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Enzymatic synthesis of AHA derivatives for cosmetic application

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Abstract

An immobilized lipase from *Candida antarctica* (Novozym 435) was used to catalyze synthesis of α-butylglucoside lactate by transesterification between α-butylglucoside and butyllactate in a solvent free medium. The influence of the reaction pressure on yield was investigated. The elimination of butanol co-product under reduced pressure resulted in the shift of the reaction equilibrium position in favor of synthesis. More than 95% of 0.5 M α-butylglucoside was converted in only 30 h. Consequently, very high α-butylglucoside lactate concentration (170 g/l) was obtained in a single batch reaction. Moreover, one purification step was enough to obtain α-butylglucoside lactate with a purity above 95% (w/w). Properties of α-butylglucoside lactate have been evaluated. This derivative is far less harmful than free lactic acid: a 0.55 M aqueous preparation is far less irritant than a lactic acid solution of a similar concentration. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Alpha-Hydroxy Acids (AHA) constitute a class of compounds which exert specific and unique effects on skin structure [1]. Their application at low concentration (under 5%) decreases intercorneocyte cohesion and induces skin peeling [2]. In particular, short chain α-hydroxy acids, such as lactic and glycolic acids, have been described as very good exfoliating and moisturizing agents [3]. Even so, when used at concentrations higher than 10%, they become irritant and penetrate too quickly into the deep epiderm [4,5]. To prevent these effects, they were grafted onto a lipophilic molecule, such as an alkyl chain [6]. Another solution is to use carbohydrate biopolymers as vectors of AHA, since they also exhibit moisturizing properties [5]. α-Butylglucoside is currently produced on an industrial scale by enzymatic conversion of maltose and butanol [7]. We therefore used this glucose derivative as a substrate for a transesterification reaction involving a lactate donor, butyllactate, and catalyzed by a lipase, Novozym 435.

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2. Experimental

2.1. Enzyme

The immobilized lipase B from *Candida antarctica* (Novozym®435) was provided by Novo Nordisk Industrie.

2.2. Substrates and solvents

L-Butyllactate was purchased from Fluka. Hexane, methanol and butanol-1 were from Prolabo. All the solvents used in chromatography were of HPLC grade.

α-Butylglucoside was produced and purified as described by Monsan et al. [7].

2.3. α-Butylglucoside lactate synthesis in stoppered flasks

Transesterification was carried out in 3 ml butyllactate containing 1.5 mmol of α-butylglucoside and 0.3 g of Novozym. The mixture was incubated at 60°C for 80 h, under magnetic stirring at 600 rpm. Samples were taken at intervals: after a 20-fold dilution in water, they were immediately filtered to eliminate the enzyme and analyzed.

2.4. α-Butylglucoside lactate synthesis under reduced pressure

α-Butylglucoside (10 mmol) was dissolved in 20 ml butyllactate, at 60°C, under specified reduced pressure, and under rotative agitation. The biocatalyst (2 g of Novozym) was then added and the reaction flask reconnected to the vacuum pump. Analysis of the mixture composition was performed regularly. Samples were prepared as described previously.

2.5. Purification

At the end of the reaction, the biocatalyst was removed by filtration.

Butyllactate was eliminated by liquid–liquid extraction with water and hexane. The mixture (1 volume) was vigorously mixed with water (1 volume) and hexane (2 volumes) for 30 min. Then, it was allowed to stand until organic and aqueous phases separated. The aqueous phase was recovered and water was evaporated at 60°C under reduced pressure. Further, hexane was added to the syrup at a volumic ratio of hexane:syrup of approximately 10:1. After intensive mixing, the upper layer (organic phase) was taken off and residual hexane was evaporated at 40°C, under reduced pressure.

2.6. Quantitative measurement of substrates and products

α-Butylglucoside, butyllactate, butanol and α-butylglucoside lactate concentrations were quantified by HPLC chromatography on a reverse phase column (Nucleosil C18, 6 μm, 250×4 mm, I.C.S., France); eluent: 50/50, methanol/water, flow rate: 0.5 ml/min, temperature: 33°C, injection volume: 10 μl, detection: refractometer (RI-Detector 8110, I.C.S., France).

2.7. Cytotoxicity assay

0.55 M lactic acid and α-butylglucoside lactate solutions were compared for their toxicity on model skin using the Episkin test system [8]. A reference sample composed of sodium dodecyl sulphate (SDS, 5 mM) was also tested. The product (100 μl/1.13 cm^3) was placed on the surface of the model skin and allowed to settle for 18 h. Product toxicity was then estimated by measuring cell viability according to the method described by Mosmann [9].

2.8. Ocular irritation assay

0.55 M and 1.1 M lactic acid and α-butylglucoside lactate solutions were compared for their toxicity on butchered beef cornea. The cornea was successively treated with Hank's balanced salt solution (HBSS), MEM culture medium (Minimum Essentiel Medium Eagle) and the product. It was finally rinsed with MEM culture medium. The test BCOP (Bovin Corneal Opacity and Permeability) was used to evaluate the in vitro ocular irritation index of the sample. This test measures two ocular irritation parameters: the opacity and the cellular permeability. The opacity is defined as the luminous transmission difference between a treated cornea and a control. The permeability is equal to the optical density (OD) measured at 490 nm with a spectrophotometer.

An in vitro irritation score has been defined with the following formula:

$$\text{Score} = \text{opacity value} + 15 \times \text{OD}.$$

3. Results and discussion

3.1. *α-Butyllactate synthesis in stoppered flasks*

α-Butylglucoside acylation with lactic acid was carried out in butyllactate at 60°C and under magnetic stirring. The immobilized lipase from *C. antarctica* was chosen as the biocatalyst because it had been described as a very regio-selective enzyme for the primary hydroxyl group of glucose [10,11]. The kinetic of *α*-butylglucoside lactate synthesis is displayed on Fig. 1. After 30 h, the reaction reaches an equilibrium. Yield cannot exceed 66%. The presence of butanol co-product limits the reaction to a maximum that cannot be theoretically exceeded in closed systems.

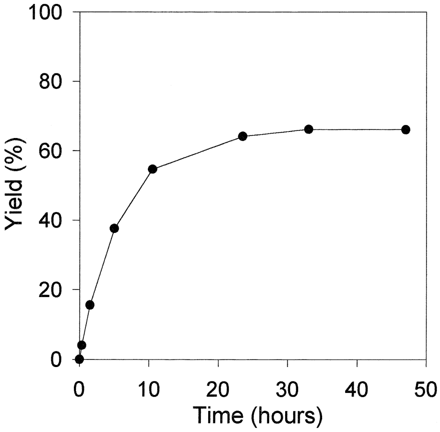

Fig. 1. *α*-Butylglucoside lactate synthesis under atmospheric pressure. The reaction was performed in butyllactate, at 60°C, in the presence of *α*-butylglucoside (0.5 M) and Novozym (100 g/l).

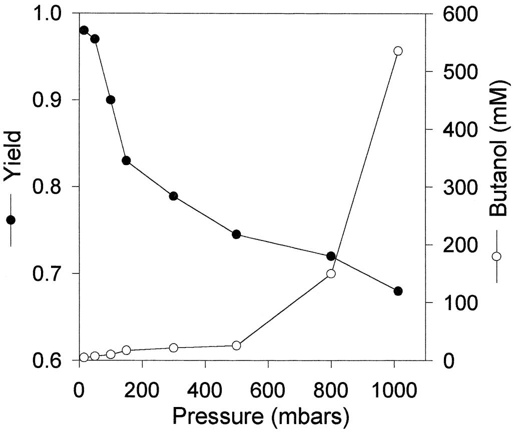

Fig. 2. Effect of reaction pressure on residual butanol concentration and on yield. *α*-Butylglucoside lactate synthesis was carried out in butyllactate, at 60°C, in the presence of Novozym (100 g/l) and *α*-butylglucoside (0.5 M); it was stopped after 80 h.

3.2. *Effect of reaction pressure on α-butylglucoside lactate synthesis*

Transesterification between *α*-butylglucoside and butyllactate is a reversible reaction:

$$\text{Butyllactate} + \alpha - \text{butylglucoside} \rightleftharpoons$$
$$\alpha - \text{butylglucoside lactate} + \text{Butanol}.$$

To shift the equilibrium position of the reaction, synthesis was performed under reduced pressure [7,11,12]. The effect of pressure on Novozym catalyzed transesterification between *α*-butylglucoside and butyllactate was investigated. Several reaction pressures were tested (between 15 and 1013 mbars). Butanol concentration and yields (calculated after 80-h reaction) were plotted against pressure. As can be seen from Fig. 2, a pressure decrease leads to an efficient evaporation of butanol co-product. The elimination of the alcohol enables the equilibrium position of the reaction to be shifted in

Table 1
α-Butylglucoside lactate effect on model skin cells and on butchered beef cornea

	Control	Reference (SDS)	Lactic acid		α-Butylglucoside lactate	
Concentration (M)	/	0.005	0.55	1.10	0.55	1.10
Model skin cell viability (%)	100	14.8	16.0	n.d.	62.4	n.d.
Ocular irritation index	0	n.d.	1.93	41.1	0	0

α-Butylglucoside lactate was produced and purified as described under Section 2.
n.d.: not determined.

favor of synthesis of α-butylglucoside lactate. As a consequence, yields are significantly improved. Under 15 mbars, α-butylglucoside conversion is higher than 95% after only 30-h reaction. High α-butylglucoside lactate concentration (170 g/l) can be reached in a single batch reaction.

3.3. Purification of α-butylglucoside lactate

α-Butylglucoside lactate was synthesized under 15 mbars in a solvent free medium consisting of butyllactate and α-butylglucoside (0.5 M). The glucose derivative substrate was completely converted into α-butylglucoside lactate. The product was finally purified by liquid–liquid extraction with water and hexane. α-Butylglucoside lactate was recovered in the aqueous phase while butyllactate partitioned into the organic phase. This single purification step was enough to retrieve α-butylglucoside lactate with a purity above 95% (w/w).

3.4. α-Butylglucoside lactate effect on skin and on cornea

α-Butylglucoside lactate (produced and purified as described previously) and lactic acid were compared for their effect on model skin and on butchered beef cornea (Table 1). At a concentration of 0.55 M, lactic acid can be classified as irritant for model skin cells since cell viability is far below 50%. On the other hand, an α-butylglucoside lactate solution of a similar concentration exerts only a slight irritant effect on skin (cell viability is between 50 and 70%). At a concentration of 1.1 M, lactic acid is irritant for cornea, whereas, α-butylglucoside lactate is completely harmless. Both tests described above clearly show that α-butylglucoside lactate is far less irritant than free lactic acid. This undoubtedly proves the value to the cosmetics industry of the synthesized product.

4. Conclusion

The present work describes an efficient procedure for α-butylglucoside lactate production and purification. A solvent-free process involving lipase B from *C. antarctica* enables almost 100% conversion of α-butylglucoside substrate into α-butylglucoside lactate, which can be recovered with a purity above 95% (w/w). The effect of pressure on yields was also studied. The evaporation of butanol under reduced pressure enables a shift in the equilibrium position of the reaction in favor of α-butylglucoside lactate formation. Under 15 mbars, at 60°C and in the presence of α-butylglucoside (0.5 M) and Novozym (100 g/l), concentrated α-butylglucoside lactate (170 g/l) can be obtained in a very short time (30 h). The purification scheme consisting in butyllactate extraction with hexane is enough to recover the product in a state of high purity.

Finally, the purified product was proved to be far less irritant than lactic acid of a similar concentration. Its value as a lactic acid vector is clear, offering the cosmetic benefits of the α-hydroxy acid (thanks to a gradual hydrolysis of the ester bond by epiderm esterases) but also eliminating the adverse effects of the free acid.

The process here described is not limited to the acylation of α-butylglucoside with lactic acid or other α-hydroxy acids. The use of α-butylglucoside as a vector could be widened to other valuable products.

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