Bioconversion of Drimenol into 3#/-Hydroxydrimanes by Aspergillus niger. Effect of Culture Additives

Hector E. Ramirez, Manuel Cortes M., and Eduardo Agosin

J. Nat. Prod., **1993**, 56 (5), 762-764• DOI:
10.1021/np50095a014 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 5, pp. 762-764, May 1993

BIOCONVERSION OF DRIMENOL INTO 3β-HYDROXYDRIMANES BY *ASPERGILLUS NIGER*. EFFECT OF CULTURE ADDITIVES

HECTOR E. RAMIREZ,* MANUEL CORTES M.,

Departamento de Química Orgánica, Facultad de Química

and EDUARDO AGOSIN

Departamento de Ingeniería Química, Facultad de Ingeniería, Pontificia Universidad Católica de Chile, Casilla 306-correo 22, Santiago, Chile

ABSTRACT.—The bioconversion of (−)-drimenol [**1**] and drimenyl acetate [**2**] into the corresponding 3β-hydroxydrimanes by *Aspergillus niger* in agitated liquid cultures was investigated. Initial hydroxylation yields of 2% and 10%, respectively, were obtained. However, drimenyl acetate hydroxylation increased to 18% when Carbopol-934 was added. The highest transformation yield (33%) was reached when an inclusion complex of drimenyl acetate to β-cyclodextrin (1:1 w/w) was added to the cultures, after 48 h of cultivation. The effect on growth and transformation yields of both additivies is discussed.

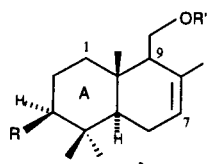
Drimenol [**1**] is a sesquiterpene alcohol isolated from the bark of *Drimys winteri* Forst (Winteraceae) (1). Recently, we have been interested in the study of microbial transformation of **1**, in order to obtain A-ring oxygenated analogues. Although several reports on the microbial transformation of terpenoids have been published (2–4), to the best of our knowledge, none of them have been conducted with **1**.

Preliminary work in this laboratory shows that limited biomass production in *Aspergillus niger* cultures (4 g/liter), as well as low solubility of **1**, could be at least partially responsible for the very low transformation yields obtained.

In this work, the effects of Carbopol and of β-cyclodextrin on the bioconversion of **2** are described. The results show that both additives significantly improved biotransformation yields, suggesting that similar strategies could be employed to optimize microbial hydroxylating procedures for a variety of terpenoids and probably other hydrophobic compounds.

RESULTS AND DISCUSSION

Preliminary incubations of **1** with *A. niger* revealed the presence of original substrate, as well as a small amount (less than 2%) of an impure, more polar metabolite **3**, which was characterized as the diacetate **5**. The identity of **5** was confirmed by careful comparison of the ^{1}H-nmr spectra with those of an authentic sample obtained by acetylation of natural **3** (6). The limited yield obtained resulted from the low solubility of **1**. Therefore, drimenyl acetate **2** was incubated with *A. niger* for 6 days. The main product obtained was isolated and identified as **4** which, in accordance with spectroscopic evidence, showed β-hydroxylation at C-3. The β-equatorial configuration of 3-OH in **4** was confirmed from 200 MHz nmr spectrum which showed the H-3 signal at δ 3.25 (1H, dd, J = 10.3, 5.5 Hz). Acetylation of **4** gave the diacetate **5**. Its ir and ^{1}H-nmr spectra were identical to those obtained from **3**.



1 R=R′=H
2 R=H, R′=Ac
3 R=OH, R′=H
4 R=OH, R′=Ac
5 R=OAc, R′=Ac

It is noteworthy that C-3 regioselectivity of this hydroxylation was similar to that observed with various kinds of sesquiterpenes (7–9). Although a significant improvement in the hydroxylation yield (10%) was obtained, the level attained was too low for an efficient biosynthetic process.

Therefore, our efforts were focused to increase the fungal biomass. Previous reports had shown that addition of an anionic polymer to the medium resulted in a significant enhancement of both the growth rate and total biomass of *A. niger* (10). Indeed, the biomass doubled, and the fungal morphology changed from globose pellets to filamentous (biochemically more active cells) after addition of Carbopol 934 (0.5% w/v final concentration). Unfortunately, the positive effect on biomass was counterbalanced by considerable difficulty in the extraction of our target compounds with organic solvents. To overcome this problem, the final concentration of this additive was reduced to 0.1%, facilitating an increase (18%) in compound **2** hydroxylation (Table 1).

TABLE 1. Effect of Adding Carbopol-934 and β-Cyclodextrin on the Bioconversion of Drimenyl Acetate [**2**] by *Aspergillus niger*.

Medium	Additive	Substrate	Yield %
Basal . . .	None	**1**	2
	None	**2**	10
	Carbopol 934[a]	**2**	18
	β-Cyclodextrin +Carbopol 934	**2**	33

[a]Other polymers including Tween-80 and Triton X-100 were less effective in promoting improvement in biomass yield.

The vast majority of organic substrates are lipophilic and sparingly soluble in H_2O. Since this was also our case, we investigated possible means to overcome the limited substrate accessibility to the biocatalyst by addition of β-cyclodextrins to the bioconversion medium (11). Initial experiments revealed that the direct addition of β-cyclodextrin to the culture medium at the beginning of the process resulted in an inhibition of fungal growth, possibly arising from non-selective inclusion in the hydrophobic cavity of essential fungal growth factors. In contrast, when this compound was added after 2 days of cultivation, microbial growth was not significantly affected. However, bioconversion yields remained low. Therefore, the additive was added as a substrate-cyclodextrin inclusion complex. Under these conditions hydroxylation yields dramatically increased, reaching 33% conversion of the starting material (Table 1). These results suggest that β-cyclodextrin would effectively enhance solubilization of drimanes in aqueous medium, probably with minor or no effects on membrane permeability.

On the basis of these studies, we can conclude that microbial hydroxylation of **1**, and particularly of **2**, may allow the production of new and valuable regio- and stereoselectively functionalized compounds. The best results were obtained for biotransformation of **2** when the culture medium contained Carbopol-934 and the substrate was added with β-cyclodextrin as a soluble complex.

EXPERIMENTAL

CHEMICALS.—Carbopol-934 and β-cyclodextrin were obtained from BF Goodrich (USA) and Cyclolab (Hungary), respectively.

SUBSTRATE PREPARATION AND COMPLEX SYNTHESIS.—Drimenol [**1**] was treated with Ac_2O in pyridine to give drimenyl acetate [**2**] (98%). The complex of drimenyl acetate-β-cyclodextrin was prepared by coprecipitation (5). Equivalent amounts of **2** and β-cyclodextrin (1:1 molar ratio) were dissolved in MeOH; the solvent was evaporated at 40° in an H_2O bath and dried in vacuo at room temperature until constant weight.

MICROORGANISM.—A local strain of *A. niger* was obtained from "Colección de Cultivos de la Fac. de Medicina, Universidad de Valparaiso."

CULTIVATION METHODS.—Mycelial inocula were prepared by suspending surface growth spores from slants in 10 ml of sterile distilled

H_2O. Spore suspensions were adjusted to a final concentration of 10^8 spores·ml^{-1}.

The culture medium employed routinely (basal) contained per liter: D-glucose 20 g, hydrolyzed casein 2.5 g (13% N), yeast extract 0.2 g, KH_2PO_4 0.6 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2$ 0.147 g. Sodium citrate (100 mM pH 6.0) was used as buffer. Carbopol-934 was added to basal medium at a 0.1% concentration (w/v). Polyethylene glycol 10,000 (0.01%, w/v) was used in all liquid cultures as an anti-foaming agent. Cultures were incubated in 150-ml and 1-liter Erlenmeyer flasks containing the medium at a 1/5 v/v ratio at 30° on a Lab-Line Environ-shaker (200 rpm). Uninoculated controls were also included. After 48 h incubation, the drimenyl acetate-β-cyclodestrin complex was added to the cultures at a final concentration of 0.5 mg/ml. After 6 days incubation, the mycelium was removed by filtration through previously tared Whatman no. 3 filter paper. The clear filtrates were extracted with EtOAc; dried mycelium was also extracted with hot EtOAc. The combined extracts were then dried over anhydrous Na_2SO_4 and evaporated to a brownish oil. All experiments were run in triplicate and repeated at least once.

PHYSICAL AND CHEMICAL ANALYSIS.—The different compounds present in the EtOAc extracts were separated on a Si gel column (70–230 mesh, 2.5 × 90 cm) with petroleum ether-EtOAc (70:30) as the eluting solvent. Ir spectra were measured on a Perkin-Elmer 1310 spectrometer (wave number in cm^{-1}).

Pure materials were identified by ^1H nmr, which were obtained on samples prepared in $CDCl_3$ containing 1% TMS as internal reference. Spectra were recorded at 200 MHz on a Bruker AC 200 FT spectrometer.

BIOCONVERSION OF DRIMENOL [1].—Substrate **1** (1 g) was dissolved in EtOH (200 µl), distributed among 5 Erlenmeyer flask cultures (1 liter), and incubated for 6 days, after which the cultures were processed as indicated above to give a very complex mixture (1.7 g) which was chromatographed to obtain 940 mg of starting material **1** and 20 mg (2%) of impure **3**. The crude diol was dissolved in dry pyridine (0.5 ml) and acetylated with Ac_2O (0.5 ml) at room temperature for 3 h to give 15 mg of the diacetate **5** as a colorless oil: ir ν max (neat) 2980, 1740, 1230; ^1H nmr (200 MHz, $CDCl_3$, TMS) δ 0.84 (3H, s), 0.86 (3H, s), 0.94 (3H, s), 1.6–1.72 (2H, m), 1.67 (3H, br s, H-12), 2.04 (3H, s, OAc), 2.05 (3H, s, OAc), 4.10 (1H, dd, J = 11.6, 5.9 Hz, H-11), 4.22 (1H, dd, J = 11.6, 3.7 Hz, H-11), 4.52 (1H, dd, J = 10.7, 5.05 Hz, H-3), 5.5 (1H, m, H-7).

BIOCONVERSION OF DRIMENYL ACETATE [2].—The drimenyl acetate (1 g)–β-cyclodextrin complex was suspended in EtOH (200 µl), distributed among 5 Erlenmeyer flask cultures (1 liter), and incubated for 6 days, after which the cultures were processed as indicated above for the biotransformation of substrate **1**, to give 630 mg of starting material **2** and 330 mg (33%) of **4**: colorless oil; ir ν max (neat) 3440, 1720, 1230; ^1H nmr (200 MHz, $CDCl_3$, TMS) δ 0.82 (3H, s), 0.86 (3H, s), 0.95 (3H, s), 1.25 (m), 1.67 (3H, br s, H-12), 2.03 (3H, s, OAc), 3.25 (1H, dd, J = 10.3, 5.5 Hz, H-3), 4.08 (1H, dd, J = 12.1, 6.0 Hz, H-11), 4.25 (1H, dd, J = 10.2, 3.2 Hz, H-11), 5.5 (1H, m, H-7). Acetylation of **4** with Ac_2O in pyridine gave the diacetate **5** identical to that obtained from **3**.

ACKNOWLEDGMENTS

This work was supported by grants N° 91/0804 and 92/0052 from the "Fondo de Desarrollo Científico y Tecnológico" (FONDECYT). We thank Dr. Eduardo Piontelli, Faculty of Medicine, University of Valparaíso, Chile, for providing the strain of *Aspergillus niger*. We are also indebted to Dr. W.A. Ayer, Department of Chemistry, University of Alberta, Canada, for ^1H-nmr spectra of compound **5**.

LITERATURE CITED

1. H.H. Appel, C.J.W. Brooks, and K.H. Overton, *J. Chem. Soc.*, 3322 (1959).
2. V. Lamare and R. Furtoss, *Tetrahedron*, **46**, 4109 (1990).
3. V. Lamare, A. Archelas, R. Faure, M. Cesario, C. Pascard, and R. Furstoss, *Tetrahedron*, **45**, 3761 (1989).
4. A. García-Granados, A. Martínez, M.E. Onorato, F. Rivas, and J.M. Arias, *Tetrahedron*, **47**, 91 (1991).
5. M.O. Ahmed, Y. Nakai, A.E.S. Aboutaleb, K. Yamamoto, A.A.Z.A. Rahman, and S.A. Saleh, *Chem. Pharm. Bull.*, **38**, 3423 (1990).
6. W.A. Ayer and P.A. Craw, *Can. J. Chem.*, **67**, 1371 (1989).
7. J.J. Hoffmann, S.D. Jolad, R.B. Bates, and F.A. Camoun, *Phytochemistry*, **27**, 2125 (1988).
8. S.A. Kouzi and J.D. McChesney, *Helv. Chim. Acta.*, **73**, 2157 (1990).
9. G. Aranda, M.S. El Kortbi, J.I. Lallemand, A. Hammoumi, I. Facon, and R. Azerad, *Tetrahedron*, **47**, 8339 (1991).
10. H. Elmayergi and J.M. Scharer, *J. Gen. Appl. Microbiol.*, **83**, 73 (1973).
11. R. Bar, *Tibtech*, **7**, 2 (1989).

Received 15 June 1992