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Microbial oxidation of terfenadine and ebastine into fexofenadine and carebastine

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Dedicated to Robert Azerad on his 65th birthday

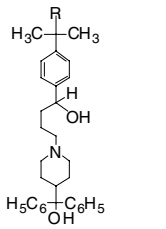
Abstract—The oxidation of *tert*-butyl-phenyl group of title compounds by some microorganisms was studied. We have optimized the conditions of culture to increase the formation of acid metabolites and to avoid the formation of side products. We showed that an oxidative activity is induced by soybean peptones in *Streptomyces platensis*. The biologically active compounds, fexofenadine and carebastine, are produced in good yield (86–95%) by *Absidia corymbifera*.

1. Introduction

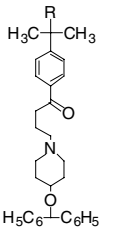
Terfenadine **1** and Ebastine **2**, two antihistaminic drugs, are oxidized into their pharmacologically active métabolites, fexofenadine **5** and carebastine **6**, respectively.[1] Cardiovascular side effects have been reported on patients treated with terfenadine (or ebastine) together with either antibacterial or antifungal drugs.[2,3] However, such adverse effects, linked to a modification in the metabolism of the antihistaminic drug, can be avoided if fexofenadine or carebastine is used as a drug.[4] Unfortunately, synthesis of these carboxylic derivatives is laborious[5] and products are obtained in low yields in comparison with those obtained for the synthesis of terfenadine and ebastine.

Since microorganisms are able to transform drugs and chemicals into metabolites identical to those observed in mammals,[6–8] the microbial oxidation of the terfenadine and ebastine *tert*-butyl groups has been investigated as an alternative method for the preparation of fexofenadine and carebastine. Namely, the fungi *Cunninghamella echinulata* var *elegans* (*C. blakesleeana*) was reported to carry out the oxidation of ebastine into carebastine.[9,10] Furthermore, processes involving the bacteria *Streptomyces platensis*,[11] *Streptomyces risomus*[12] or the fungi *C. blakesleeana*[13] and *Absidia corymbifera*[11] have been patented for the synthesis of fexofenadine from terfenadine. Finally, the hydroxylation of terfenadine into hydroxyterfenadine **3** by *C. blakesleeana*[14] and *Mortierella isabellina*[15] was also reported.

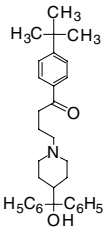
Moreover, partial phosphorylation of terfenadine on the secondary alcohol and of hydroxyterfenadine on the primary alcohol has been observed with several microorganisms.[13,11] However the phosphorylation of hydroxyterfenadine is known to be reversible and pH dependent, namely, formation of **7** occurs at pH 7–8 and hydrolysis at pH 4.[11]



1 R = CH_3
3 R = CH_2OH
5 R = COOH
7 R = $CH_2OP(O)(OH)_2$



2 R = CH_3
4 R = CH_2OH
6 R = COOH



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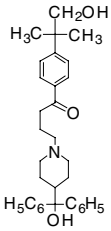

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None of the previously reported bioconversions was efficient enough to produce fexofenadine or carebastine with high yields either because transformations were incomplete or because side product formation occurred.

We report herein the comparison of several microorganisms for their ability to oxidize the *tert*-butyl group of both terfenadine and ebastine together with an optimization study of the culture conditions necessary to obtain fexofenadine and carebastine in high yields.

2. Results and discussion

It is known that the ability of microorganisms to transform organic compounds is dependent on the culture medium composition.[16,17] Particularly soybeans peptones are known to induce oxidative activity in *Streptomyces griseus*.[18] Therefore, upon screening, two strains of bacteria and three strains of filamentous fungi (Table 1) were selected and cultivated in a culture medium supplemented (YMS) or not (YM)[19] with soybean peptones to study their ability to transform terfenadine and ebastine into fexofenadine and carebastine, respectively. The kinetic study[20] indicates the fast disappearance of substrate from the incubation medium by cell adsorption because of the hydrophobic properties of chemicals. After a few hours of incubation, products were slowly brought out and appeared in the medium.

The results shown in Table 1 report the products observed in crude extracts after 96 h of incubation.[21]

The biotransformation of terfenadine by *C. blakesleeana* grown in culture medium without soybean peptones (medium YM) yield to alcohol **3** as described by Schmitz et al.[14] whereas the cells cultured in YMS medium yield to fexofenadine **5** as described by Meives.[13] Similar results were obtained for the biotransformation of ebastine **2**[10] but the hydroxylation of this ketone derivative into product **4** was more efficient than the hydroxylation of terfenadine. Anyway in all cases, products were obtained in low yields (<20–25%).

Furthermore, under the same growth conditions, terfenadine **1** and ebastine **2** were oxidized in very small amounts by *C. echinulata* and *S. risomus*.

S. platensis cells produced in the medium YM were able to transform terfenadine **1** into hydroxyterfenadine **3**. Under the conditions herein described (culture in 250 mL flask and incubation in the culture medium) compound **3** was the main product observed, and a side product was observed and identified by LC–MS and NMR as the hydroxyketone **9**[22] (10–15%).

The formation of **9** could either result from the oxidation of the secondary alcohol group of terfenadine to produce ketone **8** followed by the hydroxylation, or from the oxidation of the secondary alcohol group of hydroxyterfenadine **3**. The same oxidation of the secondary alcohol was observed in human[23] and in dog[24] metabolism of terfenadine. Therefore, the microbial biotransformations could be considered as a complementary tool in the study of drug metabolism with a particular interest in preparative scale, especially for the preparation of hydroxyketone **9** of which the chemical synthesis from fexofenadine requires laborious protection/deprotection reactions.

Ebastine was also hydroxylated into hydroxy derivative **5** but in lower amounts than **2**.

In the case of *S. platensis* cells obtained in the medium containing soybean-peptone oxidized terfenadine and ebastine, and mixtures of hydroxy and acid derivatives were obtained.

The fungi *A. corymbifera* cultured in YM medium were able to oxidize terfenadine, ebastine and mixtures of corresponding alcohol and acid were obtained. In the experiments conducted with cells obtained in YMS medium, fexofenadine and carebastine were obtained as the sole product after 96 h of incubation of terfenadine and ebastine, respectively.

Whereas no formation of acid **5** and **6** was detected when *S. platensis* was cultured in YM medium, various

Table 1. Oxidation of *t*-butyl-phenyl group of terfenadine and ebastine by some microorganisms

Microorganisms	Culture medium	Terfenadine **1**		Ebastine **2**	
		3	**5**	**4**	**6**
Cunninghamella blakesleeana ATCC 8688a	YM	+	–	+	–
	YMS	+	+	++	+
Cunninghamella echinulata ATCC 9245	YM	–	–	+	–
	YMS	+	–	+	–
Streptomyces risomus NRRL 2234	YM	–	–	–	–
	YMS	+	–	+	–
Streptomyces platensis NRRL 2364	YM	+++[a]	–	++	–
	YMS	++	++	++	++
Absidia corymbifera LCP 63 1800	YM	++	++	++	++
	YMS	–	+++	–	+++

– No product formed.
+ Product observed (<20%).
++ Alcohol and acid present (20–50%).
+++ Main or unique product observed (>75%).
[a] Contaminated with 15% of **9**.

amounts of them were observed when *S. platensis* were grown in YMS medium. This suggests that oxidation of hydroxy compound **3** and **4** was catalyzed by a soybean-peptone-inducible enzyme. Oxidation of alcohols into carboxylic acids takes place in two steps via the formation of aldehydes, and are in general catalyzed by alcohol deshydrogenase and aldehyde oxidoreductase.[25] However some examples described the cytochrome P450-dependent conversion of alcohols and aldehydes into carboxylic acid.[26] In mammalian metabolism, some cytochromes P450 are involved or supposed to be involved in the formation of fexofenadine and carebastine, namely, CYP 3A4,[27] 4F12 and 2J2.[28] As the induction of cytochrome P450 by soybean peptones has been reported in a *Streptomyces* strain,[18] it could be suggested that the inducible enzyme involved in the oxidation of hydroxyterfenadine is a cytochrome P450.

Yeasts expressing human P450 have been previously used to prepare drug metabolites. For example, an efficient method has been obtained for the preparation of 4′-hydroxydiclofenac from oxidation of diclofenac with yeast expressing cytochrome P450 2C9.[29] Likewise, terfenadine was incubated with yeast expressing cytochrome P450 3A4. Interestingly microsomes expressing CYP 3A4 were able to oxidize terfenadine but no biotransformation was observed upon incubation with their parent recombinant cells. This discrepancy is likely to be related to the incapacity of substrate to penetrate in intact yeast cells and thus points out a possible limitation in the use of yeast expressing human P450 to prepare large quantities of metabolites.

The best results were obtained with *A. corymbifera* cultured in YMS, where the complete transformations of terfenadine and ebastine were achieved. Since, culture and incubation were performed without phosphate added, no formation of phosphorylated compounds was observed. The preparative experiments carried out in 1 L of YMS culture medium on ebastine[30] (180 mg) and on terfenadine[31] (200 mg) led to 164 mg of carebastine (86% yield) and to 193 mg of fexofenadine (93% yield).

3. Conclusion

It was shown that soybean-peptones induce an oxidizing activity involved in the biotransformation of terfenadine into fexofenadine in *S. platensis*. Study is in progress to verify that the enzyme is a monooxygenase. Based on the reaction conditions, a bioconversion process was developed for the production of fexofenadine and carebastine by oxidation of terfenadine and ebastine by *A. corymbifera*. Using this methodology, synthesis of some hydroxylated intermediate analogues is in progress in order to study mammalian and microbial cytochromes P450.

Acknowledgements

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19. Liquid culture media containing (g/L) glucose 16, yeast extract 4, malt extract 10 (YM medium) and glucose 16, yeast extract 4, malt extract 10 and soybean peptones 5 (YMS medium).
20. The microorganisms were cultured in a 250 mL flask containing 100 mL of medium at 30 °C in rotatory shaker (200 rpm). After 60 h substrate was added (20 mg) in DMF (0.2 mL) and the incubations were maintained at 30 °C with orbital shaking and samples (1 mL) were withdrawn in 24 h intervals. Biomass was sedimented (15 min, 13,500 rpm), supernanants were micro-filtered (0.45 μm) and the filtrates were analyzed by HPLC.
21. The reactions were stopped after 96 h by addition of a mixture of AcOEt/Et2O/MeOH (5/5/1) (20 mL), stirred and filtered on Celite. The organic phases were dried on $MgSO_4$, concentrated and the extracts were reconstituted in acetonitrile for HPLC analysis.
22. ^{1}H NMR (CD_3OD 250 MHz) 1.2 (6H, s), 1.6–1.8 (6H, m), 1.8–2.0 (6H, m), 2.6 (1H, s), 3.3 (2H, s), 3.6 (2H, s), 7.1 (2H, d, $J = 7$ Hz), 7.2 (6H, m), 7.5 (6H, m).
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30. *A. corymbifera* LCP 62-1800 was cultivated in 2 L Erlenmeyer-flask containing 1 L culture medium (YMS) at 30 °C during 60 h and ebastine (180 mg, 0.38 mmol) was in DMF solution (2 mL). The incubation was continued for 7 days. Biomass was filtered and cells were suspended in methanol (150 mL), stirred 1 h and harvested. The filtrate was saturated (NaCl), pH adjusted at 3 with HCl, and extracted by CH_2Cl_2. The organic phase was combined, drying on $MgSO_4$ and evaporated. The residue was purified by flash chromatography: elution with CH_2Cl_2/CH_3OH 9:1 containing 1% of acetic acid afforded carebastine (164 mg, 86% yield). Mp 173–175 °C.

31. *A. corymbifera* LCP 62–1800 was cultivated at 30 °C in 2 L fermentor containing 1 L culture medium (YMS) controlled by Prelude system (Biolafitte-Moritz/Pierre Guerin). The pH set-point of 6.5 was maintained by addition of 1 N. After 48 h, terfenadine was added (200 mg) in DMF (2 mL) and incubation was continued for 5 days. Work-up was as describe for carebastine except that the filtrate was extracted with a mixture of ethyl acetate/diethyl oxyde/methanol 45/45/10. The residue was purified by flash chromatography: elution with CH_2Cl_2/CH_3OH 9:1 containing 1% of acetic acid afforded fexofenadine (195 mg, 92% yield). Mp 193–195 °C.