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Regioselectivity in the reaction of hydrolases with (*Z*,*E*)-hexa-2,4-diene-1,6-diol

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Abstract

A good regioselectivity was observed for enzymatic acetylation of (2*Z*,4*E*)-2,4-hexadiene-1,6-diol **3** as well as for enzymatic hydrolysis of (2*E*,4*Z*)-6-(acetyloxy)-2,4-hexadienyl acetate **6**. Finally, (2*Z*,4*E*)-6-hydroxy-2,4-hexadienyl acetate **5** was obtained in pure form after two successive acetylations with reversed selectivities. Its regioisomer, (2*E*,4*Z*)-6-hydroxy-2,4-hexadienyl acetate **4**, was prepared in a pure form by a non biochemical method.

Keywords: (*Z*,*E*)-Dienes; Regioselective acylation; Enzymatic acylation

1. Introduction

Enzymatic reactions have been used frequently in organic synthesis, mainly to obtain non-racemic compounds from a racemic, meso or prochiral source. Lipases were extensively used for this purpose. Enzymes are also able to perform regioselective reactions especially in the field of sugar chemistry [1]. Some separations on mixture of *Z*/*E* isomers can be carried out with enzymes, thus geraniol and nerol was separated by selective acetylation using porcine pancreatic lipase (PPL) as catalyst [2]. Previously, we had looked at enzymatic hydrolysis of (*Z*,*E*)-dimethyl-2,4-hexadienedioate and this reaction in the presence of pig liver esterase (PLE) [3] proved to proceed with a good regioselectivity. Recently, PLE was used in a regioselective hydrolysis of diesters from (*Z*)- and (*E*)-2-methylbutenedioic acids derivatives [4]. In the course of our work on preparation of dienes as useful intermediates in organic synthesis [5a,b,c] we have been interested in obtaining monoacetates derived from (*Z*,*E*)-hexa-2,4-diene-1,6-diol. This diol seemed to be a good candidate to study the enzymatic acetylation of allylic alcohols. This paper describes the acetylation of (*Z*,*E*)-hexa-2,4-diene-1,6-diol with vinyl acetate catalysed by an enzyme and the synthesis of some derivatives of this diol.

2. Results and discussion

The diol **2** [6] obtained from anhydride **1** [7] was subjected to a thermal ring opening in boiling xylene (Scheme 1). Only the (*Z*,*E*) isomer **3** was obtained according to Woodward–Hoffmann rules [8].

Acetylation of diol **3** using various enzymes (Scheme 2) and vinyl acetate as acyl donor and solvent was examined (Table 1). In all cases, the reaction was stopped when approximately equal amounts of diol **3** and diacetate **6** were observed (checked by GPC). The best result was obtained with *Candida cylindracea* lipase (CCL). In this case, after 25 h, the monoacetates **4** and **5** were accompanied neither with diacetate **6** nor with the starting material **3**. Moreover, the **4**/**5** ratio in favour of **5** was good, and the yield was excellent. This result is interesting. However, a difficulty, on the synthetic point of view, is that we did not find conditions to separate both of them. The other enzymes led to an inverted selectivity with respect to the previous one. As for the reaction of diol **3** with acetic anhydride and without

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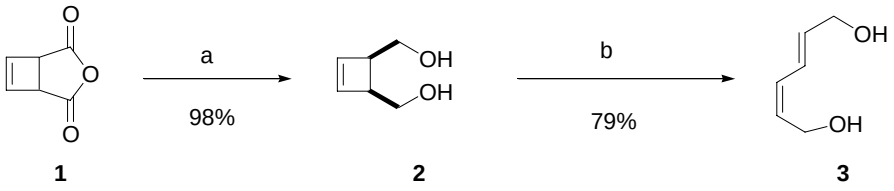
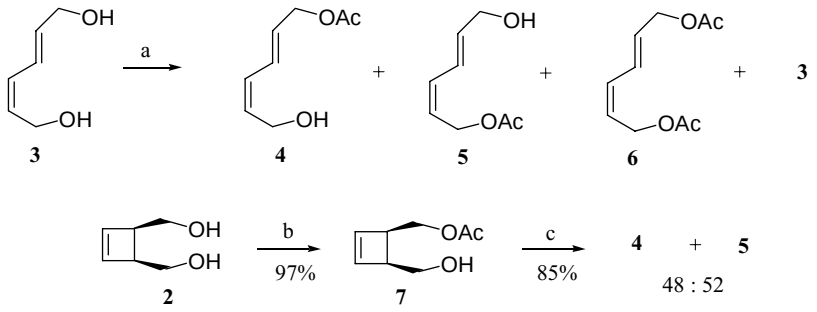


Scheme 1. (a) $LiAlH_4$; (b) xylene, reflux for 5 h and 30 min.



Scheme 2. (a) Enzyme/vinyl acetate; (b) PFL/vinyl acetate; (c) xylene, reflux for 5 h and 30 min.

enzyme, it led to a bad result: both monoacetates **4** and **5** were thus obtained in same amount and in poor yield together with the starting material **3** and diacetate **6**. Thermal opening in refluxing xylene of monoacetate **7** [9] obtained from diol **2** was not selective either and led to monoacetates **4** and **5** in a 48:52 ratio, respectively.

The enzymes used for acetylation could also be used in hydrolysis of the diacetate **6** (Table 2). It was expected that selectivity between **4** and **5** would then be inverted with respect to acetylation of diol **3**. Effectively, comparison between Tables 1 and 2 shows that the results are more or less coherent for a given lipase. However, CCL did not show any selectivity for the hydrolytic process whereas it gave an excellent result in the course of acetylation.

It should be interesting to obtain pure **5** despite the impossibility, that we experienced, to separate **4** and **5**. We then subjected the ***4*** + ***5*** mixture obtained in reaction with CCL (Table 1, entry 4) to another acetylation step in the presence of *Rhizomucor mihei* lipase (MML). As acetylation of the (*E*) moiety of **3** and hydrolysis of the (*Z*) moiety of **6** (Table 1, entry 3 and Table 2, entry 3) are strongly predominant with this lipase, it seemed probable that a large amount of **4** would be acetylated in these conditions to provide diacetate **6**, and that a large part of **5** would remain unchanged. The result was satisfying and **5** could effectively be isolated in 65% yield (or 64% overall yield for both steps) (Scheme 3).

The monoacetate **4** was prepared in a pure form using a non biochemical pathway. We took advantage of the rules for opening of cyclobutene rings [10,11].The alcohol **7** was oxidised with a Swern protocol. We converted a group with a low *outward* torquoselectivity (CH_2OH) into a group with a strong *inward* torquoselectivity (CHO). The resulting aldehyde **8** was rather unstable and not isolated; it cleanly led to the dienic (2*Z*,4*E*)-aldehyde **9**, simply by heating in refluxing diethyl ether for 1 h (Scheme 4). The reduction of this aldehyde **9** with sodium borohydride afforded the compound **4**. We noticed that the aldehyde **9** was easily converted

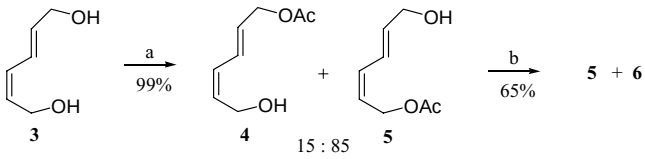
Table 1
Acetylation of diol **3** with enzyme

Entry	Enzyme	Time (h)	Ratio **3**/(**4** + **5**)/**6**	Ratio **4**/**5**	Yield (%)
1	PFL[a]	7	26/47/27	80/20	37
2	PCL[b]	9	28/39/33	65/35	31
3	MML	336	10/82/8	76/24	76
4	CCL	25	0/100/0	15/85	99
5	PPL	168	–	–	–

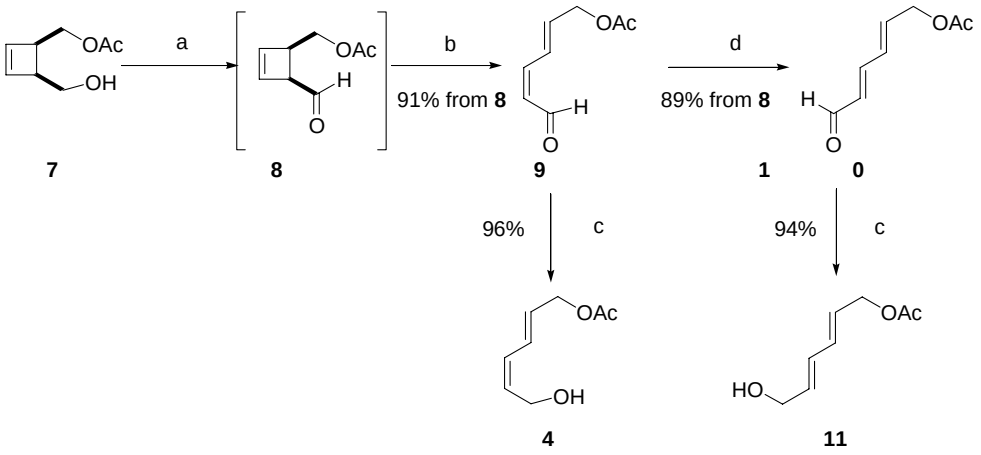
[a] *Pseudomonas fluorescens* lipase.
[b] *Pseudomonas cepacia* lipase.

Table 2
Enzymatic hydrolysis of diacetate **6**

Entry	Enzyme	Time (h)	Ratio **3**/(**4** + **5**)/**6**	Ratio **4**/**5**	Yield (%)
1	PFL	5	22/71/7	25/75	53
2	CCL	28	16/81/3	50/50	60
3	MML	28	2/98/0	20/80	93
4	PLE	3	11/87/2	80/20	70
5	PCL	7	29/69/2	35/65	52



Scheme 3. (a) CCL/vinyl acetate, RT, 25 h; (b) MML/vinyl acetate, RT, 50 h.



Scheme 4. (a) $(COCl)_2$/DMSO/CH_2Cl_2, −78 °C, 1 h; (b) diethyl ether, reflux for 3 h; (c) $NaBH_4$/THF, 30 min; (d) PTSA/$CHCl_3$, reflux for 1 h.

into its (2*E*,4*E*)-isomer **10** by reflux with chloroform and *p*−toluenesulphonic acid (*p*-TSOH) for 1 h. Reduction of **10** with sodium borohydride afforded the (*E*,*E*)-monoacetate **11**.

The pure hydroxyacetates **4** and **5**, were clearly identified by NMR (n.O.e, selective decoupling, COSY) and assignments of ^{1}H signals of both CH_2 thanks to the coupling with ^{1}H of OH in DMSO-d_6.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. IR spectra were recorded with a FT infrared spectrophotometer. Melting points are uncorrected. Elemental analyses were performed by the service of microanalyses, CNRS ICSN, Gif sur Yvette. The column chromatography were run on silica gel Gerudan SI 60, 230–400 mesh, under 1–2 bar.

3.1.1. (2Z,4E)-2,4-Hexadiene-1,6-diol *3*

A solution of diol **2** (1.2 g, 10.5 mmol) in xylene (8 ml) was refluxed for 5 h and 30 min under N_2. After removal of the solvent, the residue was purified by column chromatography (30/1, Et_2O/MeOH 95/5) to give diene **3** (950 mg, 79%) as an oil. ^{1}H NMR ($CDCl_3$): 2.48 (1H, broad s), 2.61 (1H, broad s), 4.22 (2H, dd, $J = 5.4, 1.4$), 4.32 (2H, d, $J = 6.8$), 5.65 (1H, dt, $J = 11.4, 6.8$), 5.90 (1H, dt, $J = 16.7, 5.4$), 6.13 (1H, dd, $J = 11.4, 11.4$), 6.55 (1H, ddd, $J = 16.7, 11.4, 1.4$). ^{13}C NMR ($CDCl_3$): 58.1, 62.0, 125.3, 129.7, 130.0, 134.3. IR (film) ν (cm^{-1}): 3230, 1613, 1320 and 1155. Anal. calcd. for $C_6H_{10}O_2$ (+0.1H_2O): C, 62.15; H, 8.87. Found: C, 62.17; H, 9.00.

3.1.2. General procedure for the enzymatic acetylation

A solution of diol **3** (456 mg, 4 mmol) and enzyme (20 mg) in vinyl acetate (15 ml, distilled over K_2CO_3) was stirred at RT, the progress of the reaction was monitored by GC. The solvent was evaporated and the residue purified by column chromatography. The ratio **4**/**5** was determined by ^{1}H NMR.

3.1.3. (2E,4Z)-6-(Acetyloxy)-2,4-hexadienyl acetate *6*

To a solution of diol **3** (570 mg, 5 mmol) in CH_2Cl_2 (10 ml) were added DMAP (10 mg), triethylamine (390 μl, 5.5 mmol), and then very slowly acetyl chloride (373 μl, 5.25 mmol). This mixture was stirred for 3 h before addition of saturated $NaHCO_3$ (3 ml). The organic layer was diluted with CH_2Cl_2 (50 ml) and washed successively with 1/2 saturated NH_4Cl (30 ml), water (2× 20 ml) and saturated NaCl (30 ml). After drying ($MgSO_4$) the solvent was evaporated and the residue purified by column chromatography (40/1, CH_2Cl_2/Et_2O 85/15) to give **6** as an oil (777 mg: 78%). ^{1}H NMR ($CDCl_3$): 2.07 (3H, s), 2.09 (3H, s), 4.63 (2H, d, $J = 6.2$), 4.74 (2H, d, $J = 7.1$), 5.65 (1H, dt, $J = 10.8, 7.1$), 5.85 (1H, dt, $J = 15.1, 6.2$), 6.18 (1H, dd, $J = 10.8, 10.8$), 6.58 (1H, dd, $J = 15.1, 10.8$). ^{13}C NMR ($CDCl_3$): 23.2, 65.5, 66.7, 127.9, 130.3, 132.0, 133.8, 173.0, 173.2. IR (film) ν (cm^{-1}): 3028, 2960, 1740, 1610, 1270. Anal. calcd. for $C_{10}H_{14}O_4$: C, 60.59; H, 7.12. Found: C, 60.65; H, 7.09.

3.1.4. General procedure for the enzymatic hydrolysis

To a buffered (pH 7.2) solution were added diacetate **6** (50 mg, 0.25 mmol) and enzyme (5 mg). During the reaction the pH was held at 7.2 by controlled addition of 0.5 M NaOH using an autotitrator and pH stat. The aqueous solution was extracted with Et_2O (2× 10 ml) and the combined extracts were dried ($MgSO_4$). The solvent was evaporated and the residue purified by column chromatography. The ratio **4**/**5** was determined by ^{1}H NMR.

3.1.5. (2Z,4E)-6-Hydroxy-2,4-hexadienyl acetate *5*

A solution of diol **3** (456 mg, 4 mmol) and *C. cylindracea* lipase (20 mg) in vinyl acetate (15 ml) was stirred for 25 h at room temperature. The solvent was evaporated and the residue purified by column chromatography (30/1, CH_2Cl_2/Et_2O 7/3) to give a mixture of **4** and **5** (15/85)

(615 mg, *quantitative*). This oil was dissolved in vinyl acetate (15 ml) and *R. mihei* lipase (20 mg) was added. The resulting solution was stirred for 50 h at room temperature. The solvent was evaporated to give a mixture of **5** and **6** which was purified by column chromatography (35/1, CH_2Cl_2/Et_2O 7/3) to obtain pure **5** (398 mg, 65%) as an oil. ^{1}H NMR ($CDCl_3$): 2.08 (3H, s), 3.19 (1H, s broad), 4.24 (2H, d, $J = 5.5$), 4.75 (2H, d, $J = 7.1$), 5.55 (^{1}H, dt, $J = 10.9, 7.1$), 5.94 (1H, dt, $J = 15.0, 5.5$), 6.21 (1H, dd, $J = 10.8$), 6.58 (1H, dd, $J = 15.0, 10.8$). ^{13}C NMR ($CDCl_3$): 21.2, 62.3, 65.1, 119.2, 123.2, 129.3, 135.1, 171.1. IR (film) ν (cm^{-1}): 3210, 1742, 1610 and 1270. Anal. calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.74. Found: C, 61.71; H, 7.73.

*3.1.6. (2E,4Z)-6-Oxo-2,4-hexadienylacetate **9***

To a solution of DMSO (1 ml, 14.1 mmol) in CH_2Cl_2 (34 ml) at −78 °C under N_2, oxalyl chloride (0.61 ml, 7 mmol) was added dropwise. After 5 min a solution of alcohol **7** (780 mg, 5 mmol) in CH_2Cl_2 (24 ml) was added. The resulting mixture was stirred for 1 h ($T < -50$ °C) before adding triethylamine (3.5 ml, 25 mmol) and CH_2Cl_2 (150 ml). The solution was washed successively with water (3× 50 ml) and saturated NaCl (50 ml). After drying ($MgSO_4$) the solvent was removed under reduced pressure to give **8** as an oil. This oil was immediately dissolved in Et_2O (20 ml) and refluxed for 1 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (40/1, CH_2Cl_2/Et_2O 85/15) to give **9** as an oil (698 mg: 91%). ^{1}H NMR ($CDCl_3$): 2.13 (3H, s), 4.74 (1H, dd, $J = 5.7, 1.4$), 5.93 (1H, dd, $J = 11.0, 7.7$), 6.19 (1H, dt, $J = 5.0, 5.7$), 6.94 (1H, dd, $J = 12.0, 11.0$), 7.25 (1H, ddd, $J = 15.0, 12.0, 1.4$), 10.19 (1H, d, $J = 7.7$). ^{13}C NMR ($CDCl_3$): 20.8, 63.5, 126.0, 128.1, 137.5, 145.8, 170.6, 190.3. IR (film) ν (cm^{-1}): 3055, 2963, 1735, 1695, 1240. Anal. calcd. for $C_8H_{10}O_3$: C, 62.33; H, 6.54. Found: C, 62.11; H, 6.37.

*3.1.7. (2E,4Z)-6-Hydroxy-2,4-hexadienyl acetate **4***

To a solution of **9** (617 mg, 4 mmol) in THF (8 ml) at −10 °C under N_2, $NaBH_4$ (151 mg, 4 mmol) was added by small quantities. After stirring for 30 min at −10 °C, a saturated solution of $NaHCO_3$ (1 ml) was added and the mixture was stirred for 15 min. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (80 ml). The organic layer was washed with water (2× 30 ml) and saturated NaCl (30 ml), then dried ($MgSO_4$).The solvent was removed under reduced pressure and the residue purified by column chromatography (40/1, CH_2Cl_2/Et_2O 75/25) to give **4** (597 mg, 96%) as an oil. ^{1}H NMR ($CDCl_3$): 2.09 (3H, s), 2.67 (1H, broad s), 4.34 (2H, d, $J = 6.7$), 4.63 (2H, d, $J = 6.0$), 5.68 (1H, dt, $J = 11.0, 6.7$), 5.85 (1H, dt, $J = 15.2, 6.0$), 6.10 (1H, dd, $J = 11.0, 11.0$), 6.58 (1H, dd, $J = 15.2, 11.0$).^{13}C NMR ($CDCl_3$): 20.8, 58.4, 64.6, 128.2, 128.6, 129.0, 131.2, 170.9. IR (film) ν (cm^{-1}): 3250, 1730, 1615 and 1240. Anal. calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.74. Found: C, 61.64; H, 7.56.

*3.1.8. (2E,4E)-6-Oxo-2,4-hexadienyl acetate **10***

Aldehyde **8** obtained as above, from **7** (780 mg, 5 mmol), was dissolved in $CHCl_3$ (20 ml), this solution was acidified with *p*-TsOH (10 mg) and refluxed for 1 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (40/1, CH_2Cl_2/Et_2O 80/20), to give **10** as an oil (683 mg, 89%).^{1}H NMR ($CDCl_3$): 2.11 (s, 3H), 4.72 (2H, dd, $J = 5.7, 1.4$), 6.18 (1H, dd, $J = 15.6, 7.7$), 6.26 (1H, dt, $J = 15.5, 5.7$), 6.51 (1H, ddd, $J = 15.5, 10.9, 1.4$), 7.08 (1H, dd, $J = 15.6, 10.9$), 9.58 (1H, d, $J = 7.7$).^{13}C NMR ($CDCl_3$): 20.7, 63.2, 129.8, 132.1, 137.1, 149.9, 170.1, 193.1. IR (film) ν (cm^{-1}): 3020, 1741, 1687 and 1290. Anal. calcd. for $C_8H_{10}O_3$: C, 62.33; H, 6.54. Found: C, 62.52; H, 6.59.

*3.1.9. (2E,4E)-6-Hydroxy-2,4-hexadienyl acetate **11***

With the same procedure as in Section 3.1.7, **10** (615 mg, 4 mmol) was converted to **11** (588 mg, 94%) as an oil.^{1}H NMR ($CDCl_3$): 1.73 (1H, broad s), 2.08 (s, 3H), 4.20 (2H, d, $J = 5.6$), 4.60 (2H, d, $J = 6.4$), 5.76 (1H, dt, $J = 14.5, 6.4$), 5.89 (1H, dt, $J = 14.8, 5.6$), 6.24–6.33 (2H, m). ^{13}C NMR ($CDCl_3$): 21.0, 63.1, 64.6, 126.8, 129.8, 133.4, 133.6, 170.8. IR (film) ν (cm^{-1}): 3350, 1732, 1622 and 1196. Anal. calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.74. Found: C, 61.56; H, 7.65.

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