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Regioselective Enzymatic Acylation of Methyl Shikimate. Influence of Acyl Chain Length and Solvent Polarity on Enzyme Specificity

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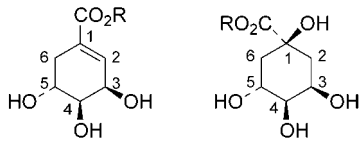
Abstract: *Candida antarctica* lipase A (CAL-A) selectively catalyzes the acylation at the secondary C-4 hydroxyl group of methyl shikimate (**2**), which possesses three secondary hydroxyl groups, the C-3 allylic one being chemically more reactive. The effect both of the acyl group of the acylating agents and of the solvent polarity has been studied. The selectivity of CAL-A is almost complete with acyl donors that possess short chains. However, when acyl donors have longer chains, better results are obtained by *C. antarctica* lipase B (CAL-B).

Shikimic acid (**1**, Figure 1) is a key intermediate in the biosynthesis of the amino acids L-phenylalanine, L-tryptophan, and L-tyrosine, as well as being a precursor to the folate coenzymes and various isoprenoid quinones.[1] The enzymes in this pathway are unique to plants, fungi, and microorganisms and are therefore important targets as potential herbicidal, antifungal, and antibacterial agents that do not affect mammals.[2] As a result, increasing effort has been directed toward the syntheses of shikimate analogues.[1c,3]

This metabolite, together with quinic acid (**3**), an alternate carbon source in the shikimate pathway, possesses several hydroxyl groups of similar reactivity in its structure, and a clear discrimination between them still remains a difficult task. Enzymatic modification[4] offers a highly efficient process compared to conventional chemical syntheses using the process of blocking/deblocking steps. This methodology has been extensively used with carbohydrates.[5] Riva and co-workers[6] have shown that lipase from *Chromobacterium viscosum* (CVL) selectively esterifies the hydroxyl group at the C-4 position of methyl quinate (**4**) and benzyl quinate (**5**). However, acylation of methyl shikimate (**2**) did not display regioselectivity with any of the enzymes tested. To our knowledge, regioselective enzymatic acylation of shikimic acid derivatives has not been described, although saturated and unsaturated esters (among them, cynnamoyl esters) of quinic and shikimic acids were shown to be plant metabolites,[7] some of them with a broad spectrum of biological activities. In our research program directed toward the syntheses of quinic and shikimic acid derivatives,[8] we describe here the regioselective enzymatic acylation of methyl shikimate and the influence of several parameters such as enzyme, solvent, temperature, and acyl chain length on the rate and regioselectivity.



1, R= H
2, R= Me

3, R= H
4, R= Me
5, R= Bn

FIGURE 1.

The three hydroxyls present in the shikimic acid lead to low solubility and reactivity, and therefore to increase the reaction rate and shift the thermodynamic equilibrium toward acylation of the substrate, activated esters were used. First, enzymatic acylation of methyl shikimate (**2**) was done in vinyl acetate as both the solvent and the acylating agent and in the presence of 4 Å molecular sieves (Scheme 1).

For the screening of suitable biocatalysts, lipases from *Candida antarctica* A (CAL-A), *C. antarctica* B (CAL-B), *Pseudomonas cepacia* (PSL-C), *C. viscosum* (CVL), and *Candida rugosa* (CRL) were tested together with the protease subtilisin (Table 1).

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SCHEME 1

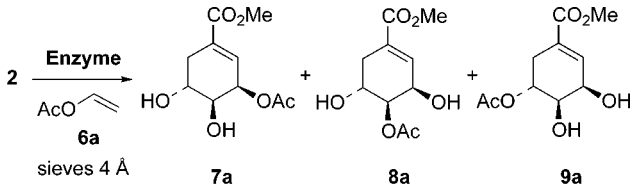

TABLE 1. Enzymatic Acylation of 2 in Vinyl Acetate at 30 °C

entry	enzyme	*t* (h)	conv (%)[a]	**7a** (%)[a,b]	**8a** (%)[a,b]	**9a** (%)[a,b]
1	CAL-A	0.75	100	17	83	
2	CAL-B	24	55	45	18	37
3	PSL-C	4.5	70	41	59	
4	Subtilisin	24	25	28	19	53
5	CVL	24	25	63	37	
6	CRL	24	45	30	38	32

[a] Based on ^{1}H NMR signal integration. [b] Percentage of regioselectivity at C-3, C-4, and C-5.

TABLE 2. Acylation of 2 with Vinyl Acetate (ratio = 1:10) Catalyzed by CAL-A at 20 °C

entry	solvent	*t* (h)	conv (%)[a]	**7a** (%)[a,b]	**8a** (%)[a,b]
1	vinyl acetate	0.75	100	10	90
2	^{t}BuOH	22	37	42	58
3	1,4-dioxane	22	90	7	93
4	toluene	3	85	5	95
5	TBME[c]	2.5	100	traces	97
6	$CHCl_3$	22	85	10	90
7	THF	22	74	12	88
8	CH_2Cl_2	22	70	25	75
9	acetone	22	52	7	93
10	MeCN	22	70	23	77

[a] Based on ^{1}H NMR signal integration. [b] Percentage of regioselectivity at C-3 and C-4. [c] *tert*-Butylmethyl ether.

Reactions were allowed to run at 30 °C until high or total conversions were achieved or, in reactions failing to achieve such conversions, for 24 h. Higher conversions were obtained with immobilized[9] biocatalysts (CAL-A, CAL-B, and PSL-C). Of the enzymes studied, CAL-A gave the best result. It catalyzed the acetylation of the C-4 hydroxyl group with high regioselectivity in less than 1 h, giving rise to the monoacetylated derivative **8a** (entry 1, Table 1). PSL-C also revealed some preference for the same hydroxyl, but the selectivity was lower (entry 3, Table 2). When CAL-B or CVL was used, both enzymes showed a slight preference for C-3 acetylation (entries 2 and 5, Table 1). However, protease subtilisin showed a certain degree of selectivity toward the C-5 position (entry 4, Table 1). In the case of CRL, an equimolecular mixture of monoacetates **7a**, **8a**, and **9a** was obtained at a low conversion (entry 6, Table 1).

To increase the selectivity exhibited by CAL-A in the enzymatic acylation and introduce acyl groups other than acetyl, an adequate solvent should be chosen. Taking into account the polar nature of the substrate, solvents of log P[10] < 2 (polar solvents) are considered to be most suitable

SCHEME 2

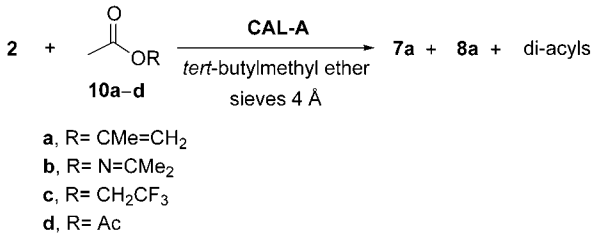

TABLE 3. Acylation of 2 with Different Acylating Agents in TBME[a] Catalyzed by CAL-A

entry	acylating agent	ratio of **2**:**10**	*T* (°C)	*t* (h)	conv (%)[b]	**7a** (%)[b,c]	**8a** (%)[b,c]	diacyls (%)[b,c]
1	**10a**	1:10	30	4.5	45	7	93	
2	**10b**	1:10	30	23	100	32	65	3
3	**10b**	1:5	30	21	100	12	75	13
4	**10c**	1:10	20	3.5	71	3	97	
5	**10d**	1:10	20	3	67		89	11
6	**10d**[d]	1:5	20	1	94		97	3

[a] *tert*-Butylmethyl ether. [b] Based on ^{1}H NMR signal integration. [c] Percentage of regioselectivity at C-3, C-4, and diacyl derivatives. [d] K_2CO_3 (0.25 equiv) was added.

for biocatalysis since they favor substrate solubility and the formation of more polar products, in this case the monoacylated species.[11] Since CAL-A is immobilized, the decrease in the catalytic activity of the enzyme in polar solvents is less pronounced. Reactions were done at 20 °C using a ratio of triol to vinyl acetate of ~1:10. This lower temperature gave rise to an increase in the selectivity shown by CAL-A in vinyl acetate (entry 1, Table 2).

In general, high conversions and regioselectivities were achieved with most of the solvents (Table 2). Excellent results were observed with CAL-A in *tert*-butylmethyl ether (TBME) and toluene (entries 4 and 5, Table 2). Thus, if the process was done in TBME, after 2.5 h the ^{1}H NMR spectrum of the reaction crude showed 97% of C-4 monoacetate **8a** and only traces of derivative **7a**.

We subsequently evaluated a variety of acylating agents in the process catalyzed by CAL-A in TBME as the solvent (Scheme 2).

If isopropenyl acetate (**10a**) was used instead of vinyl acetate, the reaction was slower and gave rise to 45% conversion after 4.5 h at 30 °C. However, the enzyme kept the excellent selectivity shown in the formation of compound **8a** (entry 1, Table 3 vs entry 5, Table 2).

Other versatile acylating agents were oxime esters.[12] In principle, acylation was run at 30 °C using a 1:10 ratio of shikimate **2** to oxime ester **10b**. In this case, total conversion was obtained after 23 h with a moderate selectivity toward the C-4 position (entry 2, Table 3). Similar behavior was observed at 20 °C. When the reaction took place in a 1:5 ratio of **2**:**10b** at 30 °C, CAL-A exhibited a moderate increase in the selectivity toward

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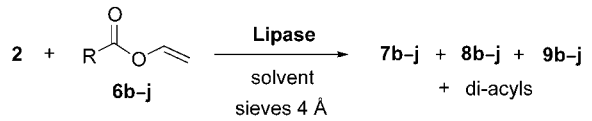
SCHEME 3



b, R= Et
c, R= ^{n}Pr
d, R= ^{n}Non
e, R= $Me(CH_2)_{10}$
f, R= $Me(CH_2)_{16}$
g, R= CH_2Cl
h, R= $CH{=}CH_2$
i, R= CH=CHMe
j, R= Ph

TABLE 4. Acylation of 2 with Vinyl Esters in TBME[a] Catalyzed by CAL-A[b]

entry	acylating agent	ratio of **2**:**6**	*T* (°C)	*t* (h)	**7** (%)[c]	**8** (%)[c]	**9** (%)[c]	diacyls (%)[c]
1	**6b**	1:5	15	0.5	10	90		
2	**6c**	1:5	15	0.66	11	70	12	7
3	**6d**	1:10	30	24	complex mixture			
4	**6e**	1:10	30	24	complex mixture			
5	**6f**	1:10	30	24	31	52	17	
6	**6g**	1:5	15	0.7	8	83	9	
7	**6h**	1:10	20	11	7	93		
8	**6i**	1:10	20	10		96		4
9	**6j**	1:10	40	8	10	90		

[a] *tert*-Butylmethyl ether. [b] Reactions were allowed to reach 100% conversion. [c] Percentage of regioselectivity at C-3, C-4, C-5, and diacyl derivatives based on ^{1}H NMR signal integration.

the C-4 position, leading to 75% yield of derivative **8a** (entry 3, Table 3). On the other hand, if 2,2,2-trifluoroethyl acetate (**10c**) or acetic anhydride (**10d**) was used as the acylating agent, CAL-A catalyzed almost exclusively the formation of C-4-monoacetate (entries 4−6, Table 3). In the case of acetic anhydride, to remove the generated acetic acid (which inhibited the enzyme), K_2CO_3 was added to the reaction mixture. No changes in the regioselectivity were observed with any of the acylating agents tested.

To take advantage of the observation that CAL-A in TBME exhibits excellent acetylation selectivity with vinyl acetate, the reaction of **2** with other vinyl esters was studied (Scheme 3).

Table 4 shows optimized conditions for each of the vinyl esters. Enzymatic acylation with vinyl propionate (**6b**) occurred with high selectivity (entry 1, Table 4) when the reaction was done at 15 °C with a 1:5 ratio of **2**:**6b**, 90% of C-4 monoacyl derivative **8b** being obtained.

If the acylating agent was vinyl butyrate (**6c**), the process took place with a moderate selectivity of 70% toward the same position mentioned above (entry 2, Table 4). However, CAL-A did not selectively catalyze the acylation with vinyl decanoate (C_{10}), vinyl laurate (C_{12}), or vinyl stearate (C_{18}) (entries 3−5, Table 4). The influence of the selectivity as a function of the acyl donor chain length is noteworthy. CAL-A is more specific toward short- rather than long-chain vinyl esters. This enzyme preference decreases in the order vinyl acetate ∼ propionate > butyrate > decanoate ∼ laurate ∼ stearate.

Although vinyl chloroacetate (**6g**) gave preferably C-4 chloroacetate **8g** (entry 6, Table 4), isolation from the reaction crude was not possible due to an acyl migration from the C-4 position to the C-3 or C-5 position during flash chromatography purification. We previously observed that in solution, the C-4 acetate (**8a**) was transformed little by little into the C-3 acetate (**7a**) until an equilibrium was achieved, in which **7a** and **8a** were the major and minor compounds, respectively. To avoid this migration, C-4 acyl derivatives should be handled very carefully using neutral silica gel for chromatography and their stay in solution should be shortened as much as possible. In the case of the C-4 chloroacetate, this migration was favored by the inductive effect of the chlorine atom.

TABLE 5. Acylation of 2 with Vinyl Stearate (ratio = 1:3) at 30 °C over 24 h Catalyzed by CAL-B

entry	solvent	conv (%)[a]	**7f** (%)[a,b]	**8f** (%)[a,b]	**9f** (%)[a,b]
1	acetone	77		100	
2	1,4-dioxane	61		100	
3	^{t}BuOH	60		100	
4	MeCN	47		100	
5	CH_2Cl_2	80	21	79	
6	$CHCl_3$	85	30	70	
7	THF	67	19	68	13
8	toluene	70	60	20	20
9	TBME[c]	50	22	32	46

[a] Based on ^{1}H NMR signal integration. [b] Percentage of regioselectivity at C-3, C-4, and C-5. [c] *tert*-Butylmethyl ether.

When the reaction was done with unsaturated esters such as vinyl acrylate (**6h**) and vinyl crotonate (**6i**), CAL-A showed excellent regioselectivity (93−96%) to the hydroxyl group at the C-4 position (entries 7 and 8, Table 4). The fact that the reaction using vinyl crotonate resulted in a higher regioselectivity than that using vinyl butyrate, which has the same chain length in the acyl moiety, revealed that CAL-A is more specific with unsaturated acyl chains.

Finally, we studied the reaction with vinyl benzoate (**6j**). Due to the lower reactivity of this acylating agent, the process did not evolve at 30 °C with either 1:5 or 1:10 ratios, and it was necessary to heat the mixture to 40 °C to reach total conversion with high selectivity toward the C-4 position (entry 9, Table 4).

Since the preference of CAL-B for long-chain acyl donors has been described in the literature,[13] and in view of the poor selectivity exhibited by CAL-A with vinyl decanoate, vinyl laurate, and vinyl stearate, the acylation of **2** performed with vinyl stearate (C_{18}) and catalyzed by CAL-B was checked. Among the solvents tested, acetone, 1,4-dioxane, *tert*-butyl alcohol, and acetonitrile gave exclusively acylation at the C-4 hydroxyl group (entries 1−4, Table 5).

Higher conversions were achieved with acetone > 1,4-dioxane ∼ ^{t}BuOH > MeCN. When the reaction took place in methylene chloride, chloroform, or THF, CAL-B showed moderate selectivities toward the same position (entries 5−7, Table 5). On the other hand, if toluene or TBME was used as a solvent, the process was not selective (entries 8 and 9, Table 5). The fact that only monoacylated products were formed can be attributed to the nature of the solvents (log $P \leq 2$), which are hydrophilic

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TABLE 6. Enzymatic Acylation of 2 with Vinyl Esters (ratio = 1:10) in Acetone at 30 °C

entry	enzyme	acylating agent	*t* (h)	conv (%)[a]	**7** (%)[a,b]	**8** (%)[a,b]	**9** (%)[a,b]
1	CAL-B	**6d**	24	53	46	47	7
2	CAL-B	**6e**	24	74	39	54	7
3	CAL-B	**6f**	24	80		100	
4	CAL-A	**6d**	18	100	60	40	
5	CAL-A	**6e**	18	100	56	44	
6	CAL-A	**6f**	18	100	12	88	
7	PSL-C	**6d**	21	65	56	44	
8	PSL-C	**6e**	21	77	40	60	
9	PSL-C	**6f**	21	80	6	94	

[a] Based on ^{1}H NMR signal integration. [b] Percentage of regioselectivity at C-3, C-4, and C-5.

solvents with an affinity for more polar molecules, in this case the monostearyl derivative. Moreover, long-chain vinyl esters are less polar than short- and medium-sized chain esters, which favors the precipitation of the monoacyl compound[13b,14] in the polar medium and avoids polyacylation and transposition reactions.

Next, we compared the specific activity of CAL-B with medium-sized vinyl esters in acetone. As shown in Table 6, the nature and the structure of the acylating agent have a strong influence on the selectivity of the acylation reaction. CAL-B did not display any remarkable selectivity with vinyl decanoate (C_{10}) or laurate (C_{12}) (entries 1 and 2, Table 6).

Additionally, we performed the acylation catalyzed by CAL-A in acetone with medium- and long-chain acyl donors. The best regioselectivity was reached with vinyl stearate, whereas the reactions with vinyl decanoate and vinyl laurate took place without selectivity (entries 4−6, Table 6). Similar results were obtained with PSL-C (entries 7−9, Table 6), which catalyzed the acylation of methyl shikimate (**2**) using vinyl stearate with excellent selectivity.

In summary, an exhaustive study of the influence of parameters such as enzyme, solvent, temperature, and acyl chain length on the selective acylation of methyl shikimate has been performed. Of note is the excellent selectivity exhibited by CAL-A toward the acylation at the most highly hindered C-4 hydroxyl group of methyl shikimate (**2**) using vinyl esters as acylating agents in *tert*-butylmethyl ether. This enzyme is more specific with short-chain than with long-chain vinyl esters. The rate of selectivity decreases in the order vinyl acetate ∼ propionate > butyrate > decanoate ∼ laurate ∼ stearate. Excellent results were also obtained with vinyl acrylate, vinyl crotonate, and vinyl benzoate. For long-chain vinyl esters, the change from *tert*-butylmethyl ether to acetone as the solvent gives rise to an increase in the regioselectivity of the enzymatic acylation. In this case, CAL-B is more specific, catalyzing the acylation of **2** with vinyl stearate exclusively at the C-4 position. By means of biocatalysts in organic solvents, novel saturated, unsaturated, and aromatic monoesters of shikimic acid have been synthesized. These can be candidates for use as potentially therapeutically useful derivatives.

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Experimental Section

General. *C. viscosum* lipase (CVL, 3800 U/mg of solid), *C. antarctica* lipase B (CAL-B, Novozym 435, 7300 PLU/g), immobilized *P. cepacia* lipase (PSL-C, 783 U/g), *C. rugosa* lipase (CRL, typeVII, 950 U/mg of solid), Subtilisin (type VIII, 12 u/mg of solid), and *C. antarctica* lipase A (CAL-A, chirazyme L-5, c-f, lyophilized, 25 U/g using 1-phenylethyl acetate) were obtained commercially. Column chromatography was performed over silica 60 Å (32−63 μm) pH 7. Analytical TLC was performed using silica 60 F_{254} aluminum sheets. The chromatograms were visualized by heating after spraying with a 5% aqueous sulfuric acid solution containing cerium sulfate (1%) and molybdophosphoric acid (2.5%). Molecular sieves (4 Å) were dried at 180 °C in a vacuum over 2 h. Methyl shikimate was dried in a high vacuum (10^{-5} mbar) before use.

Methyl Shikimate (2). To a solution of shikimic acid (2 g, 11.48 mmol) in MeOH (25 mL) was added 10 drops of concentrated HCl. The mixture was heated at 60 °C for 4 h. The solvent was evaporated to give 2.1 g (quantitative yield) of **2** as a white solid: ^{1}H NMR (MeOH-d_6, 300) δ 2.38 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}|$ = 18.2 Hz, J_{HH} = 5.5, 1.5, 1,5 Hz), 2.88 (dddd, 1H, $H_{6e'}$, $|^2J_{HH}|$ = 18.2 Hz, J_{HH} = 4.8, 2.0, 2.0 Hz), 3.88 (m, 1H, H_5), 3.93 (s, 3H, H_8), 4.18 (m, 1H, H_4), 4.56 (m, 1H, H_3), 6.98 (ddd, 1H, H_2, J_{HH} = 2.1, 1.2, 1,2 Hz); MS (ESI$^+$, *m*/*z*) 211 [(M + Na)$^+$, 100].

Enzymatic Acylation of Methyl Shikimate (2). In a standard procedure, the acylating agent, dissolved in the appropriate solvent (750 μL), was added to an Erlenmeyer flask that contained triol **2** (13 mg, 0.069 mmol), the enzyme [CAL-A (13 mg), CAL-B (13 mg), PSL-C (26 mg), subtilisin (13 mg), CVL (13 mg), or CRL (50 mg)], and 4 Å molecular sieves (13 mg) under nitrogen. The suspension was shaken at 250 rpm (the ratio of acylating agent, temperature, and time are indicated in Tables 1−6). The mixture was filtered, and the organic solvent was evaporated. Then, the reaction crude was subjected to a careful flash chromatographic column to avoid migrations from C-4 to C-3, using silica 60 Å (32−63 μm) pH 7 (40% acetone/CH_2Cl_2 for **8a**; 20% acetone/CH_2Cl_2 for **8b** and **8c**; 15% acetone/CH_2Cl_2 for **8f**; 25% acetone/CH_2Cl_2 for **8h** and **8i**; and 10% CH_2Cl_2/Et_2O for **8j**). The operation should be done within just a few minutes to minimize the contact with both the silica gel and the eluent. Then, solvents were evaporated at 20 °C under reduced pressure.

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Supporting Information Available: Complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, microanalysis, optical rotation, and MS data for major monoacylated derivatives; the level of purity is indicated by the inclusion of microanalysis and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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