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Journal of Molecular Catalysis B: Enzymatic 19–20 (2002) 203–208

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Lactones 13
Biotransformation of iodolactones

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Received 7 September 2001; received in revised form 15 May 2002; accepted 29 May 2002

Abstract

Several fungal strains were screened for their ability to transform iodolactones. The most efficient was a culture of *Botrytis cinerea*. This microorganism transformed δ-iodo-γ-lactone (**3**) into two products: hydroxy iodolactone (**5**) and hydroxy unsaturated lactone (**6**). The biotransformation of γ-iodo-δ-lactone (**4**) afforded only one product, the hydroxy iodolactone (**7**).

Keywords: Biotransformation; Dehalogenation; δ-Iodo-γ-lactone; γ-Iodo-δ-lactone; *Botrytis cinerea*

1. Introduction

Many halogenated organic compounds are used as herbicides, pesticides, refrigerants, fire retardant, solvents, degreasers and various intermediates in organic synthesis [1–3]. They have appeared as environmental pollutants as a result of their persistence, bioaccumulation or toxicity and often-limited biodegradability. One of the ways leading to their elimination from natural environment which is taken into consideration is the biodegradation via microbial transformation [4]. The first step in this process is dehalogenation. It is very important because the dehalogenated compounds are generally less toxic and more susceptible to further biodegradation.

Here we report the results of our studies on the biotransformation of iodolactones. We chose iodolactones for two reasons. Some terpenoid iodolactones exhibit antifeeding activity against the insect pests and can be used for insect pests control [5,6]. The second reason is that they can be considered as good model substrates for searching the mechanism of dehalogenation.

2. Materials and methods

2.1. Analysis

Purity of intermediates and isolated products was checked by thin layer chromatography on silica gel (DC-Alufolien Kieselgel 60 F_{254}, Merck). Chromatograms were developed in the solvent systems: hexane–acetone, 49:1 (for esters) and hexane–ethyl acetate, 20:1 (for iodolactones). The same eluents were used for preparative column chromatography performed on silica gel (Kieselgel 60, 230–400 mesh).

Gas chromatography analysis was carried out on a Hewlett-Packard 5890 instrument using a CP-cyclodextrin-B,3,6-M-19, 25 m × 0.25 mm × 0.25 μm column and on a Varian CP-3380 instrument with HP-1 column (crosslinked methyl siloxane, 25 m × 0.32 mm × 0.25 μm).

^{1}H NMR spectra were recorded in $CDCl_3$ solution on a Bruker Avance DRX 300 spectrometer. IR spectra

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1381-1177/02/$ – see front matter
PII: S1381-1177(02)00168-6

were determined with a Specord M-80 infrared spectrophotometer (Carl Zeiss, Jena, Germany). Optical rotation was measured on an Autopol IV automatic polarimeter (Rudolph).

2.2. Materials

All reagents were purchased from Fluka. The substrates for biotransformations, δ-iodo-γ-lactone (**3**) and γ-iodo-δ-lactone (**4**), were obtained by iodolactonization of (*E*)-3,3,7-trimethyl-4-octenoic acid (**2**), a product of alkaline hydrolysis of the known [7] ethyl 3,3,7-trimethyl-4-(*E*)-octenoate (**1**) (Scheme 1).

2.2.1. (E)-3,3,7-trimethyl-4-octenoic acid (2)

Ester **1** (8.6 g, 0.04 mol) was added to KOH (2.8 g) in ethanol (30 ml) solution and refluxed for 3 h. Then ethanol was evaporated in vacuo and the residue was diluted with water and organic impurities were extracted with diethyl ether. The aqueous solution was acidified (pH 4.0) with 0.01 M HCl and extracted with diethyl ether. The ethereal extract was washed with brine, dried over $MgSO_4$ and evaporated in vacuo to give the acid **2** (5.4 g, yield = 65%).

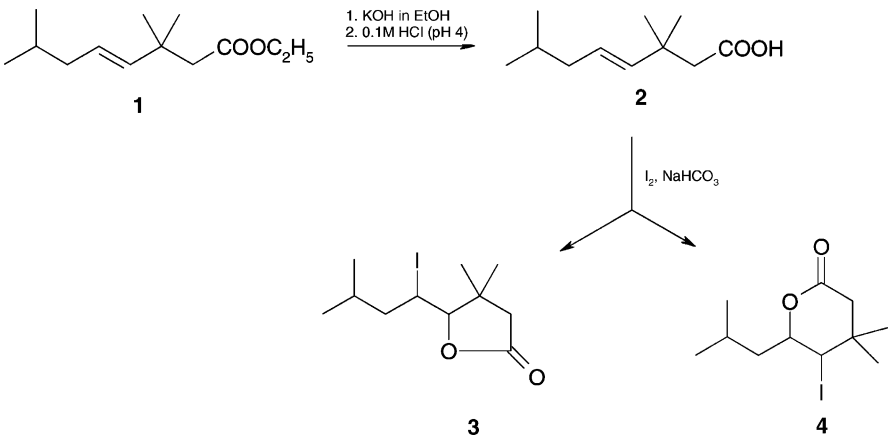
(**2**): $n_D^{20} = 1.4564$ ^{1}H NMR ($CDCl_3$) δ: 0.84 (d, J = 6.6 Hz, 6H, –CH(CH_3)$_2$), 1.13 (s, 6H, –C(CH_3)$_2$–), 1.56 (m, 1H, –CH(CH$_3$)$_2$), 1.86 (t, J = 6.6 Hz, 2H, –CH_2–CH=CH–), 2.29 (s, 2H, –CH_2–C(O)–), 5.37 (dt, J = 15.6 and 6.6 Hz, 1H, –CH$_2$–CH=CH–), 5.45 (d, J = 15.6 Hz, 1H, –CH$_2$–CH=CH–) 10.5 (br, s, 1H, COOH); IR (cm^{-1}): 1716 (s), 2600–3300 (sb), 976 (m).

2.2.2. 4,4-Dimethyl-5-(1-iodo-3-methylbutyl)tetrahydrofuran-2-one (3) and 4,4-dimethyl-6-(2-iodo-3-methylproyl)tetrahydro-2H-pyran-2-one (4)

A 0.5 M $NaHCO_3$ solution (60 ml) was added to a solution of acid **2** (5.4 g, 0.026 mol) in diethyl ether (100 ml). The mixture was stirred at room temperature for 30 min and then refluxed. To the refluxing mixture was gradually added a solution of KI (20.8 g, 0.12 mol) and I_2 (10 g, 0.08 mol) in water (100 ml). The mixture was stirred under reflux for 10 h. After cooling, it was diluted with diethyl ether (50 ml) and washed with $Na_2S_2O_3$ solution. The ethereal solution was washed with saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated in vacuo to give 4.3 g of a mixture of crude iodolactones **3** and **4** (yield 56%). Separation by column chromatography (silica gel, hexane–ethyl acetate, 20:1) afforded pure products: **3** (2.9 g) and **4** (1.4 g). Their physical and spectral data are as follows.

(**3**): (Oily liquid); ^{1}H NMR ($CDCl_3$) δ: 0.79 and 0.92 (two d, J = 6.5 Hz, 6H, –CH(CH_3)$_2$), 1.11 and 1.42 (two s, 6H, –C(CH_3)$_2$–), 1.62–1.82 (two m, 2H, –CH_2–CHJ–), 1.89 (m, 1H, –CH(CH$_3$)$_2$) 2.31 and 2.44 (two d, J = 17.2 Hz, 2H, –CH_2–C(O)–, AB system), 3.91 (ddd, J = 11.1, 9.4 and 3.4 Hz, 1H, –CH$_2$CHI–), 4.36 (d, J = 9.4 Hz, 1H, >CH–O–); IR (cm^{-1}): 1796 (s), 1172 (s), 996 (s).

(**4**): Melting points 50–53 °C; ^{1}H NMR ($CDCl_3$) δ: 0.92 and 0.93 (two d, J = 6.4 Hz, 6H, –CH(CH_3)$_2$), 1.10 and 1.14 (two s, 6H, –C(CH_3)$_2$–), 1.67 and 1.98 (three m, 3H, –CH_2–CH(CH$_3$)$_2$), 2.38 and 2.71 (two d, J = 17.2 Hz, 2H, –CH_2–C(O)–, AB system), 3.92



Scheme 1.

(d, $J = 10.9$ Hz, 1H, –C*H*I–), 4.46 (ddd, $J = 11.1$, 10.9 and 1.8 Hz, 1H, >C*H*–O–); IR (cm^{-1}): 1740 (s), 1172 (s), 1012 (s).

3. Biotransformations

Most of strains used in this work are from local origin: *Beauveria bassiana* (AM 446), *Botrytis cinerea* (AM 235), *Fusarium culmorum* (AM 3/1), *Fusarium semitectum* (AM 20), *Fusarium solani* (AM 203) and *Rhodotorula rubra* (AM 4) are from the collection of the Institute of Biology and Botany, Medical University, Wrocław, *Trichotecium roseum* (AR 11) comes from the collection of the Department of Phytopathology, Agricultural University, Wrocław, *Yarrovia lipolytica* (A 101) is from the collection of the Institute of Microbiology, Agricultural University, Wrocław. *Pycnidiella resinae* (16050) was obtained from the collection of the Department of Phytopathology, Agricultural University, Kraków.

Cultivation of microorganisms was carried out on Sabouraud agar containing aminobac (5.0 g), peptone K (5.0 g), glucose (40.0 g) and agar (15.0 g) in distilled water (1 l), at 28 °C and stored in refrigerator at 4 °C.

3.1. Screening procedure

The microorganisms were cultivated at 22 °C in 300 ml Erlenmayer flasks containing 50 ml of Sabouraud medium (aminobac (5.0 g), peptone K (5.0 g), and glucose (40.0 g) in distilled water (1 l)). After 3 days of growth, 10 mg of substrate in 0.5 ml of acetone was added to the shaken cultures. The incubation was continued for 14 days. The products were extracted with diethyl ether after 5, 9 and 14 days and analyzed by TLC (silica gel, hexane–ethyl acetate, 20:1) and gas chromatography (HP-1 column). The results of GC analyses are presented in Table 1 (for lactone **3**) and Table 2 (for lactone **4**).

3.2. Preparative biotransformation

Iodolactones **3** or **4** (300 mg in 3 ml of acetone) was added to 3-day cultures of *B. cinerea* (three flasks with 300 ml of the medium in each) prepared as described for the screening procedure. After 5 days of shaking the products were extracted with diethyl ether. The ethereal solutions were evaporated in vacuo and the crude product mixtures were separated by column chromatography (silica gel, hexane–ethyl acetate, 20:1). Preparative biotransformation of δ-iodo-γ-lactone (**3**) afforded 35 mg of substrate, 72 mg of hydroxy iodolactone **5** and 74 mg of hydroxy unsaturated lactone **6**. Their physical and spectral data are as follows.

Table 1
Composition (in percentage according to GC) of the product mixtures of biotransformation of iodolactone (**3**)

Entry	Strain	Time (days)	Products (%)		
			(**3**)	(**5**)	(**6**)
1	*Beauveria bassiana*	5	100	0	0
2		9	94	6	0
3		14	93	7	0
4	*Botrytis cinerea*	5	18	41	41
5		9	6	23	71
6		14	0	5	95
7	*Fusarium culmorum*	5	100	0	0
8		9	100	0	0
9		14	22	78	0
10	*Fusarium semitectum*	5	100	0	0
11		9	98	2	0
12		14	80	20	0
13	*Fusarium solani*	5	76	24	0
14		9	73	24	3
15		14	44	53	3
16	*Pycnidiella resinae*	5	80	11	9
17		9	78	11	11
18		14	78	5	17
19	*Rhodotorula rubra*	5	100	0	0
20		9	86	8	6
21		14	86	7	7
22	*Trichotecium roseum*	5	100	0	0
23		9	98	2	0
24		14	97	3	0
25	*Yarrovia lipolytica*	5	100	0	0
26		9	81	17	2
27		14	56	42	2

3.2.1. 4,4-Dimethyl-5-(1-iodo-3-hydroxy-3-methylbutyl)tetrahydrofuran-2-one (**5**)

(Oily liquid); $[\alpha]_D^{29} = 6.2$, ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 1.20 and 1.25 (two s, 6H, –C(CH_3)$_2$–), 1.30 and 1.45 (two s, 6H, –C(OH)(CH_3)$_2$–),

Table 2
Composition (in percentage according to GC) of the product mixtures of biotransformation of iodolactone (**4**)

Entry	Strain	Time (days)	Products (%)	
			(**4**)	(**7**)
1	*Beauveria bassiana*	5	75	25
2		9	68	32
3		14	66	34
4	*Botrytis cinerea*	5	88	12
5		9	79	21
6		14	39	61
7	*Fusarium culmorum*	5	40	60
8		9	40	60
9		14	40	60
10	*Fusarium semitectum*	5	96	4
11		9	90	10
12		14	77	23
13	*Fusarium solani*	5	76	24
14		9	68	32
15		14	51	49
16	*Pycnidiella resinae*	5	85	15
17		9	80	20
18		14	80	20
19	*Rhodotorula rubra*	5	98	2
20		9	98	2
21		14	96	4
22	*Trichotecium roseum*	5	89	11
23		9	78	22
24		14	72	28
25	*Yarrovia lipolytica*	5	68	32
26		9	52	48
27		14	40	60

1.70 (s, 1H, –O*H*), 2.32 (dd, J = 15.9 and 7.4 Hz, 1H, one of –CH_2–CH(*J*)–), 2.58 (dd, J = 15.9 and 3.1 Hz, 1H, one of –CH_2–CH(*J*)–), 2.56 and 2.61 (two d, J = 17.2 Hz, 2H, –CH_2–C(O)–, AB system), 4.15 (ddd, J = 9.4, 7.7 and 3.1 Hz, 1H, –C*H*I–), 4.38 (d, J = 9.4 Hz, 1H, >C*H*–O–); IR (cm^{-1}): 3500 (sb), 1788 (s), 1290 (s), 1228 (s), 1204 (s), 1180 (s).

3.2.2. 4,4-Dimethyl-5-(3-methyl-1-buten-1-yl)-tetrahydrofuran-2-one (6)

(Oily liquid); $[\alpha]_D^{28}$ = 10.1, (c = 1.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.98 and 1.14 (two s, 6H, –C(CH_3)$_2$–), 1.32 (s, 6H, –C(OH)(CH_3)$_2$–), 1.54 (s, 1H, –O*H*), 2.35 and 2.38 (two d, J = 16.9 Hz, 2H, –CH_2–C(O)–, AB system), 4.49 (d, J = 6.8 Hz, 1H, >C*H*–O–), 5.65 (dd, J = 15.6 and 6.8 Hz, 1H, –CH$_2$C*H*=CH–), 5.94 (d, J = 15.6 Hz, 1H, –CH$_2$CH=C*H*–); IR (cm^{-1}): 3476 (sb), 1788 (s), 1656 (m), 1292 (s), 1204 (s), 1132 (s), 971 (s).

Preparative biotransformation of 300 mg of γ-iodo-δ-lactone (**4**) after 5 days gave the mixture of substrate **4** (80 mg) and hydroxy iodolactone **7** (84 mg). The physical and spectral data are given below.

3.2.3. 5-Iodo-4,4-dimethyl-6-(2-hydroxy-2-methylpropyl)-tetrahydro-2H-pyran-2-one (7)

Melting points 100–103 °C; $[\alpha]_D^{29}$ = −1.1, (c = 3.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 1.11 and 1.16 (two s, 6H, –C(CH_3)$_2$–), 1.23 (s, 1H, –O*H*), 1.28 and 1.32 (two s, 6H, –C(OH)(CH_3)$_2$–), 2.44 and 2.70 (two d, J = 17.2 Hz, 2H, –CH_2–C(O)–, AB system), 1.87 (dd, J = 15.0 and 9.6 Hz, 1H, one of –CH_2–), 2.47 (d, J = 15.0 Hz, 1H, one of –CH_2–), 3.98 (d, J = 11.9 Hz, 1H, –C*H*I–), 4.93 (dd, J = 11.0 and 9.6 Hz, 1H, >C*H*–O–); IR (cm^{-1}): 3476 (sb), 1740 (s), 1256 (s), 1144 (s), 1028 (s).

4. Results and discussion

The substrates for biotransformation δ-iodo-γ-lactone (**3**) and γ-iodo-δ-lactone (**4**) were obtained from known [7] ester **1** according to Scheme 1. The ester **1** was transformed into acid **2** in 65% yield by alkaline hydrolysis with ethanolic KOH solution. The lactone ring was closed by iodolactonization of the acid **2** carried out according to the procedure described by Mori and Nakazano [8]. A mixture of δ-iodo-γ-lactone (**3**) (66%) and γ-iodo-δ-lactone (**4**) (34%) was obtained (Scheme 1). Pure lactones were separated by column chromatography.

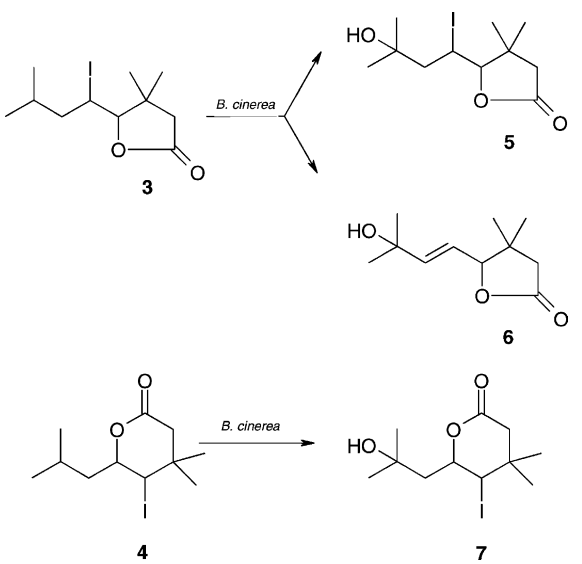
The five-membered lactone ring in **3** was confirmed by an absorption band at 1796 cm^{-1} in the IR spectrum and a doublet (J = 9.4 Hz) at 4.36 ppm (H-5) in the ^{1}H NMR spectra. The presence of the δ-lactone ring in **4** was confirmed by an absorption band at 1740 cm^{-1} in the IR spectrum, the doublet (J = 9.4 Hz) of H-5 at 3.92 ppm and the multiplet (ddd, J = 10.8, 11.1 and 1.8 Hz) of H-6 at 4.46 ppm in the ^{1}H NMR spectrum.

A preliminary screening experiment (monitoring by TLC and GC) showed that all fungi species from

among the nine tested transformed iodolactone **3** (Table 1). Four of them: *B. cinerea*, *F. culmorum*, *F. solani* and *Y. lipolytica* transformed the substrate to a high extent. In the case of *B. bassiana*, *F. culmorum*, *F. semitectum* and *T. roseum* the formation of only one product—hydroxy iodolactone (**5**) was observed. Strains: *B. cinerea*, *F. solani*, *P. resinae*, *R. rubra* and *Y. lipolytica* were able to dehalogenate iodolactone (**3**) into hydroxy unsaturated lactone (**6**).

The analysis of the dependence of the composition of product mixture obtained from the biotransformation of **3** with *B. cinerea* on time (entries 4–6) indicates that the unsaturated hydroxy lactone **6** is probably formed from hydroxy iodolactone **5** by dehydrohalogenation. So the first transformation of iodolactone **3** is its hydroxylation and then the dehydrohalogenation of hydroxy lactone formed (Scheme 2). The first biotransformation–hydroxylation is well known in the literature. There are many examples of biohydroxylation of ketones [9–12], lactones [13,14], hydrocarbons [15,16], steroids [17], and other natural and non-natural compounds [18]. In opposite to this we have not found in the literature any information about the second transformation–dehydrohalogenation carried out by microorganisms.

It is noteworthy that this microbial dehydrohalogenation leads to a disubstituted double bond in α-, β-position to the furanone ring, whereas the chemical dehydrohalogenation with DBU gave the unsaturated lactone with a trisubstituted double bond exocyclic to the lactone ring [19].



Scheme 2.

Each one of the tested fungal strains was able to transform γ-iodo-δ-lactone (**4**) (Table 2). Screening experiments indicated that only one product from **4** was formed: hydroxy iodolactone **7**.

The most efficient was again *B. cinerea* (according to GC over 60% after 14 days, entry 6) and the least efficient: *R. rubra* (according to GC only 4% after 14 days, entry 21).

Preparative biotransformation of lactones **3** and **4** were carried out with *B. cinerea*. Biotransformation duration of 5 days for **3** and 14 days for **4** was selected, because after that time all products and substrates are present in the reaction mixture. That gave the possibility to isolate unreacted substrate and all products in reasonable amounts and check the enantioselectivity of biotransformation.

GC analysis (carried out on a chiral column) of the product mixture obtained from a preparative biotransformation of **3** indicated that it contains unreacted substrate ($[\alpha]_D^{28} = 2.2$, $c = 3.54$, $CHCl_3$) with (+) enantiomer (ee 6%) predominating, and hydroxy iodolactone **5** and hydroxy unsaturated lactone **6** with (+) enantiomers predominating (ee 12 and 23%, respectively).

Structures of lactones **5** and **6** were established on the basis of IR and ^{1}H NMR data. The IR spectrum of **5** showed the presence of a tertiary hydroxy group (absorption bands 3476, 1292 and 1132 cm^{-1}) and a lactone ring (1788 cm^{-1}) in the molecule. In the ^{1}H NMR spectrum the methyl groups at C-3′ in the side chain gave a singlet at 1.32 ppm. The multiplet pattern of the protons of the furanone ring confirmed that the lactone ring was not affected by biotransformation. The double bond in lactone **6** was confirmed by the presence of the multiplets of vinyl protons at 5.65 and 5.94 ppm. The coupling constant ($J = 15.6$ Hz) indicates the *E* configuration of the double bond formed.

The separation of product mixture obtained from biotransformation of lactone **4** afforded the unreacted substrate with predominance of enantiomer (−) (ee 16%) ($[\alpha]_D^{28} = -2.0$, $c = 2.68$, $CHCl_3$) and hydroxy iodolactone with predominance of enantiomer (−) (ee 14%), too.

The spectral data of the product of this biotransformation (**7**) proved that it is hydroxy iodolactone with a tertiary hydroxy group (ν = 3476, 1256 and 1144 cm^{-1}). The unchanged δ-lactone ring in this product was confirmed by the absorption band at 1740 cm^{-1} in the IR spectrum and the pattern of the multiplets of –CHI– and >CH–O– protons at 3.98 and 4.93, respectively, in the ^{1}H NMR spectrum.

5. Conclusions

To give a summary of the results presented above it can be pointed out the following.

1. *Botrytis cinerea* caused effective dehalogenation of δ-iodo-γ-lactone (**3**) by hydroxylation then dehydrohalogenation.
2. The enantioselectivity of both processes was low (ee from 12 to 23%).
3. The γ-iodo-δ-lactone was only hydroxylated by this strain in the same conditions.

Acknowledgements

This work was supported by the State Committee for Scientific Research (KBN), Grant No. 6 PO6B 031 20.

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