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Lipase-catalyzed Regioselective Protection of Hydroxyl Groups in Aromatic Dihydroxyaldehydes and Ketones

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Abstract *Pseudomonas cepacia* lipase catalyzes the acetylation in organic solvent of dihydroxyaldehydes and ketones using vinyl acetate as acyl donor The method is completely regioselective and allows to obtain partially acetylated compounds different from those obtained by enzymic hydrolysis of polyacetoxy arylaldehydes and ketones

INTRODUCTION

In order to obtain selectively acylated acetophenones as starting material for the synthesis of biologically active polyphenolic compounds, Vs Parmar *et al* [1a,b] have resorted to the lipase catalyzed regioselective deacylation of polyacetoxy acetophenones. We have now examined the possibility of preparing selectively protected aromatic dihydroxyaldehydes and ketones by direct, irreversible acetylation[2a-c] using vinyl acetate as acyl donor and a lipase as catalyst.

RESULTS AND DISCUSSION

Compounds **1-7** dissolved in a 9 1 (vol·vol) mixture of cyclohexane/*tert*-amyl alcohol were treated with five molar equivalents of vinyl acetate in the presence of *Pseudomonas cepacia* lipase adsorbed on Celite This enzyme in preliminary experiments proved to be more efficient than *Chromobacterium viscosum* lipase that we had used in previous work on the biocatalyzed esterification of monohydric phenols [3] At the end of the reaction the enzyme was removed by filtration and the products separated by column chromatography. Assignment of the structure of the monoacetates (Table 1) rests on the following arguments.

i) compounds **1a-7a** shows in the ir spectrum a strong band at 1640-1665 cm^{-1} characteristic of *o*-hydroxy substituted aromatic aldehydes and ketones,[4] ii) compounds **11b** and **12b** show a sharp peak between 3520 and 3540 cm^{-1} indicating a free hydroxyl at position *ortho* to the alogen,[5] while this peak is absent in the spectrum of **11a** and **12a**, iii) in the NOESY spectrum of **11b** and **12b** cross-peaks are observed between the acetate protons and the aromatic protons at positions 3 and 5

The results of the experiments of enzyme catalyzed acetylation of the substrates taken into consideration in the present work are summarized in Table 1 2',5'-Dihydroxyacetophenone (**1**), 2',5'-dihydroxypropiophenone (**2**) and 2,5-dihydroxybenzaldehyde (**3**) showed complete regioselectivity, all of them undergoing acetylation exclusively at position 5 to give respectively 5'-acetoxy-2'-hydroxyacetophenone (**1a**), 5'-acetoxy-2'-hydroxypropiophenone (**2a**) and 5-acetoxy-2-hydroxybenzaldehyde (**3a**) As much selectivity was found in the acetylation of 2,3-dihydroxybenzaldehyde (**4**) which gave only 3-acetoxy-2-hydroxybenzaldehyde (**4a**), however with a reaction rate definitely slower Also 2',4'-dihydroxyacetophenone (**5**), 2',4'-dihydroxypropiophenone (**6**) and 2,4-dihydroxybenzaldehyde (**7**) all reacted regioselectively to give the corresponding 4-acetyl derivatives **5a**, **6a** and **7a**, respectively It was observed in these last cases that the reaction, in the standard conditions, did not proceed beyond ca 25% conversion, a more acceptable conversion requiring repeated additions of enzyme This anomalous behaviour seems to be associated with the presence in the molecule of a hydroxyl at *para* to the carbonyl group. Indeed, also the simplest compounds of this type, 4-hydroxybenzaldheyde (**8**) and 4'-hydroxyacetophenone (**9**) react with comparable conversion Also in these cases conversion could be increased by repeated additions of the enzyme

In a recent study[1a,b] of the regioselective deacylation of polyacetoxy acetophenones catalyzed by porcine pancreas or *Candida cylindracea* lipases, the authors suggest that the enzyme binds to the carbonyl in such a way as to inhibit the hydrolysis of the acetoxyl *ortho* to the carbonyl group and at the same time to place the other acetoxyls near the appropriate portion of the active site of the lipase, so making their hydrolysis easier Since an experiment of transesterification catalyzed by *Pseudomonas cepacia* lipase in 2',5'-diacetoxyacetophenone (a substrate which is reported to give 2'-acetoxy-4'-hydroxy-acetophenone with high regioselectivity when porcine pancreas or *Candida cylindracea* lipase are used as catalyst) proved to be poorly selective, we concluded that in the case in hand an explanation of the regioselectivity had to be looked for elsewhere. Since chelation of the carbonyl with the hydroxyl group in *ortho* appeared a valid alternative, we were induced to examine the behaviour of 3,4-dihydroxybenzaldehyde (**10**), which was expected to react with poor, if any, regioselectivity Unfortunately, in the condition of our experiments, **10** proved to be completely unreactive Hence, we considered two more 2,4-dihydroxybenzene incapable of chelation, i e 2,4-dihydroxychlorobenzene (**11**) and 2,4-dihydroxybromobenzene (**12**). These substrates both reacted with moderate selectivity, thus confirming the opinion that in polyhydric arylaldehydes and ketones chelation is

Table 1 Transesterification of substituted dihydroxybenzenes mediated by *Pseudomonas cepacia* lipase in cyclohexane/*tert*-amyl alcohol [a]

Substrate	time (h)	conversion (%)	Product/s (%)
2',5'-Dihydroxyacetophenone **1**	12	97	5'-Acetoxy-2'-hydroxyacetophenone **1a**
2',5'-Dihydroxypropiophenone **2**	24	93	5'-Acetoxy-2'-hydroxypropiophenone **2a**
2,5-Dihydroxybenzaldehyde **3**	24	78	5-Acetoxy-2-hydroxybenzaldehyde **3a**
2,3-Dihydroxybenzaldehyde **4**	48	76	3-Acetoxy-2-hydroxybenzaldehyde **4a**
2',4'-Dihydroxyacetophenone **5**	24	20	4'-Acetoxy-2'-hydroxyacetophenone **5a**
2',4'-Dihydroxypropiophenone **6**	24	20	4'-Acetoxy-2'-hydroxypropiophenone **6a**
2,4-Dihydroxybenzaldehyde **7**	24	22	4-Acetoxy-2-hydroxybenzaldeyde **7a**
4-Hydroxybenzaldehyde **8**	24	25	4-Acetoxybenzaldehyde **9a**
4'-Hydroxyacetophenone **9**	24	26	4'-Acetoxyacetophenone **10a**
3,4-Dihydroxybenzaldeyde **10**	24		no product/s
2,4-Dihydroxychlorobenzene **11**	12	75	2-Acetoxy-4-hydroxychlorobenzene **11a** (21)
			4-Acetoxy-2-hydroxychlorobenzene **11b** (73)
			2,4-Diacetoxychlorobenzene **11c** (6)
2,4-Dihydroxybromobenzene **12**	12	82	2-Acetoxy-4-hydroxybromobenzene **12a** (26)
			4-Acetoxy-2-hydroxybromobenzene **12b** (62)
			2,4-Diacetoxybromobenzene **12c** (12)

[a] Substrate 1 mMol (0.2 mMol with **10**), vinyl acetate 460 µL (5 Mol eqs), 100 mg adsorbed *Pseudomonas cepacia* lipase (20 mg with **10**), solution *tert*-amyl alcohol/cyclohexane 10/90 (20mL), 40°C, 300 rpm Conversion and yelds were determined by gas-chromatography or ^{1}H-nmr analyses

crucial in determining the regioselectivity.

In conclusion, in aromatic dihydroxy aldehydes and ketones the hydroxyl other than the one at position *ortho* to the carbonyl is selectively acylated using vinyl acetate as acyl donor and *Pseudomonas cepacia* lipase as catalyst. Moreover, since the regiopreference in acetylation is the same as that observed in the lipase mediated hydrolysis of peracetates of polyacetoxyaryl aldehydes and ketones (although different lipases were used in the two procedures),[1a,b] direct acetylation allows to obtain partially acetylated compounds different from those obtained by peracetylation followed by enzymic alcoholysis

EXPERIMENTAL

General Methods

Gc analyses were carried out on a HP-5 %5 phenylmethylsilicone capillary column 25 m x 0.2 mm i d., N_2 as gas carrier, injector temperature 200 °C, flame ionization detector TLC was performed on Kieselgel 60 F254 pre-coated silica gel plates All compounds were detected by UV light (254 nm) or by spraying with a chromogenic reagent (10% solution of $Ce(SO_4)_2$ in 1M H_2SO_4 or a 1 5% solution of $FeCl_3$ in water). All chemicals were analytical grade. Cyclohexane and *tert*-amyl alcohol were kept overnight on 3Å molecular sieves before use. Compounds **1-12** were obtained commercially Lipase from *Pseudomonas cepacia* was obtained from Amano International Enzyme Co., and used adsorbed on Celite IR spectra were recorded in $CHCl_3$ using a Perkin Elmer FT-IR 1720X spectrometer NMR were measured in $CDCl_3$ on a Bruker AC250 spectrometer, and chemical shifts are reported as ppm (δ) downfield from TMS

Preparation of immobilized enzyme

Pseudomonas cepacia lipase (4 g) was dissolved in 0 1 N phosphate buffer (pH 7.0, 10 ml) and stirred with celite Hyflo Super Cel (10 g) The mixture, spread on a Petri dish, was left to dry for 24 h at room temperature, then kept overnight under reduced pressure The catalytic activity of the adsorbed enzyme in the esterification of 2,5-dihydroxybenzaldehyde was about 5-fold higher than that of the enzyme "straight from the bottle"

Enzyme-catalyzed esterification of compounds 1-12

In a standard experiment 1 mMol of substrate (0.2 mMol with **10**) was dissolved in 20 mL of a 9·1 (vol/vol) mixture of cyclohexane and *tert*-amyl alcohol Adsorbed *Pseudomonas cepacia* lipase (generally 100 mg, 20 mg with **10**) and 460 µL of vinyl acetate were added and the suspension was incubated at 40 °C with continuous shaking (300 rpm). The conversion of the substrate at appropriate time intervals (12-48 hs) was determined by GC or ^{1}H-nmr analysis. Finally the reaction was quenched by filtering off the enzyme and the filtrate dried in vacuo The residue was chromatographed on silica gel column using hexane/dichloromethane as eluent and the identity of the product(s) ascertained by spectroscopic analysis or comparison with authentic samples.

In further experiments with compounds **5-9** the reaction time was increased (72 hs) and two further additions of enzyme (100 mg each) ware made after 24 and 48 hs of incubation The conversion were in the range 55-65%

Alcoholysis of 2',5'-diacetoxyacetophenone

2',5'-Diacetoxyacetophenone (**1c**) (0 1 mMol) was dissolved in 2 mL of a mixture 9:1 (vol/vol) cyclohexane/*tert*-amyl alcohol After addition of adsorbed *P cepacia* lipase (10 mg) and 45 µL of *n*-butanol (5 Mol eqs) the mixture was incubated at 40 °C with shaking (300 rpm) After 5 mins the reaction was stopped by removing the enzyme ^{1}H-nmr analysis of the reaction mixture showed complete conversion of the substrate and the presence of 5'-acetoxy-2'-hydroxyacetophenone (28% yield), 2'-acetoxy-5'-hydroxy-acetophenone (65% yield) and 2',5'-dihydroxyacetophenone (7% yield)

Spectral and analytical data of previously unreported compounds

5'-Acetoxy-2'-hydroxyacetophenone (**1a**): ^{1}H NMR: δ 2.30 (s, -OCOCH$_3$), 2.57 (s, -COCH$_3$), 6 97 (d, J=9.0, H-3'), 7.19 (dd, J=2.7 and 9.0, H-4'), 7.34 (d, J=2.7, H-6'); ^{13}C NMR: ppm 20.9, 26.6, 119.2, 122.6, 124 8, 130.14, 141.9, 159.9, 169.7, 203.8; IR: ν_{max} 1760, 1651, 1427, 1370, 1323 cm^{-1}; Anal Calcd for $C_{10}H_{10}O_4$· C, 61.85; H, 5.15; found: C, 61.96; H, 5.20.

5'-Acetoxy-2'-hydroxypropiophenone (**2a**)· ^{1}H NMR. δ 1.10 (t, J=7.1, -CH$_2$CH$_3$), 2.19 (s, -OCOCH$_3$), 2.86 (q, J=7.1, -CH$_2$CH$_3$), 6.65 (d, J=7.5, H-3'), 7.08 (dd, J=2.7 and 7 5, H-5'), 7.36 (d, J=2.7, H-6'); ^{13}C NMR ppm 7.9, 20 8, 31.5, 118.7, 119.2, 121.8, 129 7, 141.9, 159.9, 169.5, 206.2; IR: ν_{max} 1763, 1651, 1623, 1587, 1484, 1428, 1370, 1280, 1173, 1124, 1014 cm^{-1}; Anal Calcd for $C_{11}H_{12}O_4$. C, 63.45, H, 5.77; found. C, 63.30, H, 5 86

5-Acetoxy-2-hydroxybenzaldehyde (**3a**) ^{1}H NMR: δ 2.30 (s, -OCOCH$_3$), 6.98 (d, J=9.0, H-3), 7.24 (dd, J=2 0 and 9.0, H-4), 7 31 (d, J=2.0, H-6), 9 83 (s, -CHO); ^{13}C NMR· ppm 20.3, 118.6, 120.2, 125 3, 130.6, 142.9, 159.2, 169.5, 195.7; IR: ν_{max} 1762, 1665, 1589, 1483, 1371, 1319, 1287, 1207, 1142, 1014 cm^{-1}, Anal. Calcd for $C_9H_8O_4$: C, 60 00, H, 4.44, found· C, 59.88; H, 4 39

3-Acetoxy-2-hydroxybenzaldehyde (**4a**). ^{1}H NMR δ 2 26 (s, -OCOCH$_3$), 6 91 (dd, J=7 8 and 7.8, H-5), 7.22 (dd, J=1 5 and 7 8, H-4), 7 37 (dd, J=1 5 and 7 8, H-6), 9.80 (s, -CHO); ^{13}C NMR: ppm 20 4, 119 4, 121 7, 129 8, 130 9, 138 8, 153 3, 168 5, 196.3, IR ν_{max} 1763, 1662, 1458, 1372, 1360, 1281, 1207 cm^{-1}, Anal Calcd for $C_9H_8O_4$ C, 60 00, H, 4 44, found: C, 60 40, H, 4 51.

4'-Acetoxy-2'-hydroxyacetophenone (**5a**) ^{1}H NMR δ 2 30 (s, -OCOCH$_3$), 2 59 (s, -COCH$_3$), 6 66 (dd, J=2 0 and 8 9, H-5'), 6 72 (d, J=2 0, H-3'), 7 73 (d, J=8.9, H-6'); ^{13}C NMR ppm 21.1, 26.5, 111 1, 112 9, 125 5, 131 9, 156.6, 163 8, 168.5, 203 5, IR nmax 1768, 1641, 1515, 1370, 1326, 1212, 1157, 1131 cm^{-1}; Anal Calcd for $C_{10}H_{10}O_4$ C, 61 85; H, 5 15, found C, 61 96, H, 5 21

4'-Acetoxy-2'-hydroxypropiophenone (**6a**) ^{1}H NMR: δ 1.22 (t, J=7.0, -CH$_2$CH$_3$), 2.29 (s, -OCOCH$_3$), 2.98 (q, J=7.0, -CH$_2$CH$_3$), 6.64 (dd, J=2 0 and 9.0, H-5'), 6.72 (d, J=2.0, H-3'), 7.77 (d, J=9.0, H-6'); ^{13}C NMR: ppm 8.2, 21.1, 31.6, 111.2, 112.8, 117.2, 131 0, 156.4, 163 9, 168.5, 206.1, IR ν_{max} 1763, 1642, 1504, 1371, 1212, 1156, 1129, 1015 cm^{-1}; Anal. Calcd for $C_{11}H_{12}O_4$· C, 63 45, H, 5 77, found. C, 63.33; H, 5.83

4-Acetoxy-2-hydroxybenzaldehyde (**7a**) ^{1}H NMR δ 2 31 (s -OCOCH$_3$), 6.74 (d, J=2 0, H-3), 6.78 (dd, J=2.0 and 8.1, H-5), 7 56 (d, J=8.1, H-6), 9.84 (s, -CHO), ^{13}C NMR. ppm 21.0, 110 6, 113.8, 118.6, 134 9, 157.8, 164 3, 168.6, 195.5; IR ν_{max} 1762, 1657, 1637, 1592, 1504, 1448, 1373, 1329, 1212, 1148, 1118, 1013 cm^{-1}; Anal Calcd for $C_9H_8O_4$ C, 60 00, H, 4.44, found: C, 60.13, H, 4 50

2-Acetoxy-4-hydroxychlorobenzene (**11a**) ^{1}H NMR. δ 2 35 (s, -OCOCH$_3$), 6 58 (bd, H-3), 6.60 (dd, J=2 5 and 8.3, H-5), 7 23 (d, J=8 3, H-6); ^{13}C NMR: ppm 20 6, 111 1, 114 4, 121 7, 130 4, 147 1, 155 1, 169 0, IR. ν_{max} 1770, 1705, 1607, 1485, 1372, 1318, 1140, 1124, 1040, 1115 cm^{-1}, Anal Calcd for $C_8H_7O_3Cl$ C, 51 50, H, 3 78, found· C, 51.40, H, 3 73

4-Acetoxy-2-hydroxychlorobenzene (**11b**). ^{1}H NMR: δ 2.27 (s, -OCOCH$_3$), 6.62 (dd, J=2.6 and 8.6, H-5), 6 74 (d, J=2 6, H-3), 7 27 (d, J=8.6, H-6); ^{13}C NMR: ppm 20.9, 110 0, 114.5, 117 2, 129 2, 150 1, 151 9, 169 5; IR· ν_{max} 3536, 1762, 1599, 1485, 1371, 1318, 1212, 1144, 1127, 1014 cm^{-1}, Anal Calcd for $C_{18}H_7O_3Cl$: C, 51.50; H, 3 78; found: C, 51 38; H, 3 74

2,4-Diacetoxychlorobenzene (**11c**). ^{1}H NMR δ 2.22 (s, -OCOCH$_3$), 2 29 (s, -OCOCH$_3$) 6 95 (dd, J=2.5 and 8.4, H-5), 6.97 (d, J=2.5, H-3), 7 39 (d, J=8.4, H-6); ^{13}C NMR. ppm 20.2, 20.6, 117.3, 120.0, 123.6, 130.0, 147 0, 149.3, 167 7 168.4; IR: ν_{max} 1768, 1593, 1482, 1418, 1371, 1258, 1212, 1146, 1064, 1047, 1015 cm^{-1}; Anal. Calcd for $C_{10}H_9O_4Cl$· C, 52.53; H, 3.97; found: C, 52.66; H, 3.92

2-Acetoxy-4-hydroxybromobenzene (**12a**) ^{1}H NMR δ 2.36 (s, -OCOCH$_3$), 6.57 (dd, J=2.5 and 8 0, H-5), 6 59 (bd, H-3), 7.38 (d, J=8 0, H-6), ^{13}C NMR: ppm 20.9, 106.2, 111 3, 115 0, 133.4, 153 5, 156 0, 169 1, IR: ν_{max} 1770, 1592, 1480, 1432, 1372, 1300, 1166 cm^{-1}; Anal Calcd for $C_8H_7O_3Br$. C, 41.59; H, 3.05; found: C, 41.71; H, 3.10.

4-Acetoxy-2-hydroxybromobenzene (**12b**) ^{1}H NMR δ 2.28 (s, -OCOCH$_3$), 6 58 (dd, J=2 6 and 8 7, H-5), 6 77 (d, J=2.6, H-3), 7 43 (d, J=8 7, H-6); ^{13}C NMR: ppm 21.0, 106.9, 109 8, 115 0, 132.2, 150 9, 152.9, 169 2, IR ν_{max} 3517, 1758, 1709, 1600, 1480, 1424, 1370, 1315, 1114, 1120, 1038, 1014 cm^{-1}; Anal Calcd for $C_8H_7O_3Br$· C, 41.59, H, 3 05, found: C, 41.72, H, 3.11.

2,4-Diacetoxybromobenzene (**12c**) ^{1}H NMR: δ 2.22 (s, -OCOCH$_3$), 2 30 (s, -OCOCH$_3$), 6.89 (dd, J=2.0 and 9 0, H-5), 6 97 (d, J=2.0, H-3), 7.56 (d, J=9.0, H-6); ^{13}C NMR: ppm 20 4, 20.7, 112 4, 117 4, 120 4, 134 0, 148 3, 150 1, 167 7, 168 3; IR: ν_{max} 1773, 1590, 1477, 1416, 1371, 1258, 1212, 1145, 1125, 1044, 1015 cm^{-1}, Anal. Calcd for $C_{10}H_9O_4Br$ C, 43 98, H, 3.32; found. C, 44 11, H, 3 39

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