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Enzymatic synthesis of derivatives of vitamin A in organic media

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Abstract

The present article provides an enzymatic method of retinol esterification to reduce photodestruction and irritation problems characteristic of retinol. More specifically, it relates to a method of synthesising retinyl adipate, retinyl succinate, retinyl oleate and retinyl lactate greatly appreciated by cosmetic manufacturer. Desired compounds can be synthesised directly using *Candida antarctica* lipase and *Rhizomucor miehei* lipase in organic media.

Keywords: Retinol; Enzymatic synthesis; Lipase; Cosmetics

1. Introduction

Vitamin A (retinol) and derivatives (retinyl esters, retinoic acid, retinaldehyde), are of great commercial potential in cosmetics and pharmaceuticals such as skin care products. This water-insoluble vitamin has been shown to enhance gap junction communication in a dose-dependent manner, and may promote normal cell growth [1,2]. Vitamin A is necessary for maintenance of healthy epithelial tissue and can prevent the inception or progress of skin cancers by stimulating normal cell differentiation. Deficiency of vitamin A is usually accompanied by a number of severe diseases [3,4]. Consequently, in certain conditions extra amounts are needed in order to cure or prevent certain of these diseases. But vitamin A is very unstable (readily oxidised in air and inactivated by UV light) and difficult to formulate satisfactorily for topical application. Moreover, vitamin A is irritating to the skin. Consequently, to reduce photodestruction and irritation problems, vitamin A may be stabilised by conversion to vitamin A ester (retinyl ester).

Various chemical methods have been published describing the synthesis of retinyl esters. However, retinol degradation often is important, leading to low yields. Moreover, these methods often have the disadvantage that they produce amounts of undesired residual chemical reactants in the final products such as salts.

In view of the attractiveness of retinyl esters as cosmetic agents, as therapeutic and nutritional additives, enzymatic methods can be suggested to produce retinyl esters by a ‘‘biological’’ process. Hydrolytic enzymes such as

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lipases in particular, have received special attention because of their effectiveness in regioselective and enantioselective esterifications and transesterifications of organic acids and alcohols in organic solvents [5,6]. Applications of such enzymatic reactions have been considered, for example, in the synthesis of flavor [7–9] and fatty-acid esters [10,11], in the regioselective acylation of carbohydrates [12], in the chemoselective acylation of amino-sugar derivatives [13,14] and in the synthesis of lactones [15], peptides [16], and chiral drugs [17].

The present article presents a preliminary study of the enzymatic retinol esterification using lipases in organic media.

2. Experimental

2.1. Biological and chemical material

Novozym® SP 435 (lipase from *Candida antarctica* immobilised on an acrylic resin), Lipozyme® (lipase from *Rhizomucor miehei* immobilised on an anionic macroporous resin, Duolite 568N), were from Novo Industries (Denmark). Porcine pancreatic lipase, *C. cylindracea* lipase and *Rhizopus arrhizus* lipase, were from Sigma (USA).

The solvents, all analytical grade, retinol, dimethyl adipate and dimethyl succinate, monomethyl succinate, succinic acid, methyl oleate and L-methyl lactate were from Sigma.

2.2. General procedure for the enzymatic reaction

Reactions were conducted in screw-cap glass vials containing 0.5 mmol of retinol **1**, 0.5 mmol of acyl donor, 100 mg of lipase and 0.25 g of 4-Å molecular sieves with 5 ml of solvent. The reaction mixture was stirred under positive argon pressure at 55°C and protected from light. After 50 h the biocatalyst and the molecular sieve were removed by filtration.

2.3. HPLC analysis

Analyses were performed with an HPLC system from Hewlett Packard (processor, pump, UV detector and injector model 1100, differential refractometer (Waters) model 410), equipped with an Ultrasep C18 (250 × 4 mm, 6 μ) reverse phase column from ICS, France. 25 μl of the appropriate dilution of the reaction mixture were injected. A mixture of methanol/water/acetic acid, 90/10/0.3 (v/v/v) was used as eluent at 40°C and a flow rate of 1 ml/min. Products were detected using a UV detector at 280 nm and a differential refractometer. The samples were quantitated by means of calibration curves with pure reagent.

2.4. Purification of reaction products

At the end of the reaction, the biocatalyst was removed by filtration and the solvent evaporated under reduced pressure. The remaining oil was purified by HPLC preparative using an Ultrasep C18 (250 × 8 mm, 6 μ) reverse phase column from ICS, France. A mixture of methanol/water/acetic acid, 90/10/0.3 (v/v/v) was used as eluent at 40°C and a flow rate of 3 ml/min. Products were detected using a UV detector at 280 nm.

2.5. Structural analysis

^{13}C and ^{1}H Nuclear Magnetic Resonance spectra were recorded on a JEOL-JNM LA400 (400 MHz) spectrometer (Laboratoire Commun d'Analyse, Université de La Rochelle), with an internal reference of tetramethylsilane. IR spectra were recorded on a Perkin Elmer Paragon 1000PC instrument in NaCl plate. Mass spectra were recorded on a Varian MAT311 in the ''Centre Régional de Mesure Physiques de L'Ouest'' (CRMPO), Université de Rennes, France.

(Cad: carbon of adipate; Csu: carbon of succinate; Col: carbon of oleate; Cla: carbon of lactate.)

(Had: proton of adipate; Hsu: proton of succinate; Hol: proton of oleate; Hla: proton of lactate.)

*2.5.1. Retinyl methyl succinate (**3a**)*

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1}, and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 173 and 172.5 (2Co*su* ester), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 51.2 (CH_3O), 33.0, 33.3 (CH_2–CO*su*), 23.8 ($2\times CH_2$ *su*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3–C=), 1.89 (3H, s, CH_3–C=), 4.68 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, $2\times$ CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 2.3 (4H, m, 2 CH_2CO*su*), 3.56 (3H, s, OCH_3 *su*); $C_{25}H_{36}O_4$ requires M, 400.2613, found M^+, 400.2618; *m/z* 400 (M^+, 3.16%), 268 (M^+–$C_5H_8O_4$, 4.17%), 43 (C_3H_7, 100%).

*2.5.2. Retinyl succinate (**3b**)*

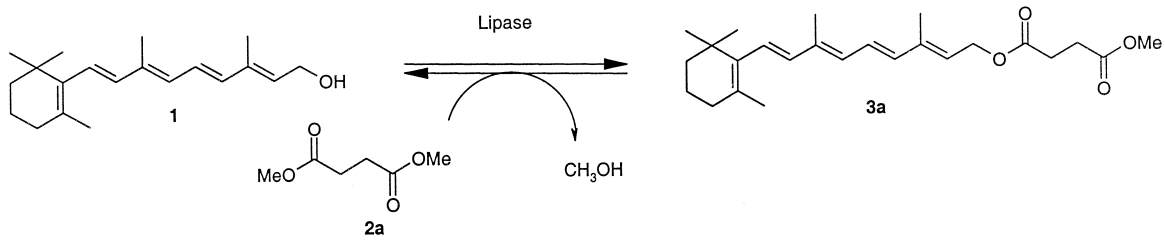
IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1}, ν(CO–O acid) = 1725 cm^{-1} and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 173 (CO*su* ester), 170 (Co*su* acid), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 33.0, 33.3 (CH_2–CO*su*), 23.8 ($2\times CH_2$ *su*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3–C=), 1.89 (3H, s, CH_3–C=), 4.68 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, $2\times$ CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 2.3 (4H, m, 2 CH_2CO*su*), 3.56 (3H, s, OCH_3 *su*); $C_{24}H_{34}O_4$ requires M, 386.2456, found M^+, 386.2462; *m/z* 386 (M^+, 6.32%), 268 (M^+–$C_4H_6O_4$, 5.22%), 43 (C_3H_7, 100%).

*2.5.3. Retinyl methyl adipate (**3c**)*

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1} and ν(CO–O ester) = 1737 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 173 and 172.5 (2CO*ad* ester), 138.4, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 51.2 (CH_3O), 33.0, 33.3 (CH_2–CO*ad*), 23.8 ($2\times CH_2$ *ad*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3 to C=), 1.89 (3H, s, CH_3–C=), 4.68 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, $2\times$ CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 1.50 (4H, m, CH_2–CH_2 *ad*), 2.3 (4H, m, 2 CH_2CO*ad*), 3.56 (3H, s, OCH_3 *ad*); $C_{27}H_{40}O_4$ requires M, 428.2926, found M^+, 428.2935; *m/z* 428 (M^+, 7.52%), 268 (M^+–$C_7H_{12}O_4$, 3.26%), 43 (C_3H_7, 100%).

*2.5.4. Retinyl L-lactate (**3d**)*

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1}, ν(OH) = 3400 cm^{-1} and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 174.5 (CO*la* ester), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.8 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 ($2\times CH_3$), 12.5 ($2\times CH_3$ cyclohexenyl), 33.9 (C cyclohexenyl), 65.9 (CH*la*), 20.4 (CH_3 *la*); ^1H NMR/DMSO, 6D (δ in ppm): 0.98 (6H, s, $2\times CH_3$ cyclohexenyl), 1.42 (2H, m, CH_2 cyclohexenyl), 1.56 (2H, m, CH_2 cyclohexenyl), 1.99 (2H, m, CH_2 cyclohexenyl), 1.67 (3H, s, CH_3–C=), 1.84 (3H, s, CH_3–C=),



Scheme 1.

1.89 (3H, s, CH_3–C=), 4.71 (2H, d, CH_2O), 5.41 (1H, t, CH=), 5.62 (1H, t, CH=), 6.13 (2H, 2d, 2 × CH=), 6.32 (1H, d, CH=), 6.66 (1H, dd, CH=), 4.13 (1H, m, CH*lac*), 1.23 (3H, d, CH_3*lact*); $C_{23}H_{34}O_3$ requires M, 358.25078, found M^+, 358.2522; *m/z* 358 (M^+, 13.52%), 268 (M^+–$C_3H_6O_3$, 52.87%), 45 ($C_2H_5O_1$, 100%), 43 (C_3H_7, 70.48%).

*2.5.5. Retinyl oleate (**3e**)*

IR: ν(CH) = 2800–2900 cm^{-1}, ν(=CH) = 3100 cm^{-1} and ν(CO–O ester) = 1740 cm^{-1}; ^{13}C NMR/DMSO, 6D (δ in ppm): 174 (CO*ol* ester), 138.7, 137.3, 135.9 and 128.9 (C=), 137.2, 136.0, 130.3, 126.3, 125.8 and 125.14 (CH=), 60.5 (CH_2O), 39.5, 32.5 and 18.7 (CH_2 cyclohexenyl), 21.5 (CH_3), 28.8 (2 × CH_3), 12.5 (2 × CH_3 cyclohexenyl), 33.9 (C cyclohexenyl), 130 (2 CH=CH*ol*), 22.7–35.8 (14 CH_2*ol*), 14 (CH_3*ol*); $C_{38}H_{62}O_2$ requires M, 550.4749, found M^+, 550.4754; *m/z* 550 (M^+, 10.86%), 268 (M^+-$C_{18}H_{34}O_2$, 4.17%), 43 (C_3H_7, 100%).

3. Results and discussion

The synthesis of retinyl methyl succinate **3a** from retinol **1** and dimethyl succinate **2a**, was chosen as the model reaction (Scheme 1). The *C. antarctica* lipase (Novozym®) and *R. miehei* lipase (Lipozyme®) were selected as catalysts because these immobilised enzymes are very efficient to the synthesis by reverse hydrolysis in organic media. The reaction was run in screw-cap glass vials containing 0.5 mmol of retinol **1**, 0.5 mmol of dimethyl succinate **2a**, 100 mg of lipase and 0.25 g of 4-Å molecular sieves with 5 ml of different solvents for 50 h at 55°C. From HPLC analysis, the decrease of retinol **1** concentration was seen to be concomitant with the synthesis of **3a**. In the absence of the enzyme, product **3a** did not appear. The

Table 1
Analytical yields of retinyl methyl succinate synthesis in different solvents
All the reactions were carried out at 55°C, with 100 mM of retinol, 100 mM of dimethyl succinate and 10 g/l of lipase in the presence of 4-Å molecular sieves (0.25 g/5 ml solvent). The degree of conversion was determined by HPLC on the base of the substrates' disappearance. No acylation was observed without lipase.

Solvent	Yield (%) with *C. antarctica* lipase	Yield (%) with *R. miehei* lipase	Reaction time (h)
Hexane	75	77	50
4-Methyl-2-pentanone	26	20	50
t-Amyl-alcohol	24	17	50
Dioxane	8	2	50
2-Butanone	0	0	50
Cyclohexanone	0	0	50

Table 2
Esterification of retinol with various acyl donors, catalysed by *R. miehei* lipase
All the reactions were carried out at 55°C, in hexane or in *t*-amyl-alcohol/hexane* (2/3, v/v), with 100 mM of retinol, 100 mM of acyl donor and 10 g/l of lipase in the presence of 4-Å molecular sieves (0.25 g/5 ml solvent). The degree of conversion was determined by HPLC on the base of the substrates' disappearance. No acylation was observed without lipase.

Acylating agent	Solvent	Yield after 50 h (%)	Product
Succinic acid	*t*-Amyl-alcohol/hexane*	4	Retinyl succinate **3b**
Monomethyl succinate	*t*-Amyl-alcohol/hexane*	44	Retinyl methyl succinate **3a**
Dimethyl adipate	Hexane	83	Retinyl methyl adipate **3c**
Methyl lactate	Hexane	86	Retinyl L-lactate **3d**
Methyl oleate	Hexane	90	Retinyl oleate **3e**

isolation, purification and characterisation of product **3a** confirmed that **3a** corresponded to retinyl methyl succinate.

The different yields obtained depending on the nature of solvents and on the nature of lipase, are reported in Table 1. When cyclohexanone and 2-butanone are used as reaction solvents, lipase activity is not detectable. In all the other tests, retinol esterification occurs with variable yields, depending on the solvent and lipase used. The highest yield is obtained in hexane. Three other commercial lipases (porcine pancreatic lipase, *C. cylindracea* lipase and *Rhi. arrhizus* lipase), were tested under the same conditions but no significant reaction was observed.

In addition, using *R. miehei*, other acyl donors were used to produce a wide variety of retinyl esters of great interest in cosmetic formulations. The results are presented in Table 2. Reactions with succinic acid and monomethyl succinate are carried out in *t*-amyl-alcohol/hexane system (2/3, v/v), in order to solubilise acyl donors which are insoluble in hexane. With the exception of monomethyl succinate, we observed excellent yields (more than 80%) for all the methyl ester donors. On the other hand, with succinic acid and monomethyl succinate, we found a lower reactivity than with the methyl ester donors.

In order to improve the system performance and to shift the reaction equilibrium towards synthesis, all the reactions were run in *t*-amyl-alcohol at 90°C under reduced pressure. The co-produced methanol was then removed more rapidly than under atmospheric pressure. We used *C. antarctica* lipase. This one has already shown its efficiency in similar conditions. However, in these conditions, the retinol degradation increases more rapidly than the retinol acylation. Indeed, retinol methyl succinate formation is competitive with the destruction of retinol characterised by the production of several compounds more hydrophilic than retinol.

4. Conclusion

An efficient method has been developed for the lipase-catalysed synthesis of retinyl esters such as retinyl succinate, retinyl adipate, retinyl oleate and retinyl lactate. To our knowledge, this enzymatic process is the first that describes retinyl esters synthesis with such excellent yields. In addition, the retinyl lactate produced may be considered as an excellent carrier of lactic acid. Indeed, in the presence of esterase-type epidermal enzymes, they can undergo hydrolysis, thus releasing lactic acid progressively. Derived from fruit and dairy products, alpha hydroxy acids are widely included in cosmetics as exfoliants. The most commonly used are lactic acid and glycolic acid, both of which seem to exert slight but significant effects in reducing skin discolorations and roughness when applied in a cream [18,19]. Significant irritation is often associated with the use of alpha hydroxy acids alone. Esters of retinol and alpha

hydroxy acids are unusually effective as skin conditioners, with significant reductions in the irritation problems characteristic of retinol and alpha hydroxy acids in nonesterified form [20].

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