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Tetrahedron: *Asymmetry* 15 (2004) 3743–3749

Enantioselective hydrolysis of δ-acetoxy-γ-lactones

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Received 4 August 2004; accepted 25 October 2004

Abstract—Both enantiomers of 5-(1′-acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one **1a** have been prepared with high enantioselectivity by microbial resolution using cultures of *Fusarium solani* and *F. tricinctum*. Enantioselectivity of lipolitic activity of *F. solani* and *F. tricinctum* was tested on four homologues of δ-acetoxy-γ-lactones (±)-**1b**–**e**. Absolute configurations of the products were determined by spectroscopic methods (^{1}H NMR and CD) and an X-ray diffraction study.

1. Introduction

Lactone functionality is present in a large variety of biologically active compounds and natural products.[1] Physiological activity of γ-butyrolactones often depends on the configuration of the stereogenic centres and on the enantiomeric purity of the compounds. For example, in the case of insect sex pheromones, the presence of even a small amount of the opposite enantiomer can greatly reduce the biological activity of a compound.[2a] We are interested in synthesis of terpenoid lactones being analogues of natural compounds, isolated from insects, microorganisms or plants.[1] Previous studies on the correlation between the configuration of terpenoid lactones and their feeding deterrent activity showed that often only one enantiomer of a racemic mixture exhibits high biological activity.[2,3] For this reason, we are interested in obtaining pure enantiomers of some hydroxy and acetoxy lactones. This goal can be achieved by an asymmetric synthesis or by resolution of racemic mixtures of hydroxy or acetoxy lactones.

Herein we report on the resolution of racemic δ-acetoxy-γ-lactone (±)-**1a** using lipases or esterases as well as the whole microorganisms. The absolute configurations of (+)-**1a** and (−)-**1a** have also been established. Then lipolitic activity of two selected biocatalysts (*Fusarium solani* and *F. tricinctum*) was tested on four analogous acetates.

2. Results and discussion

2.1. Synthesis of 5-(1′-acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one (±)-1a

Acetate (±)-**1a** was obtained in the reaction with acetyl chloride from hydroxy lactone (±)-**2a** (Scheme 1). The racemic δ-hydroxy-γ-lactone (±)-**2a** was prepared in a four step synthesis.[5b] The key step of the synthesis, the orthoacetate modification of the Claisen rearrangement of allyl alcohols, afforded (*E*)-γ,δ-unsaturated ethyl ester, which was next oxidised with *m*-chloroperbenzoic acid. The final step, the lactonisation of the epoxy ester, was carried out in aqueous acid conditions. The δ-hydroxy-γ-lactone was then purified by column chromatography.

2.2. Resolution of the racemic mixture of 5-(1′-acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one (±)-1a and the determination of absolute configuration

At the beginning of our studies on the resolution of the racemic mixture, five commercially available hydrolases: esterase from pig liver, lipase from *Aspergillus niger*, lipase from *Candida cyclodracea*, esterase from hog liver immobilised on Eupergit®C, lipase from *Porcine Pancreas* were tested. Following the procedure used previously,[4] the enzymatic hydrolysis of (±)-**1a** was carried out either in aqueous solution or in various other media of relatively low water content. On the whole, the screening tests on these enzymes afforded isomers of δ-hydroxy-γ-lactones and δ-acetoxy-γ-lactones with enantiomeric excesses not exceeding 40%, thus making the products useless for our investigation.

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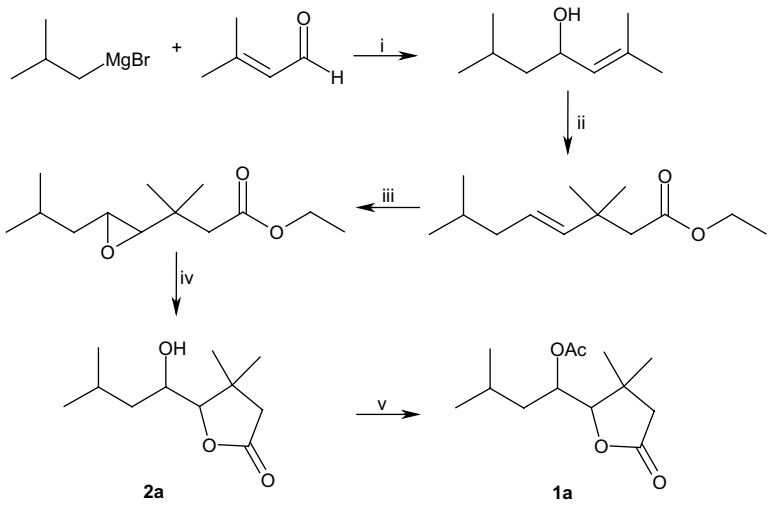
0957-4166/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2004.10.023

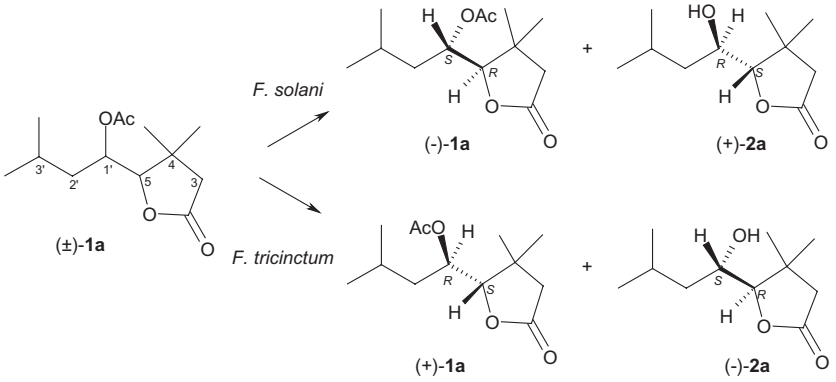


Scheme 1. Reagents and conditions: (i) Mg, Et_2O (70%); (ii) $CH_3C(OC_2H_5)_3$, C_2H_5COOH, 138 °C (75%); (iii) MCPBA, CH_2Cl_2 (89%); (iv) THF/H_2O/$HClO_4$ (10:5:0.1), 40 °C (80%); (v) CH_3COCl, py (93%).

We next decided to work with the whole microorganism and to search for 'novel' microbial lipases that would be more enantioselective for δ-acetoxy-γ-lactones. The ability of the several following species: *Rhodotorula rubra*, *Daedaleopsis confragosa*, *F. solani*, *Nocardia rubra*, *Aphanoclaudium album*, *Nigrospora oryzae*, *Epithydrium rosinea* and seven species of *Fusarium* to perform enantioselective hydrolysis of racemic (±)-**1a** was checked.

Finally, the two species of *Fusarium*: *F. solani* and *F. tricinctum* were selected for further investigation of the rate and enantioselectivity of the hydrolysis, leading to the resolution of racemic 5-(1′-acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one (±)-**1a** (Scheme 2, Table 1).

Microbial preparative resolution was carried out on δ-hydroxy-γ-lactone (±)-**1a** using the culture of *F. solani*



Scheme 2.

Table 1. Microbial hydrolysis of (±)-**1a**—composition of crude product mixtures

Microorganism	Time (days)	**1a**:**2a**[a] (%)	**1a**		**2a**	
			Ee[a] (%)	Enantiomer	Ee[a] (%)	Enantiomer
F. solani	1	91:9	13.4	(−)-(1′*S*,5*R*)	18.6	(+)-(1′*R*,5*S*)
	2	87:13	16.8	(−)-(1′*S*,5*R*)	51.2	(+)-(1′*R*,5*S*)
	4	43:57	69.6	(−)-(1′*S*,5*R*)	41.0	(+)-(1′*R*,5*S*)
	6	33:77	91.6	(−)-(1′*S*,5*R*)	43.0	(+)-(1′*R*,5*S*)
	7*	29:71	93.2	(−)-(1′*S*,5*R*)	40.0	(+)-(1′*R*,5*S*)
F. tricinctum	2	47:53	74.5	(+)-(1′*R*,5*S*)	56.3	(−)-(1′*S*,5*R*)
	4	42:58	74.1	(+)-(1′*R*,5*S*)	53.2	(−)-(1′*S*,5*R*)
	6*	21:79	100	(+)-(1′*R*,5*S*)	27.2	(−)-(1′*S*,5*R*)

* Chromatogram from preparative biotransformation before chromatography purification.
[a] Determined by GC.

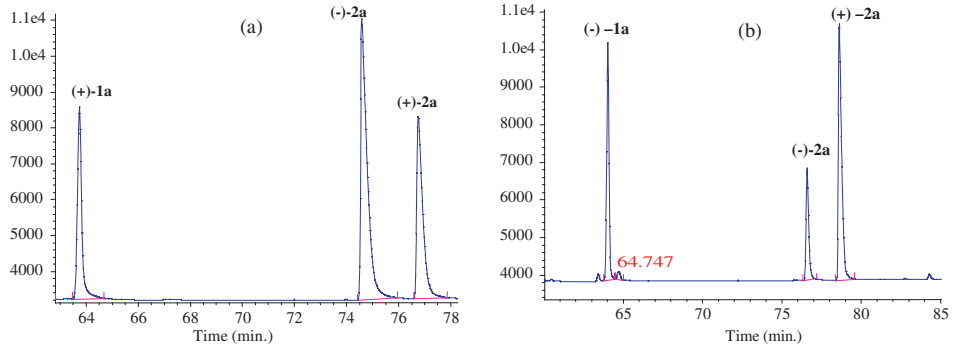


Figure 1. Chromatograms of crude product mixtures of preparative hydrolysis of (±)-**1a**: (a) with *F. tricinctum*, (b) with *F. solani*.

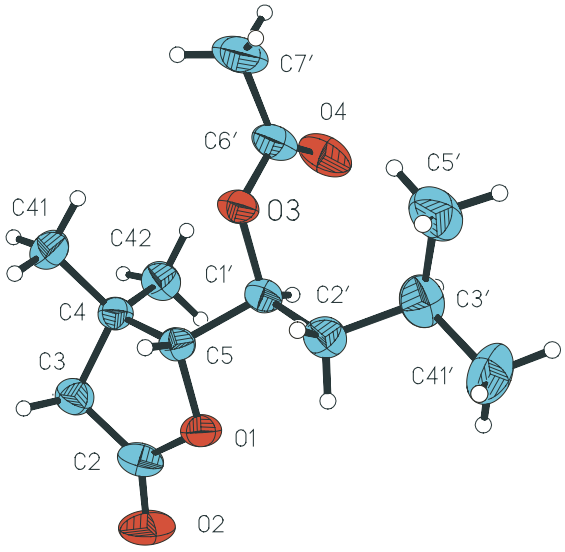


Figure 2. Molecular structure of **1a** with crystallographic numbering.

or *F. tricinctum*. In the first transformation with *F. solani*, we obtained unreacted (−)-**1a** isomer {ee = 93%, $[\alpha]_D^{24.1} = -31.1$}; and δ-hydroxy-γ-lactone **2a** with 40% enantiomeric excess of the (+)-isomer. After a 7-day hydrolysis of (±)-**1a** with *F. tricinctum*, the (+)-isomer was isolated {ee = 100%, $[\alpha]_D^{21.1} = +30.6$} and δ-hydroxy-γ-lactone **2a** with the (−)-isomer predominating (27.2% ee).

The enantiomeric excesses of the (+)- and (−)-isomers of 5-(1′-acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one **1a** were determined directly by means of gas chromatography, using a chiral phase column coated with modified γ-cyclodextrins (Fig. 1).

Analysis of the molecular structure led to the conclusion that compound **1a** forms two enantiomers: 1′*R*,5*S* or 1′*S*,5*R*, respectively. The absolute configuration of the second stereogenic centre was established by the X-ray diffraction study (Fig. 2).[6]

This, together with the Cotton effect measurement, allowed us to assign the (5*S*)-configuration, because the lactone exhibited a positive Cotton effect of n–π* transition measured at 213 nm for (+)-**1a** ($\Delta\varepsilon = +0.9$), which is in accordance with the literature data.[7] The effect for the (−)-1-Ac $\Delta\varepsilon$ is equal to −1,0. The measurements were taken on compounds (+)-**1a** (ee = 100%) and (−)-**1a** (ee = 93.2%), the absolute configurations of which were determined as 1′*R*,5*S* and 1′*S*,5*R*, respectively.

Pure enantiomers of δ-acetoxy-γ-lactone (+)-**1a** and (−)-**1a** were hydrolysed enzymatically (using esterase from hog liver) to the corresponding δ-hydroxy-γ-lactone **2a**.

2.3. Investigation of the enantioselectivity of lipolitic activity of *F. solani* and *F. tricinctum* using δ-acetoxy-γ-lactones (±)-1b–e

Enantioselectivity of the lipolitic activity of *F. solani* and *F. tricinctum* was tested on four homologues of δ-acetoxy-γ-lactones (±)-**1b–e**. Unlike δ-acetoxy-γ-lactone **1a**, these compounds contain only one methyl group at C-4, and this additional stereogenic centre is responsible

Table 2. Compositions of the crude product mixtures of preparative microbial hydrolysis (determined by GC)

Substrate	Microorganism	Time (days)	Conversion (%)	δ-Acetoxy-γ-lactones		δ-Hydroxy-γ-lactones	
				Ee	Configuration	Ee	Configuration
(±)-**1b**	*F. solani*	4	27:73*	95.3	(−)-(1′*S*,4*R*,5*R*)	20.8	(+)-(1′*R*,4*S*,5*S*)
(±)-**1b**	*F. tricinctum*	2.5	25:75	100	(+)-(1′*R*,4*S*,5*S*)	26.6	(−)-(1′*S*,4*R*,5*R*)
(±)-**1c**	*F. solani*	6	20:80*	100	(−)-(1′*S*,4*S*,5*R*)	33.0	(+)-(1′*R*,4*R*,5*S*)
(±)-**1c**	*F. tricinctum*	6	45:55	22.6	(+)-(1′*R*,4*R*,5*S*)	3.7	(−)-(1′*S*,4*S*,5*R*)
(±)-**1d**	*F. solani*	10	20:80	95.0	(−)-(1′*S*,4*R*,5*R*)	54.8	(+)-(1′*R*,4*S*,5*S*)
(±)-**1d**	*F. tricinctum*	4	15:85*	97.2	(+)-(1′*R*,4*S*,5*S*)	15.1	(−)-(1′*S*,4*R*,5*R*)
(±)-**1e**	*F. solani*	9	20:80	100	(−)-(1′*S*,4*S*,5*R*)	20.2	(+)-(1′*R*,4*R*,5*S*)
(±)-**1e**	*F. tricinctum*	8	42:68	11.9	(+)-(1′*R*,4*R*,5*S*)	26.2	(−)-(1′*S*,4*S*,5*R*)

* Chromatogram from preparative biotransformation before chromatography purification.

for formation of *trans*-(±)-**1b** and (±)-**1d**) and *cis*-(±)-**1c** and (±)-**1e** isomers. Acetates (±)-**1b–e** were obtained in the reaction with acetyl chloride from corresponding hydroxy lactones (±)-**2b–e**.

A series of racemic δ-hydroxy-γ-lactones (±)-**2b–e** were prepared from isobutyl bromide **2** or **2c** or neopentyl bromide **2d** or **2e** with crotonaldehyde in the same way as compound **2a** (Scheme 1).[5] Acid lactonisation of racemic epoxy esters gave a mixture of *trans*-**2b** and **2d** and *cis*-**2c** and **2e** δ-hydroxy-γ-lactones. The mixture of the diastereoisomers was then separated by column chromatography.

Pure enantiomers of δ-hydroxy-γ-lactones (+)-**2b–e** and (−)-**2b–e** (above 93% ee), used for identification, were synthesised in the same way as lactones **2a** (Scheme 1), but starting from corresponding (*R*)- or (*S*)-enantiomer of the allyl alcohol. The racemic mixture of these alcohols was resolved by the Sharpless asymmetric epoxidation. Configurations of the products were determined by spectroscopic methods (^{1}H NMR, CD). The absolute configuration of compound **2d** was identified with the aid of X-ray diffraction study.[5]

In the following experiments, four racemic δ-acetoxy-γ-lactones **1b–e** were resolved using cultures of *F. solani* and *F. tricinctum*. The results of these biotransformations are presented in Table 2.

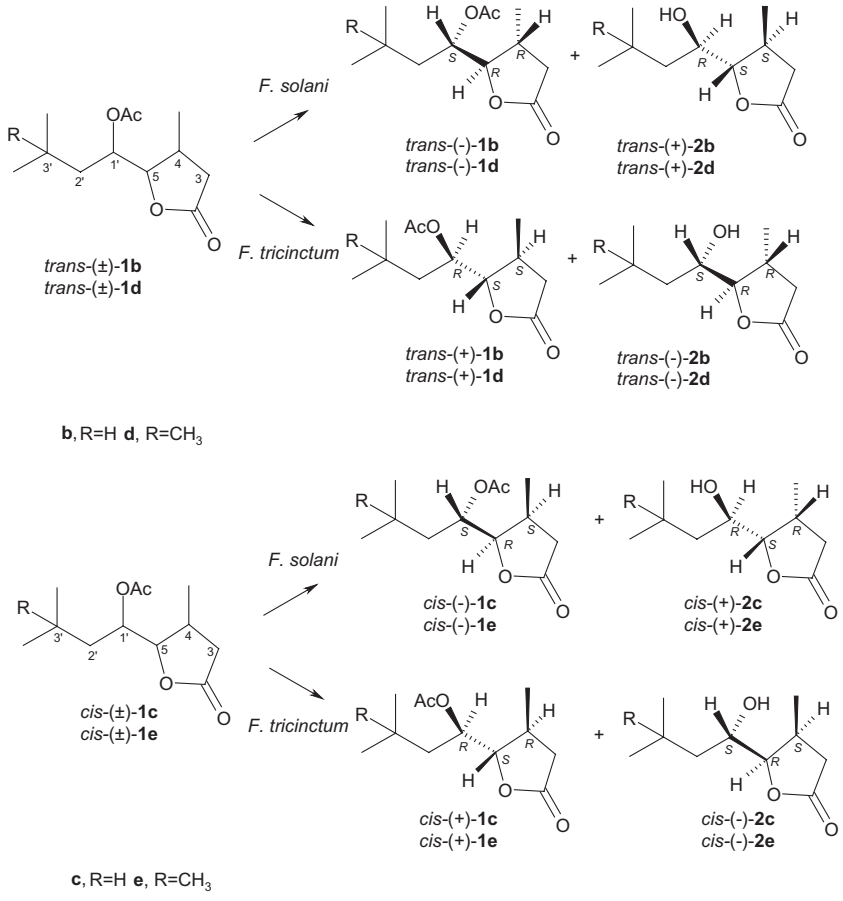
In accordance with our earlier experiments, the cultures of *F. solani* hydrolysed (1′*R*,5*S*)-enantiomer faster while the residual (1′*S*,5*R*)-enantiomer was isolated and purified. The absolute configuration at C-4 did not have an influence on the enantioselectivity of the hydrolysis. The rates of the hydrolyses were different for the *trans*- and *cis*-isomers of δ-acetoxy-γ-lactones, depending on the presence of the additional methyl group at C-3′ Scheme 3.

In the same way, used cultures of *F. solani* we obtained (−)-(1′*S*,4*R*,5*R*)-**1b** ee = 95.3%, $[\alpha]_D^{23.2} = -26.1$; (−)-(1′*S*,4*S*,5*R*)-**1c** ee = 100%, $[\alpha]_D^{24.1} = -34.9$; (−)-(1′*S*,4*R*,5*R*)-**1d** ee = 95.0% and (−)-(1′*S*,4*S*,5*R*)-**1e** ee = 100%.

Similar hydrolytic activity was observed when using the cultures of *F. tricinctum*, where *trans*-isomers of (−)-**1b** and (−)-**1d**, with an absolute configuration 1′*S*,*4S*,5*R*, were hydrolysed faster than the opposite ones. Different results were observed when *cis*-(±)-**1c** and *cis*-(±)-**1e** were used as the substrates. In these cases, we detected an almost equal rate of hydrolysis of both the (+)- and (−)-*cis*-isomers.

3. Conclusion

The results of our research showed that the pure enantiomers of optically active 5-(1′-acetoxy-3′-methyl-



Scheme 3.

butyl)-4,4-dimethyl-tetrahydrofuran-2-one **1a** can be obtained by resolution of racemic δ-acetoxy-γ-lactones using the cultures of *F. solani* and *F. tricinctum*. These results were compared with the utility of these two species for the resolution of δ-acetoxy-γ-lactones (±)-**1b–e**. It was observed that the rate of transformation decreases in the presence of an additional methyl group either in the lactone ring **1a** or in the chain **1d** and **1e** compared with δ-acetoxy-γ-lactones **1b**. Furthermore, the hydrolysis of racemic *cis*- and *trans*-isomers proceeds in a different way. Pure enantiomers of (−)-**1b**, (−)-**1c**, (−)-**1d** and (−)-**1e** have been prepared highly enantioselectively by microbial resolution using *F. solani* culture. Enantioselectivity of lipolitic activity of *F. tricinctum* was significant only for *trans*-(±)-**1b** and (±)-**1d** isomers.

4. Experimental

4.1. General

The composition of crude mixtures was analysed by TLC and GC. TLC was carried out using silica gel 60, 0.2 mm thick plates with hexane/isopropanol/acetone/ethyl acetate (60:3:1:1) as eluent. The same eluent was applied in the course of preparative column chromatography (silica gel: 60 230–400 mesh) for separation and purification of final products. Yields of acetates and products of microbial resolution were calculated after chromatography purification. Conversion percent and enantiomeric excesses of the lactones were determinated by capillary GC using chiral columns: CP-cyclodextrin, 25 m × 0.25 mm × 0.25 μm; Chirasil-Val-L, 25 m × 0.25 mm × 0,17 μm. GC. Gas chromatography (GC) analysis was carried out on a Hewlett Packard 5890A Series II instrument: fitted with a flame ionisation detector.

The structure of δ-acetoxy-γ-lactones was determinated based on ^{1}H NMR and IR spectra. ^{1}H NMR spectra were recorded at 300 MHz using $CDCl_3$ solutions on a Bruker Avance DRX 300 spectrometer. IR spectra were measured on a Specord M-80 infrared spectrophotometer (Carl Zeiss, Jena, Germany). Optical rotations were measured on an Autopol IV automatic polarimeter (Rudolph) using chloroform solutions at concentrations given in g/100 cm^3. Melting points (uncorrected) were measured on Boetius apparatus.

4.2. Biocatalysts

Enzymes: esterase from pig liver, lipase from *A. niger*, lipase from *C. cyclodracea*, esterase from hog liver immobilised on Eupergit®C were purchased from Fluka, while lipase from *Porcine Pancreas* was produced by Sigma Chemical Co.

The following microorganisms: *R. rubra* AM4, *F. solani* AM203, *A. album* AM417, *N. oryzae* AM8, *F. avenaceum* AM1*F. culmorum* AM196*F. oxysporum* AM145 were obtained from the collection of Institute of Biology and Botany, Medical University of Wrocław. *F. tricinctum* 395, *N. rubra* 2, *E. rosinea* 16048, *Daedaleopsis* 15969, *F. equiseti* 400 and *F. semitectum* 16 were obtained from the Agricultural University of Wrocław.

4.3. Substrates

4.3.1. Preparation of δ-hydroxy-γ-lactones (±)-2a–e. Syntheses of δ-hydroxy-γ-lactones (±)-**2a–e**, mechanism of lactonisation, as well as spectral and physical data have been presented in previous work.[5]

Enantiomers of δ-hydroxy-γ-lactones **2b–e** were used as standards for GC analysis with chiral columns. The method of the syntheses of optically active isomers and the configurations of stereogenic centres were established on the basis of ^{1}H NMR and CD spectral data, which have already been presented.[5]

4.3.2. Preparation of δ-acetoxy-γ-lactones (±)-1a–e. The δ-hydroxy-γ-lactones (1.0 g, 5 mmol of **2a**,**d**,**e**: 5.4 mmol of **2b**,**c**) were added to the stirred solution of acetyl chloride (1.4 cm^3, 8 mmol), pyridine (1.5 cm^3) and dry diethyl ether (20 cm^3). When the reaction was complete (TLC), 100 cm^3 of diethyl ether was added and the mixture washed with 20% HCl solution. The ethereal layer was separated and the aqueous layer extracted with diethyl ether (3 × 50 cm^3). The combined ethereal extract was washed with acid solution, saturated $NaHCO_3$ solution and brine and dried over $MgSO_4$. After solvent evaporation, the crude product was purified by column chromatography. The obtained physical and spectral data of δ-acetoxy-γ-lactones (±)-**1b–e** are as follows:

5-(1′-Acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one 1a: yield: 93%; mp: 41 °C; ^{1}H NMR (δ, ppm): 0.92 and 0.94 (2d, J = 6.2 Hz, 6H, $(CH_3)_2CH$–), 1.09 and 1.22 (2s, 6H, >C$(CH_3)_2$), 1.49–1.69 (m, 3H, $(CH_3)_2CHCH_2$–), 2.08 (s, 3H, –C(O)CH_3) 2.32 and 2.42 (2d, J = 17.0 Hz, 1H and J = 17.0 Hz, 1H, –CH_2C(O)–), 4.08 (d, J = 7.3 Hz, 1H, >C*H*O–), 5.16 (ddd, J = 9.3; 7.3; 2.9 Hz, 1H, –C*H*(OAc)–); ^{13}C NMR (500 MHz) (δ, ppm): 21.0; 21.6; 21.7; 23.6; 24.1; 26.6; 39.2; 41,0; 45.0; 70.3; 87.9; 169.8; 175.0; (IR (film, cm^{-1}): 1792 (s), 1752 (s).

***trans*-5-(1′-Acetoxy-3′-methylbutyl)-4-methyl-tetrahydrofuran-2-one 1b**: yield: 94 %; n_D^{20} = 1.4441; ^{1}H NMR (δ, ppm): 0.92 and 0.94 (2d, J = 6.0 Hz, 6H, $(CH_3)_2CH$–), 1.18 (d, J = 6.8 Hz, 3H, –CH(CH_3)–), 1.39 and 1.60 (2ddd, J = 14.1; 9.5; 4.9 Hz, 1H and J = 14.1; 7.0; 1.7 Hz, 1H, –CH_2CH(OAc)–), 1.55–1.91 (m, 1H, $(CH_3)_2CH$–), 2.08 (s, 3H, –C(O)CH_3), 2.16 and 2.75 (2dd, J = 17.5; 8.9 Hz, 1H and J = 17.5; 7.2 Hz, 1H, –CH_2C(O)–), 2.44–2.49 (m, 1H, –C*H*(CH$_3$)–), 4.4 (dd J = 5.8 and 4.0 Hz, 1H, >C*H*(O–)–) 5.15 (dt, J = 9.5; 4.0 Hz, 1H, –C*H*(OAc)–); IR (film, cm^{-1}): 1792 (s),1752 (s), 1236 (s), 1024 (s).

***cis*-5-(1′-Acetoxy-3′-methylbutyl)-4-methyl-tetrahydrofuran-2-one 1c**: yield: 92%; bp: 89/3.5 mmHg; n_D^{20} = 1.4481; ^{1}H NMR (δ, ppm): 0.94 and 0.95 (2d, J = 6.4 Hz, 6H, $(CH_3)_2CH$–), 1.07 (d, J = 7.0 Hz, 3H, –CH(CH_3)–),

1.43 and 1.55 (2ddd, $J = 13.9$; 9.8; 4.5 Hz, 1H and $J = 13.9$; 9.8; 2.7 Hz, 1H, –CH_2CH–), 1.78–1.91 (m, 1H, $(CH_3)_2$CH–), 2.08 (s, 3H, –C(O)CH_3), 2.25 and 2.72 (2dd, $J = 19.8$; 6.7 Hz, 1H and $J = 19.8$; 6.9 Hz, 1H, –CH_2C(O)–), 2.59–2.68 (m, 1H, –CH(CH_3)–), 4.41 (dd $J = 7.0$; 5.0 Hz, 1H, >CH (O–)–) 5.16 (ddd, $J = 9.5$; 7.0; 2.9 Hz, 1H, –CH(OAc)–); IR (film, cm^{-1}): 1792 (s), 1752 (s), 1236 (s), 1024 (s).

***trans*-5-(1′-Acetoxy-3′,3′-dimethylbutyl)-4-methyl-tetrahydrofuran-2-one 1d**: yield: 90%; $n_D^{20} = 1.4451$; ^{1}H NMR (δ, ppm): 0.92 (s, 9H, (CH_3)$_3$C–), 1.2 (d, $J = 6.8$ Hz, 3H, –CH(CH_3)–), 1.44 and 1.62 (2dd, $J = 15.2$; 1.9 Hz, 1H and $J = 15.2$; 8.9 Hz, 1H, –CH_2CH(OAc)–), 2.06 (s, 3H, –C(O)CH_3), 2.16 and 2.73 (2dd, $J = 17.5$; 8.8 Hz, 1H and $J = 17.5$; 7.8 Hz, 1H, –CH_2C(O)–), 2.32–2.46 (m, 1H, –CH(CH_3)–), 4.06 (dd, $J = 6.3$; 3.8 Hz, 1H, –CH(O–)–) 5.17 (ddd, $J = 8.9$; 3.8; 1.9 Hz, 1H, –CH(OAc)–), IR (film, cm^{-1}): 1796 (s), 1752 (s), 1240 (s), 1028 (s).

***cis*-5-(1′-Acetoxy-3′,3′-dimethylbutyl)-4-methyl-tetrahydrofuran-2-one 1e**: yield: 93%, $n_D^{20} = 1.4453$, ^{1}H NMR (δ, ppm): 0.93 (s, 9H, (CH_3)$_3$C–), 1.13 (d, $J = 6.9$ Hz, 3H, –CH(CH_3)–), 1.55 and 1.69 (2dd, $J = 15.0$; 2.1 Hz, 1H and $J = 15.0$; 9.3 Hz, 1H, $(CH_3)_3CCH_2$–), 2.05 (s, 3H, –C(O)CH_3), 2.22 and 2.70 (2dd, $J = 16.5$; 7.4 Hz, 1H and $J = 16.5$ and 5.6 Hz, 1H, –CH_2C(O)–), 2.67–2.72 (m, 1H, –CH(CH_3)–), 4.4 (dd, $J = 5.6$; 5.5 Hz, 1H, >CH(O–)–), 5.27 (ddd, $J = 9.3$; 5.5; 2.1 Hz, 1H, –CH(OAc)–); IR (film, cm^{-1}): 1792 (s), 1752 (s), 1240 (s), 1048 (s).

4.4. Enzymatic hydrolysis

A typical enzymatic hydrolyse procedure of δ-acetoxy-γ-lactone **1a**: 10 mg of enzyme was added to 3 cm^3 of phosphorus buffer (7.2) solution or one of the following organic solvents: $C_6H_5CH_3$, c-C_6H_{12}, CH_3CN, $(C_2H_5)_2O$, $CH_3CO_2CH_3$ or THF containing 20 mg of substrate. The suspension was stirred both at room temperature and at 36 °C. The samples were collected and filtered over Celite. The Celite was additionally washed with diethyl ether and the crude mixture was analysed by GC.

4.5. General procedure for whole cell microbial hydrolysis

Screening procedure: The microorganisms were cultivated at 25 °C in 300 cm^3 Erlenmeyer flasks containing 75 cm^3 of one of the following nutrients: 1% solution of peptone and glucose (3%) or potatoes extract obtained from 800 g potatoes (washed, peeled, cut up and gently boiled in 1000 cm^3 of distilled water). After 3–5 days of growth, 10 mg of a substrate in 0.5 cm^3 of acetate was added to shaken cultures. After 2, 4, 6, 8 days the products were extracted with diethyl ether (30 cm^3) and analysed by TLC and GC.

(5*R*)-((1′*S*)-Acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one (−)-1a: The δ-acetoxy-γ-lactone (±)-**1a** (250 mg of diluted in 2.5 cm^3 acetone) was added in equal portions to five Erlenmeyer flasks containing 75 cm^3 cultures of *Fusarum solani*. After a 6-day hydrolysis of (±)-**1a** the products were extracted with diethyl ether (3×50 cm^3 each flask) and dried ($MgSO_4$). The solvent was evaporated off and the crude product mixture was separated by column chromatography. After purification there was obtained 48.2 mg (20% yield) of unreacted (−)-**1a** isomer [ee = 93%, $[\alpha]_D^{24.1} = -31.1$ (***c*** 2.68, $CHCl_3$)], 126.9 mg of δ-hydroxy-γ-lactone **2a** with 40% of enantiomeric excess of the (+) isomer and 30 mg of not separated products.

Following the same procedure, there were obtained:

(*S*)-((1′*R*)-Acetoxy-3′-methylbutyl)-4,4-dimethyl-tetrahydrofuran-2-one (+)-1a: After 7-days hydrolysis with *F. tricinctum* there was obtained 37.3 mg, yield (15%) ee = 100%, $[\alpha]_D^{21.1} = +30.6$ (c 2.52, $CHCl_3$).

***trans*-(5*R*)-((1′*S*)-Acetoxy-3′-methylbutyl)-(4*R*)-methyl-tetrahydrofuran-2-one (−)-1b**: After 4-days hydrolysis with *F. solani* there was obtained 58 mg yield (23%) of (−)-**1b**, ee = 95.3%, $[\alpha]_D^{23.2} = -26.1$ (c 2.44, $CHCl_3$).

***cis*-(5*R*)-((1′*S*)-Acetoxy-3′-methylbutyl)-(4*S*)-methyl-tetrahydrofuran-2-one (−)-1c**: After 6-days hydrolysis with *F. solani* there was obtained 43 mg yield (17%) of (−)-**1c** ee = 100%, $[\alpha]_D^{24.1} = -34.9$ (c 2.05, $CHCl_3$).

***trans*-(5*S*)-((1′*R*)-Acetoxy-3′,3′-dimethylbutyl)-(4*S*)-methyl-tetrahydrofuran-2-one (+)-1d**: After 4-days hydrolysis with *F. tricinctum* there was obtained 27.5 mg yield (11%) of (+)-**1d** ee = 97.2%, $[\alpha]_D^{22.6} = +31.5$ (c 3.08, $CHCl_3$).

Acknowledgements

This work was supported by the Polish State Committee for Scientific Research, Grant No. 6 P06B 031 20 and 060/T09/2001/15. The authors thank Professor Jacek Gawroński (A. Mickiewicz University, Poznań) for the CD spectra measurements.

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