Chemo-enzymatic epoxidation of β-caryophyllene mediated lipases and by mycelium-bound lipases

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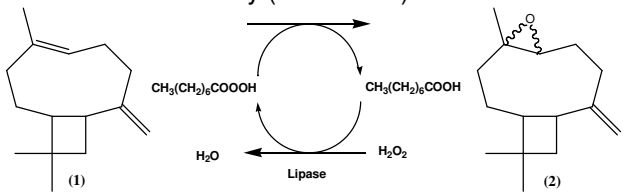
INTRODUCTION

A "green" method available for the epoxidation of alkenes (terpenes), based on the perhydrolysis of carboxylic acids and esters, is through the use of lipases (triacylglycerol hydrolases, 3.1.1.3.) in the presence of an oxidizing agent.[1,2] In this study, twelve commercial lipases from different sources, that is, *Candida antarctica* B (CAL-B, 10,000 PLU/g); *Burkholderia cepacia* (PS-C Amano® I, 1.638 U/g); PS Amano® SD; 30,000 U/g; PS Amano®, 30,000 U/g; PS Amano® IM, 500u/g; PS-C Amano® II, 1,000 U/g; *Rhizopus oryzae* (150 u/mg); *Candida rugosa* (30,000 u/g); *Pseudomonas fluorescens* (26,600 U/g); *Aspergillus niger* (120,000 u/g); *Rhizomucor miehei* (5-6 BAUN/g); and *Mucor miehei* (5-6 BAUN/g), were used in the chemo-enzymatic epoxidation of caryophyllene (0.6 mL, 2.5 mmol) using aqueous hydrogen peroxide (1.2 mL, 30%) as the oxidant agent and octanoic acid (0.16 mL, 1 mmol) as the acyl donor in dichloromethane at ~25°C. Also, two native lipases from *A. niger* (LAN 18.2 U/mL*)* and *Rhizopus oligosporus, (*LRO 14.9 U/mL*)* isolated from microorganisms of a soil from the Bueno Brandão region (MG, Brazil)[3], and nine mycelium-bound lipases from Amazon region fungi (UEA_01, UEA_06, UEA_07, UEA_23, UEA_27, UEA_28, UEA_41, UEA_53 and UEA_115)[4], were screened in this study (**Scheme 1**).



Scheme 1 Chemo-enzymatic epoxidation of caryophyllene

RESULTS AND DISCUSSION

The epoxidation reaction was maintained under orbital agitation (150 rpm). Aliquots were withdrawn at predetermined times and the epoxy-caryophyllene (**2**) was quantified by gas chromatography. In the reactions catalyzed by 50 mg of commercial lipases, (**2**) was obtained in conversion degrees of 16 to 27%. Similar results were obtained with the use of LAN (20%) and LRO (23%). The epoxide (**2**) was obtained in >99.9% conversion in a period of 24 h using CAL-B. In the case of the Amazonian fungi, the influence of time was then evaluated and the results are presented in **Table 1**.

Table 1. Degree of conversion (%) of caryophyllene (**1**) into epoxide **(2)**.

Mycelium	24 h	72 h	120 h	168 h
UEA_01	14	38	43	49
UEA_06	16	49	59	64
UEA_07	13	38	37	40
UEA_23	8	47	49	55
UEA_27	2	2	8	8
UEA_28	11	42	40	45
UEA_41	10	44	47	48
UEA_53	21	34	41	41
UEA_115	15	29	40	46

* Reaction conditions: caryophyllene (2.5 mmol), H_2O_2 (5 mmol, 30%), octanoic acid (1 mmol), mycelium-bound lipases (100 mg), dichloromethane (10 mL), 24 h, r.t

After 24 h of reaction, the conversion degrees were in the range of 2-21%. For UEA_06 and UEA_53 the results were similar to those obtained using commercial lipases. After 168 h, the best conversion was achieved using UEA_06 (64%), followed by UEA_23 (55%), UEA_01 (49%) and UEA_41 (48%).

CONCLUSION

The results obtained are promising and showed the importance of evaluating different sources of biocatalysts for each specific substrate and type of reaction. Depending on the catalyst and reaction time, the epoxide (**2**) was obtained with moderate to good conversion degrees.

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