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Lipase-catalyzed highly enantioselective kinetic resolution of racemic α-hydroxy butenolides

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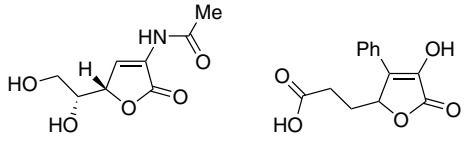
Abstract—The cyclization of 1,3-bis(trimethylsilyloxy)-1,3-butadienes **2a**–**e** with oxalyl chloride afforded functionalized γ-alkylidene butenolides **3a**–**e** while their subsequent hydrogenation gave racemic α-hydroxy butenolides **4a**–**e**. These were subjected to lipase-catalyzed kinetic resolutions in a biphasic mixture composed of phosphate buffer and 20% toluene. The highest enantioselectivities (E = 67–100) were achieved using lipase from *Burkholderia cepacia* (Amano PS).

1. Introduction

Butenolides represent pharmacophoric groups of a variety of natural products. For example, drugs such as (+)-leptosphaerin[1] or compound WF-3681, which represents an aldose reductase inhibitor produced by *Chaetomella raphigera*[2] (Scheme 1), possess a butenolide core structure. Butenolides are also important building blocks for the synthesis of natural products. For example, they have been used during the synthesis of (+)- and (−)-eldanolide,[3a] the antileukaemic lignans (+)-*trans*-burseran,[3b] (−)-isostegane,[3b] (+)- and (−)-steganacin,[3c] (−)-verrucarinolactone[3d] and chrysanthemic acid analogues.[3e]



(+)-Leptosphaerin WF-3681

Scheme 1. Examples of butenolides.

We have recently reported a new synthesis of α-hydroxy-γ-alkylidenebutenolides by Lewis acid catalyzed cyclization of 1,3-bis(trimethylsilyloxy)-1,3-butadienes with oxalyl chloride.[4] This method was applied to the synthesis of racemic α-hydroxy butenolides based on diastereoselective hydrogenations.[5] Herein, we report the enzymatic resolution of these compounds, which provides a convenient access to enantiomerically pure α-hydroxy butenolides.[6]

2. Results and discussion

Racemic butenolides **4a**–**e** were synthesized according to the literature:[4b,5] The cyclization of 1,3-bis-silyl enol ethers **2a**–**e** prepared from the β-keto esters **1a**–**e** with oxalyl chloride afforded the γ-alkylidene butenolides **3a**–**e**. Hydrogenation (H_2, Pd/C)[7] of the latter gave racemic α-hydroxy butenolides **4a**–**e** (Scheme 2).

Different lipases and esterases[8] were investigated for their activity and selectivity on racemic **4a** (Scheme 3). Out of these, lipase from *Burkholderia cepacia* (Amano PS, Amano) was found to hydrolyze the substrate with high enantioselectivity (E >100, Table 1) in a biphasic mixture composed of phosphate buffer (10 mM, pH 7.5) and 20% (v/v) toluene at 30 °C. For the isolation and determination of the enantiomeric excess, the non-converted ester **4a** was recovered from the reaction

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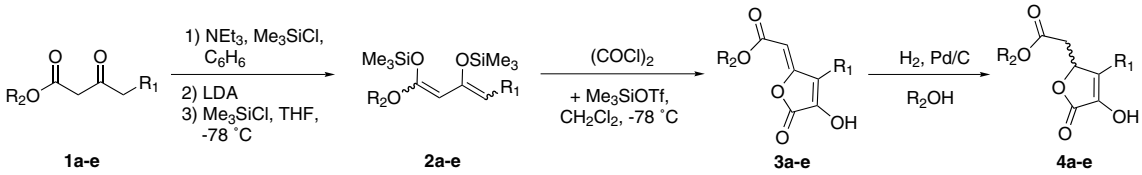
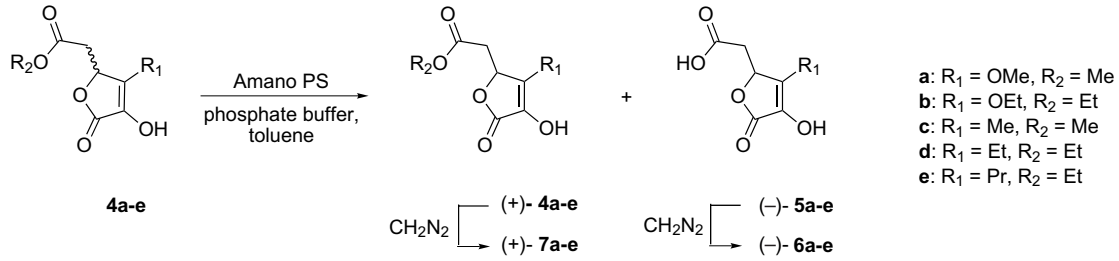


Scheme 2. Synthesis of butenolides **4a**–**e**.



Scheme 3. Lipase-catalyzed kinetic resolution of **4a**–**e** and derivatization of the acids **5a**–**e** produced. Please note that compounds **6a** and **7a**, as well as **6c** and **7c**, are identical.

Table 1. Kinetic resolution of racemic butenolides **4a**–**e** with lipase from *Burkholderia cepacia* (Amano PS) in small-scale reactions

Compound	Time (h)	Conversion[a] (%)	Enantiomeric excess		E[a]
			ee_S[b] (%)	ee_P[b,c] (%)	
4a	6	50	98	97	>100
4b	8	51	94	90	67
4c	50	50	93	93	94
4d	41	49	94	99	>100
4e	26	50	98	97	>100

[a] Calculated according to Chen et al.[9]
[b] Determined by GC with chiral column.
[c] Of methyl esters **6a**–**e**.

mixture by extraction with methylene chloride so that its enantiomeric excess could be determined directly by GC analysis. For the analysis of the formed free acid **5a**, the mixture was acidified and the acid extracted with diethyl ether. The free acid was then converted to the corresponding methyl ester **6a** with diazomethane, whereby the hydroxy group at the α-position was also methylated (Scheme 3). Using this protocol, butenolides **4b**–**e** were also subjected to lipase-catalyzed resolutions and analyzed in a similar manner (Table 1). In the cases of non-hydrolyzed **4b**–**4e**, these were also methylated with diazomethane on an analytical scale yielding **7b**–**7e** in order to facilitate GC separation of the enantiomers.

This revealed that the butenolides with an alkoxy side chain (**4a**–**b**) were hydrolyzed much faster (6–8 h) than the compounds bearing an alkyl side chain (**4c**–**e**, 26–50 h). Enantioselectivities were excellent ($E = 94$ to >100) in all cases except for **4b** ($E = 67$). In this case, slow autohydrolysis was observed, which may explain the decrease in enantiomeric excesses.

Preparative-scale experiments using 0.5 mmol of racemic butenolide had been carried out with **4a** and **4d** so far. The isolated yields were moderate to good and the enantioselectivities similar to those observed in the small-scale reactions (Table 2). Specific rotations were determined for the remaining substrates **4a** and **4d** and the free acids **5a** and **5d**, which were synthesized for the first time. The determination of the absolute configurations of all optically active compounds is currently under investigation.

3. Conclusions

In this paper, we have demonstrated that an efficient kinetic resolution of α-hydroxy butenolides can be achieved using lipase catalysis. Thus, these important building blocks are now available in their enantiomerically pure forms and can serve as a basis for natural product synthesis.

4. Experimental

4.1. General

Commercial enzyme preparations were used as supplied. Specific rotations were recorded on a Perkin–Elmer

Table 2. Kinetic resolution of racemic butenolides **4a** and **4d** with lipase from *Burkholderia cepacia* (Amano PS) in preparative scale

Compound	Remaining substrate			Product			E[d]
	Yield (%)	Ee_S[a] (%)	$[\alpha]_D^{21}$[b]	Yield (%)	Ee_P[a,c] (%)	$[\alpha]_D^{21}$[b]	
4a	60	95	+40	43	92	−28	90
4d	90	97	+21	60	97	−26	>100

[a] Determined by GC with chiral column.
[b] Specific rotation[10] recorded at 21 °C in MeOH or EtOH, $c = 1$ (see Experimental).
[c] Of methyl esters **6a** and **6d**.
[d] Calculated according to Chen et al.[9]

polarimeter 241 at 589 nm in MeOH (free acids and methyl esters) or EtOH (ethyl esters) at $c = 1$. ^{1}H and ^{13}C NMR-spectra were recorded in d_6-acetone or CD_3OD on a 300 or 600 MHz (Bruker) instrument.

4.2. Synthesis of the α-hydroxy butenolides

The α-hydroxy butenolides **4a–e** were prepared according to literature.[4b,5]

4.3. General procedure for the enzyme-catalyzed kinetic resolutions

For small-scale reactions substrates **4a–e** (0.025 mmol) and lipase Amano PS from *Burkholderia cepacia* (300 U, 10 mg) were dissolved in phosphate buffer (400 μL, 10 mM, pH 7.5) and toluene (100 μL). The mixtures were shaken in a thermoshaker (Eppendorf, Hamburg, Germany) at 30 °C and 1000 rpm. After certain time intervals, samples (100 μL volume) were taken, and the same amount of distilled water was added. The samples were extracted, vortexed and centrifuged (1 min, 13,000 rpm) before GC analysis (see below).

Enzymatic reactions on a preparative scale were performed in 50 mL flasks with 0.5 mmol substrate, 6000 U of lipase Amano PS dissolved in 8 mL phosphate buffer (10 mM, pH 7.5) and 2 mL toluene. The mixtures were stirred in a water bath at 30 °C until 50% conversion was reached. After addition of the same amount (10 mL) of distilled water, substrate and product were isolated and separated by extraction. The remaining ester was extracted with methylene chloride (5 × 5 mL) and after acidification with 2 M HCl [(2 mL, 10% (v/v)], the free acid was extracted from the mixture with diethyl ether (5 × 5 mL). The organic phases were dried, filtrated and the excess solvent removed in vacuo.

4.3.1. (+)-(4-Hydroxy-3-methoxy-5-oxo-2,5-dihydrofuran-2-yl)-acetic acid methyl ester 4a. Yield: 60%; 95% ee; $[\alpha]_D^{21} = +40$ (c 1, MeOH). For spectroscopic data, see Ref. 5.

4.3.2. (−)-(4-Hydroxy-3-methoxy-5-oxo-2,5-dihydrofuran-2-yl)-acetic acid 5a. Yield: 43%; 92% ee; $[\alpha]_D^{21} = -28$ (c 1, MeOH); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 2.44$ (1H, dd, $J = 16.3$, 8.7 Hz, CH_2), 2.87 (1H, dd, $J = 16.3$, 3.6 Hz, CH_2), 4.17 (3H, s, CH_3O), 5.01 (1H, dd, $J = 8.7$, 3.6 Hz, CH). A small amount of hydroxy acid resulting from lactone cleavage was present.

4.3.3. (+)-(3-Ethyl-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)-acetic acid ethyl ester 4d. Yield: 90%; 97% ee; $[\alpha]_D^{21} = +21$ (c 1, EtOH). For spectroscopic data, see Ref. 5.

4.3.4. (−)-(Ethyl-4-hydroxy-5-oxo-2,5-dihydrofuran-2-yl)-acetic acid 5d. Yield: 60%; 97% ee; $[\alpha]_D^{21} = -26$ (c 1, MeOH); ^{1}H NMR (600 MHz, d_6-acetone): $\delta = 1.16$ (3H, t, $J = 7.8$ Hz, CH_3), 2.26 (1H, m, CH_2CH_3), 2.45 (1H, dd, $J = 16.2$, 8.4 Hz, CH_2CH), 2.54 (1H, m, CH_2CH_3), 2.98 (1H, dd, $J = 16.2$, 3.0 Hz, CH_2CH), 5.24 (1H, dd, $J = 8.4$, 3.0 Hz, CH); ^{13}C NMR (150 MHz, d_6-acetone): $\delta = 12.1$ (CH_3), 18.2 (CH_2), 38.3 (CH_2), 77.0 (CH), 135.5 and 138.8 (C=C), 160.1 (CH_2C), 171.1 (CO_2H).

4.4. Chiral GC analysis

For GC analysis, free acids **5a–e** were converted into the corresponding methyl esters **6a–e** by the addition of an etheric solution of diazomethane. Excess reagent and solvent were removed under nitrogen and the residue dissolved in 10 μL methylene chloride for GC analysis.

GC analyses were carried out on a Shimadzu GC-14A gas chromatograph with a chiral column (heptakis-(2,3-di-*O*-acetyl-6-*O*-tertbutyldimethylsilyl)-β-cyclodextrin). Separation protocols and retention times for the compounds **4a–e** and **5a–e** had to be determined first (Table 3). Esters **4b–e** were also reacted with diazomethane in order to methylate the hydroxyl group at the α-position **7b–e** resulting in a better separation. Injection and detection temperature were always set to 220 °C.

Table 3. Chiral GC analysis

Compound	T_{Column} (°C)	Retention time[a] (min)
4a	160	18.5/21.1
6a	150	16.5/18.5
6b	145	21.8/24.2
6c[b]	145	15.7/17.5
6d	140	19.6/20.6
6e	135	35.2/36.5
7b	145	27.4/30.1
7c[b]	145	15.7/17.5
7d	140	24.1/25.1
7e	135	43.9/45.1

[a] Retention times of the two enantiomers.
[b] Compounds **6c** and **7c** are of identical structure.

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