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Lactones 12.[☆] Enzymatic lactonization of γ,δ-epoxy esters by the apple fruit and Jerusalem artichoke bulb

Teresa Olejniczak, Agnieszka Mironowicz, and Czesław Wawrzeńczyk[*]

Department of Chemistry, Agricultural University, Norwida 25, 50-375 Wrocław, Poland

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Abstract

The enzymatic lactonization of three acyclic γ,δ-epoxy esters (ethyl 3,7-dimethyl-4,5-epoxyoctanoate, ethyl 3,7,7-trimethyl-4,5-epoxyoctanoate, and ethyl 3,3,7-trimethyl-4,5-epoxyoctanoate) by apple fruit (*Malus silvestris*) and Jerusalem artichoke bulb (*Helianthus tuberosus* L.) was investigated. The substrates were transformed into a mixture of isomeric δ-hydroxy-γ-lactones and γ-hydroxy-δ-lactones. The γ-lactones (yields ranging from 45–70%) predominated over δ-lactones (yields ranging from 8–40%). The composition of the product mixture depended on the structure of substrate as well as the biocatalyst. The enzymatic system in these biocatalysts also exhibited diastereoselectivity and enantioselectivity.

Keywords: *Malus silvestris*; *Helianthus tuberosus*; Biotransformations; Lactonization; γ,δ-Epoxy esters; δ-Hydroxy-γ-lactones; γ-Hydroxy-δ-lactones

1. Introduction

Lactones are present in many plants [2–4], where they are usually components of essential oils. They often contribute to odor properties of plants, their flowers, and fruits [5]. Lactones, especially sesquiterpenoid ones, also exhibit specific and useful

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[*] Corresponding author. Fax +4871-3284124.
E-mail address: c-waw@ozi.ar.wroc.pl (C. Wawrzeńczyk).

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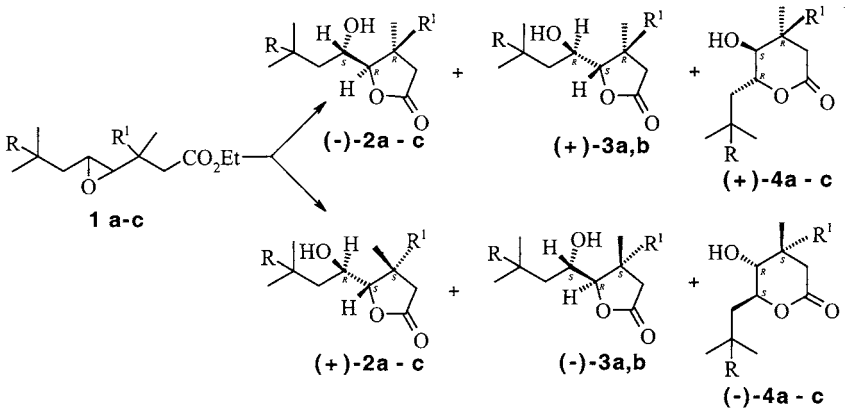
biological activities. They are commonly known for their cytostatic and antimicrobial [6,7] activity. Many of them have feeding deterrent activity against insects [8–10]. We are interested in the synthesis of terpenoid lactones because of this last activity. We have obtained over 50 lactones, which exhibit high activity, comparable with natural antifeedants [1,11,12]. We have observed that the feeding deterrent activity, like other biological activities, depends on the configuration of chiral centers present in the molecule of the tested compounds. It is therefore important in biological studies to test for activity using pure enantiomers. They can be obtained by resolution of racemic samples or via asymmetric synthesis.

As part of our ongoing studies in this area we have focused our efforts on an efficient and stereoselective synthesis of hydroxy lactones from three γ,δ-epoxy esters (ethyl 3,7-dimethyl-4,5-epoxyoctanoate, ethyl 3,7,7-trimethyl-4,5-epoxyoctanoate, and ethyl 3,3,7-trimethyl-4,5-epoxyoctanoate **1a–c**, Scheme 1) using biocatalysts of the lactonization processes. Recently [13,14] we reported the results of lactonization of racemic γ,δ-epoxy esters using microorganisms. It was found that some strains transformed these substrates into δ-hydroxy-γ-lactones with satisfactory yield and high enantioselectivity (ee ~70%).

Here, we present results of lactonization of γ,δ-epoxy esters by enzymatic systems of apple fruit (*Malus silvestris*) and bulbs of Jerusalem artichoke (*Helianthus tuberosus*). Our rationale for using apple fruit and artichoke bulb in this process is based on the fact that lactones are present in these plants [3,4]. Therefore they should have an enzymatic system able to synthesize lactones. The choice of *H. tuberosus* bulb flesh and *M. silvestris* fruit flesh for the biotransformation was also suggested by results obtained in the transformation of ketones, racemic esters, and alcohols using these systems [15,16].



a, R = H , R¹ = H **b**, R = CH₃ , R¹ = H **c**, R = H , R¹ = CH₃

Scheme 1.

2. Materials and methods

2.1. Analytical methods

Gas chromatographic analyses were performed on a Hewlett–Packard 5890 instrument, FID, carrier gas—H_2 at 2 ml min^{-1}, using the following Chrompack WCOT capillary columns: HP-1 (Crosslinked Methyl Siloxane; 25 m × 0.32 mm × 0.52 μm), Chirasil-Val-L (25 m × 0.25 mm × 0.12 μm) for **2a**, **3a**, **4a** (column temp. 142°/1 min; negative gradient 0.1°/min, injector temp. 150°, detector temp. 300°), Chirasil Dex CB (25 m × 0.25 mm × 0.25 μm) for **2b**, **3b**, **4b** (column temp. 134°/0 min; 0,1°/1 min; injector temp. 150°, detector temp. 300°), and CP-cyclodextrin-B-2,3,6-M-19 (25 m × 0.25 mm × 0.25 μm) for **2c** and **4c** (column temp. 143°/1 min; 0.1°/1 min; injector temp. 150°, detector temp. 300°); split ratio 150. Analytical TLC was carried out on silica gel Kieselgel 60 F_{254} (Merck) with hexane –Me_2CO–*iso*-PrOH–EtOAc (60:1:3:1) as the developing system.

2.2. Substrates and standards

Racemic epoxy esters **1a–c** were substrates in the biotransformations carried out. Their syntheses as well as spectral and physical data are presented elsewhere [17]. Enantiomers of hydroxy lactones **2a–c**, **3a**, **b**, and **4a–c** [13,18,19] were used as standards for GC analysis with chiral columns. Optically active isomers of **2a**, **b**, **3a**, **b**, and **4a**, **b** were obtained by lactonization of diastereoisomeric epoxy esters with a fixed configuration at C-3 [13]. Enantiomers of hydroxy lactone **2c** were obtained via the hydrolysis of its racemic acetate with *Fusarium tricinctum* or *Fusarium solani* [18,19]. Upon hydrolysis of the acetate of δ-lactone **4c** with these microorganisms, only the enantiomerically enriched isomers of **4c** were obtained [19]. Configurations of chiral centers in the studied compounds were established on the basis of ^1H NMR and CD spectral data [13].

2.3. Non-enzymatic lactonization of *1a*

In order to determine the pH of the biotransformation medium for which acidic lactonization is not significant, the following experiment was carried out. A solution of epoxy ester **1a** (0.001 mol) in acetone (1 ml) was added to conical flask containing an aqueous solution (50 ml) of tartaric acid with defined pH (1.6; 2.6; 3.6; 4.5; 5.8; 6.8) and the reaction mixture was shaken for 48 h at room temperature. After that time the reaction mixture was extracted with ethyl ether and the composition of product mixture was determined by GC using the HP-1 column.

2.4. Biocatalysts

Apples (*M. silvestris*) of 'Gloucester' variety were received from the Institute of Pomology, Wróblowice near Wrocław. Topinambur (*H. tuberosus*) was obtained from the Experiment Station of Agricultural University, Wrocław.

2.5. Biotransformation

Healthy bulbs from the Jerusalem artichoke or apples were comminuted using an electric mixer for 2 min. Subsequently, 20 ml of the pulp (3.5–4.0 g dry wt.) was placed in an Erlenmeyer flasks containing 50 ml of sodium phosphate buffer (pH 4.5 or 7.0). An aqueous solution of NaOH (0.1 M) was added to the apple pulp to maintain pH at 4.5 or 7.0. This pulp with 0.1 mM of substrate (dissolved in 0.5 ml acetone) was shaken for 48 h. Then the products were extracted with ethyl ether. The enantiomeric composition of product mixture was analyzed by GC with using chiral columns mentioned above.

3. Results and discussion

The biotransformation of **1a–c** by apples and Jerusalem artichoke was preceded by determining up the optimal conditions for the experiment. One of the most important factors, which could affect the lactonization reaction, is the pH of the medium. In an acidic environment, γ,δ-epoxy esters are readily lactonized without the help of enzymes. Acidic lactonization of racemic epoxy esters **1a** and **1b** gave a mixture of two racemic γ-lactones (**2a**, **3a** and **2b**, **3b**) and one racemic δ-lactone (**4a**, **4b**), respectively (Scheme 1). The epoxy ester **1c** afforded the racemic γ-lactone **2c** and δ-lactone **4c** using the same conditions [17].

In order to exclude the non-enzymatic lactonization process, we investigated the dependence of the course of the lactonization of **1a–c** on the pH of the medium. Lactonization of epoxy ester (**1a**) was carried out in tartaric acid solutions at pH values ranging from 1.6 to 6.8. The results presented in Fig. 1 depict that at a pH above 4.5, the epoxy ester (**1a**) does not undergo lactonization. At the pH below 4.5 the proportion of γ-, δ-lactones obtained from **1a** varies with the pH of the reaction medium (Fig. 1).

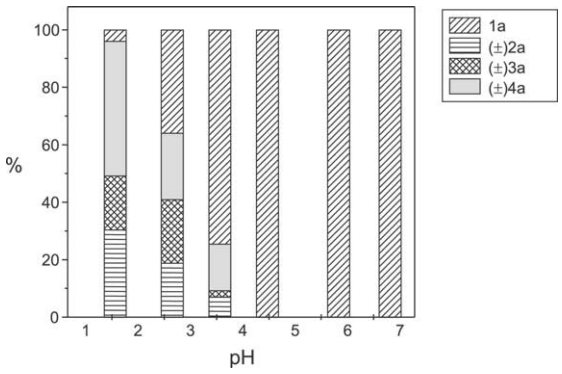


Fig. 1. Composition of product mixture of acidic lactonization of **1a** at various pH of the reaction medium.

Taking into consideration the value of pH (4.5) described here and the natural apple pulp pH (3.5), we added 0.1 M aqueous solution of NaOH to the apple pulp, thus obtaining pH 4.5. The pulp prepared in this way was placed in the solutions of sodium phosphate buffer of different pH values (4.5 and 7.0), and incubated with an appropriate substrate. The time of incubation was 48 h.

The compositions of the product mixture of biotransformations with the enzymatic system of apple *Gloucester* are presented in Table 1.

Epoxy esters **1a** and **1b** were transformed with similar efficiency. In sodium phosphate buffer (pH 7.0), 65 and 72% of epoxy ester was transformed into hydroxy lactones, respectively. Similar transformations (84–74%) were achieved when the sodium phosphate buffer (pH 4.5) was used. Epoxy ester **1c** was lactonized to a lesser extent, only 20% at pH 7.0 and 66% at pH 4.5. Generally, when sodium phosphate buffer at pH 4.5 was used, lactonization proceeded to a greater extent than in the experiments using a buffer of pH 7.0.

All epoxy esters were transformed mainly into δ-hydroxy-γ-lactones. In the case of epoxy ester **1a**, the δ-hydroxy-γ-lactone, made up 56–71% of the product mixture with a predominance of the *cis* isomer (**3a**). The lactonization of **1b** afforded a mixture with an almost equimolar contribution of all lactones formed. In the case of the biotransformation of epoxy ester **1c** at pH 4.5, the γ-hydroxy-δ-lactone **4c** was a major (46%) product (entry 6). The product mixture contained also 20% of δ-hydroxy-γ-lactone (**2c**) and 34% of unreacted substrate.

Analysis of the enantioselectivity of lactonization carried out by GC (using chiral columns) indicates that the (−) enantiomers of *trans* γ-lactones **2a** and **2b**, (+) enantiomers of *cis* γ-lactones **3a** and **3b** and (+) enantiomers of δ-lactones **4a** and **4b** were formed predominantly. All these enantiomers have the *R* configuration at C-4. The best reaction enantioselectivity of lactonization was observed for biotransformation of **1b**. In the experiment at pH 7.0 (entry 3), the (−) isomer of *trans* δ-hydroxy-γ-lactone (**2b**) was formed with 74% ee and with 70% ee at pH 4.5 (entry 4). The comparison of enantioselectivity of lactonization of esters **1a**, **1b**, and **1c** indicates that the additional methyl group at C-7 (epoxy ester **1b**) increases enantioselectivity whereas an additional methyl group at C-3 (**1c**) leads to a decrease in enantioselectivity.

Table 1
Composition (in % according to GC) of the product mixture after biotransformation of **1a–c** with apple pulp (*M. silvestris*)

Entry	pH	Substrate	**1a–c**	**2a–c**		**3a,b**		**4a–c**	
				(−)	(+)	(−)	(+)	(−)	(+)
1	7.0	**1a**	35	17	7	12	20	3	6
2	4.5	**1a**	16	17	9	18	27	4	9
3	7.0	**1b**	28	20	3	8	14	10	17
4	4.5	**1b**	26	17	3	10	14	12	18
5	7.0	**1c**	80	7	7	—	—	3	3
6	4.5	**1c**	34	11	9	—	—	24	22

The enzymatic system of the Jerusalem artichoke was next examined for its ability to lactonize epoxy esters. The pH of a comminuted Jerusalem artichoke bulb is 4.5. Hence the biotransformation was carried out in the solutions of sodium phosphate buffers at pH 4.5, 7.0 and in water without buffer. Other conditions of biotransformations were the same as those used for lactonization with apple pulp.

The greatest extent of lactonization reaction was observed for epoxy ester **1a**. Additional methyl groups at C-3 (**1c**) and particularly at C-7 (**1b**) caused a decrease in the efficiency of the transformation (Table 2). Starting epoxy esters **1c** (32–47%) or **1b** (62–75%) were still present in the product mixture even after 2 days of biotransformation.

The results presented in Table 2 indicate that the composition of product mixtures did not depend on the pH of the medium. They are almost the same for experiments carried out in sodium phosphate buffers (pH 4.5 and 7.0) and in pure water (pH 7). This observation could suggest that activity and enantiospecificity of lactonizing enzymes of artichoke bulb do not depend on pH in range 4.5–7.0 or on the ionic strength of the reaction medium.

The enantioselectivity of lactonization with this enzymatic system was low for the epoxy ester **1a** (ee below 25%) and moderate for **1b** and **1c** (ee reaches 40%). Similar to lactonization with apple pulp, in the biotransformation of epoxy esters **1a** and **1b** with an artichoke bulb, the (+) enantiomers of *cis* γ-lactones **3a** and **3b** and δ-lactones **4a** and **4b** were the predominant isomers obtained. In contrast to biotransformation with apple enzymes, the (+) enantiomers of *trans* γ-lactones **2a** and **2b** prevailed over the (−) isomers.

In conclusion, it was shown for the first time, that enzymes present in apple fruit as well as in artichoke bulbs could be used as an effective agent for the lactonization of acyclic γ,δ-epoxy esters. Unfortunately the diastereospecificity of these enzymes towards the epoxy esters **1a** and **1b** as well as enantiospecificity towards epoxy ester **1c** is low. These observations preclude the use of these two systems in larger biotransformation reactions.

Table 2
Composition (in% according to GC) of the product mixture after biotransformation of **1a–c** with artichoke bulb (*H. tuberosus*)

Entry	pH	Substrate	**1a–c**	**2a–c**		**3a,b**		**4a–c**	
				(−)	(+)	(−)	(+)	(−)	(+)
1	7.0	**1a**	0	26	27	18	21	4	4
2	4.5	**1a**	0	25	27	14	23	4	7
3	Water	**1a**	0	24	28	16	23	4	5
4	7.0	**1b**	62	7	13	5	10	1	2
5	4.5	**1b**	75	3	9	3	5	2	3
6	Water	**1b**	62	6	15	5	9	1	2
7	7.0	**1c**	47	13	30	—	—	7	3
8	4.5	**1c**	7	14	37	—	—	24	18
9	Water	**1c**	32	12	31	—	—	17	8

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