secoiridoid glucosides. We describe herein the acetylation, at 20 °C in THF as solvent and vinyl acetate or ethyl acetate as acetyl group sources, catalysed by CAL, of the aglycone moiety of 10-hydroxyoleoside dimethyl ester (**1**) to give 10-acetoxyoleoside dimethyl ester (**2**) and the acetylation of **2** to give the compounds 6'-O-acetyl-10-acetoxyoleoside dimethyl ester (**3**), also present in *Jasminum odoratissimum*⁴⁾ and 3'-O-acetyl-10-acetoxyoleoside dimethyl ester (**4**). The reaction of either **3** and **4** in the conditions above described yields 3',6'-O-diacetyl-10-acetoxyoleoside dimethyl ester (**5**). Compounds **4** and **5** have not been previously described and their structures were established by ¹H-, ¹³C-NMR, COSY, HSQC, HMQC, ROESY NMR and HR-FAB-MS spectroscopy. Chart 1 depicts the reactions catalyzed by CAL.

Acetylation of 1 The results of the acetylation of **1**, as a function of the time of reaction and the nature of the acyl donor are shown in Table 1. Vinyl acetate produced a total regioselective and almost quantitative conversion to compound **2**. After 2 h, small amounts of compounds **3**, **4** and **5** were detected, which means that the enzyme has began to use compound **2** as a substrate as may be seen in the next section. Ethyl acetate reacted more slowly, but after 24 h, acetylation of the aglycone moiety occurred almost completely, with no further acetylation in the glycosidic ring. It should be noted

that the regioselective enzymatic esterification of the aglycone moiety of natural glucosides is quite unusual³⁾ and this is the first time that this has been described.

Acetylation of 2 The results of the reaction when **2** was used as a substrate of CAL are summarized in Table 2. In this case, again vinyl acetate is more reactive, though ethyl acetate was at the same time, acyl donor and solvent.

Acetylation of the glycosidic ring was slower and less regioselective than that of the aglycone. The enzyme did not discriminate between 3' and 6' sites and, after 10 h, both positions were acetylated. The results that are shown in Chart 1 were in agreement with the formation of **5** from **3** or **4**, and not directly from **2**. Consequently, **3** or **4** as substrates of the enzyme should lead to **5**. Table 3 summarizes the acetylation with vinyl acetate, that took place at the 3'-position when 6'-position was occupied and *vice versa*.

3'-O-Acetyl-10-acetoxyoleoside Dimethyl Ester (4) This compound was obtained as a yellowish oil. [α]_D²⁵ –130° (*c*=0.1, CDCl₃). IR (NaCl) $\nu_{\max}$ cm⁻¹: 3443, 1731, 1634, 1438, 1374, 1250, 1079, 757. The HR-FAB-MS gave a molecular mass [M+Na]⁺ of 541.1522, in accordance with the formula C₂₂H₃₀O₁₄Na. In the ¹H-NMR spectrum, which is given in Table 4, the value at δ 4.97 assigned to the H-3' must be especially regarded. This value corresponds, approximately, to a down-field shift of 1.5 ppm with respect to the average value for this proton in a non-acetylated glucoside derivative (**1**), caused by the presence of an acetoxy group at C-3'.⁴⁾ The rest of the values in this spectrum were in the same range than those for the starting compound. COSY, HMBC and HSQC experiments gave information which supported the proposed structure.

3',6'-O-Diacetyl-10-acetoxyoleoside Dimethyl Ester (5) This compound was isolated, as in the above case, as an oily yellowish substance. [α]_D²⁵ –150° (*c*=0.1, CDCl₃). IR (NaCl) $\nu_{\max}$ cm⁻¹: 3466, 1739, 1635, 1438, 1371, 1236, 1082, 1038,

Table 1. Time-Course of the Acetylation of **1** Catalysed by CAL

Acetyl donor	Conversion (%)					
	1 h	2 h	3 h	6 h	7 h	24 h
Vinyl acetate	98 (2)	90 (2) 10 (3+4+5)	85 (2) 15 (3+4+5)			
Ethyl acetate	50 (2)	60 (2)	70 (2)	80 (2)	90 (2)	98 (2)

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Table 2. Time-Course of the Acetylation of **2** Catalysed by CAL

Acetyl donor	Conversion (%)		
	5 h	22 h	48 h
Vinyl acetate	30 (3)	10 (3)	5 (3)
	20 (4)	10 (4)	5 (4)
	5 (5)	80 (5)	90 (5)
Ethyl acetate	5 (3)	10 (3)	20 (3)
	5 (4)	10 (4)	20 (4)
		15 (5)	30 (5)

Table 3. Time-Course of the Acetylation of **3** and **4** Catalysed by CAL Using Vinyl Acetate as Acetyl Donor

Substrate	Conversion (%)		
	5 h	22 h	48 h
3	20 (5)	60 (5)	80 (5)
4	10 (5)	50 (5)	75 (5)

Chart 1. Lipase-Catalyzed Acetylation of Secoiridoid Glucosides

758. The HR-FAB-MS gave a mass $[M+Na]^+$ of 583.1571, corresponding to the formula $C_{24}H_{32}O_{15}Na$, which may imply the presence of an additional acetyl group, with respect to compound **3** or **4**. This hypothesis was confirmed by the observation in the 1H -NMR spectrum the down-field shift of signals assignable to the proton H-3' and to the two protons of the methylene group at C-6' of the glucoside moiety, with

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

	4		5	
	δ_H	δ_C	δ_H	δ_C
1	5.81 (s)	93.7	5.76 (s)	93.2
3	7.47 (s)	153.2	7.47 (s)	153.0
4		108.1		108.2
5	4.00 (dd, 8.0, 4.0)	31.2	4.01 (dd, 9.0, 3.9)	30.9
6a	2.88 (dd, 16.0, 4.0)	40.1	2.83 (dd, 15.3, 4.0)	39.8
6b	2.45 (dd, 16.0, 8.0)		2.42 (dd, 15.3, 9.6)	
7		171.4		166.3
8	6.10 (t, 6.4)	123.8	6.08 (t, 6.6)	123.9
9		131.7		131.5
10a	4.81 (dd, 16.0, 8.0)	60.5	4.74 (dd, 13.5, 5.6)	60.6
10b	4.72 (dd, 16.0, 4.0)		4.84 (dd, 13.5, 7.6)	
11		166.3		153.0
1'	4.91 (d, 7.8)	100.1	4.89 (d, 7.2)	99.6
2'	3.61—3.73 (H-2' + H-4')	71.7	3.60—3.70 (m)	74.6
3'	4.97 (t, 9.3)	78.1	4.99 (t, 7.3)	77.2
4'	3.61—3.73 (H-2' + H-4')	69.3	3.58 (m)	68.7
5'	3.51 (m)	76.4	3.60—3.70 (m)	71.6
6'a	3.95 (d, 12)	62.0	4.45 (d, 12.3)	62.8
6'b	3.82 (dd, 12.0, 4.0)		4.33 (d, 12.3)	
10-CH ₃ CO	2.06 (s)	20.9, 170.8	2.06 (s)	20.8, 171.1
3'-CH ₃ CO	2.18 (s)	21.0, 172.6	2.18 (s)	21.0, 172.3
6'-CH ₃ CO			2.11 (s)	20.9, 171.5
7-OCH ₃	3.65 (s)	51.6	3.65 (s)	51.7
11-OCH ₃	3.73 (s)	51.9	3.74 (s)	51.5

regard to that of the starting compound.

Experimental

General Experimental Procedures 1H - and ^{13}C -NMR spectra were measured on a Bruker AMX-400 spectrometer using TMS as an internal standard. MS were obtained using a Micromass Autospec spectrometer and IR spectra were obtained on a Bruker IFS 28/55 (FTIR) spectrometer. HPLC separations were performed on a JASCO PU-1580 apparatus equipped with a JASCO UV-5755 UV detector, using the following columns: Supelcosil LC-18 (250×4.6 mm i.d., 5 μ m) for analytical purposes (MeOH/H₂O 1 : 1, flow rate: 3 ml/min; t_R of **1**: 5.51 min, **2**: 7.90 min, **3**: 11.04 min, **4**: 9.67 min, **5**: 12.10 min) and Kromasil C8 (250×10 mm i.d., 5 μ m) or Kromasil Si (250×10 mm i.d., 5 μ m) for preparative purposes. The percentages of conversion which are shown in the Tables 1, 2 and 3 are approximate estimations in the area of the peaks of the chromatogram.

Enzymatic Reactions The substrate for the reaction was obtained from *Jasminum odoratissimum* following the method described in a previous paper.¹⁾ The enzyme used was immobilized lipase from *Candida antarctica* (Novozyme Corp.) from Sigma. In a typical experiment 50 mg of lipase were added to the reaction mixture which consisted of 10 mg of substrate in 1.5 ml of THF and 0.35 ml of vinyl acetate or ethyl acetate. Acetylation of compounds **2**, **3** and **4** with ethyl acetate did not require THF as a solvent. In any case, both the solvent and the acyl donors must be previously dried over molecular sieves 3 Å. Incubation was carried out at 20 °C with magnetic stirring, and the reaction was monitored by HPLC. In each reaction, the products were purified by HPLC, through a Kromasil Si column and *n*-hexane : EtOAc : MeOH (30 : 60 : 10) as eluent. Compounds **2** and **3** were identified by comparison with authentic samples, isolated from *J. odoratissimum*.

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