Synthesis and Biotransformation of Tetrahydroquinoline by *Mortierella isabelina*

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Tetrahydroquinoline (**1**), prepared by a traditional synthetic method, the imino Diels-Alder reaction, was biotransformed by *Mortierella isabelina* to afford a new compound, **2**, characterized by spectroscopic methods.

Key words: *Mortierella isabelina*, Biotransformation, Tetrahydroquinoline, Imino Diels-Alder Reaction

Introduction

Quinoline and its derivatives are a family of compounds intensely studies because of their wide distribution and range of activities. They influence the cardiovascular system (Bell *et al.,* 1989) and show sedative (Lukevics *et al.*, 1997), antidepressant (Oshiro *et al.*, 2000), anticonvulsive (Guan *et al.*, 2007), analgesic and anti-inflammatory (Savini *et al.,* 2001), cytotoxic (Chen *et al.*, 2002), antitumour (Dallavalle *et al.*, 2001), antiviral (Benard *et al.,* 2004), antibacterial (Kidwai *et al.,* 2000), fungicide (Tsushima *et al.*, 1989), and herbicide activities (Vasiliev *et al.*, 2004). In this group of compounds tetrahydroquinoline (THQ) is a molecule with great diversity of substitution patterns and a wide range of biological activities, including enzyme inhibitory, antimicrobial, cytotoxic and antiulcerogenic activities (Monsees *et al.,* 1998; Katritzky *et al.*, 1996). A series of dihydrobenzopyrans and tetrahydroquinolines was synthesized and pharmacologically tested for their ability to inhibit the P-glycoprotein-mediated daunomycin efflux in multidrug resistant CCRF-CEM vcr 1000 cells. Several compounds exhibited activities in the range of the reference compounds verapamil and propafenone (Hiessbock *et al.*, 1999). Many methods of synthesis have been reported for this kind of molecules (Abele *et al.*, 2005), the methodology of imino Diels-Alder reaction (Kouznetsov *et al.*, 2004, 2006) is the one which has shown the best performance and is characterized by using an environment friendly catalyst.

One of the problems that have became apparent in the tetrahydroquinolines synthesis is their relative low solubility in aqueous systems. It can be improved by incorporating hydroxy groups as substituents. The incorporation of these groups at specific positions by organic synthesis is sometimes quite difficult depending on the size of the molecule and some characteristics of spatial distribution of those. This problem can be solved by using microorganisms capable of biotransformation and introduction of functional groups to the original substrate.

The use of microorganisms in chemistry is not a new issue. Bacteria and fungi have been used to produce chemicals, pharmaceuticals and perfumes for decades.

Biotransformations have a number of advantages compared to the corresponding chemical methods: they act not only region- and stereospecific but are also enantiospecific, allowing the production of chiral products from racemic mixtures (Leresche and Meyer, 2006). Microbial processes have been used to introduce hydroxy groups at difficult positions on many compounds. Chemical-microbiological methods constitute an alternative way to obtain new polyoxygenated compounds from abundant products (Garbarino *et al.*, 2001). Biotransformations are usually carried out in

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aqueous systems and at pH 7.0, avoiding the use of harmful solvents common in conventional synthesis.

Material and Methods

General experimental procedures

Melting points were determined on a Koffler hot stage apparatus (Electrothermal 9100) and are uncorrected. IR spectra were recorded on a Nicolet Nexus FT-IR instrument as potassium bromide pellets. The NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 400 NMR spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C NMR, respectively; TMS was used as an internal standard and chemical shifts (δ) and J values are reported in ppm and Hz, respectively. MS spectra were measured with a Varian unit at 70 eV. Silica gel 60 (Merck, 230–240 mesh particle size) was used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis. Final purification of all products for elemental analyses was done by recrystallization.

Synthesis of tetrahydroquinoline

Compound **1** was prepared by three-component imino Diels-Alder reaction. A mixture of aryl amine (2.8 mmol) and aldehyde (3.4 mmol) in anhydrous CH_3CN (15 mL) was stirred at room temperature (RT) for 30 min. $BiCl_3$ (20 mol%) was added. Over a period of 20 min, a solution of nitro-1-vinyl-2-pyrrolidone (NVP) (5.5 mmol) in CH_3CN (10 mL) was added dropwise. The resulting mixture was stirred for 10–14 h. After completion of the reaction as indicated by TLC, the reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate (EtOAc) (3 x 15 mL). The organic layer was separated dried (Na_2SO_4) and, concentrated *in vacuo*, and the resulting product was purified by column chromatography (silica gel, petroleum ether, EtOAc) to afford pure compound **1**; 250 mg of **1** were obtained: Colourless crystals; m.p. 148–150 °C. – IR (KBr): ν = 3355, 2950, 1671, 1605 cm^{-1}. – ^1H NMR (400 MHz, $CDCl_3$): δ = 7.26 (1H, s, 2'-H), 7.27 (1H, d, J = 7.1 Hz, 6'-H), 7.23 (1H, t, J = 7.6 Hz, 5'-H), 7.14 (1H, d, J = 7.1 Hz, 4'-H), 7.06 (1H, t, J = 7.6 Hz, 7-H), 6.88 (1H, br.d, J = 7.6 Hz, 8-H), 6.71 (1H, dt, J = 8.1, 1.0 Hz, 6-H), 6.59 (1H, d, J = 7.8 Hz, 5-H), 5.73 (1H, t, J = 9.1 Hz, 4-H), 4.56 (1H, t, J = 7.1 Hz, 2-H), 4.04 (1H, br.s, NH), 3.26–3.21 (2H, m, 1”-H), 2.53–2.43 (2H, m, 3”-H), 2.38 (3H, s, 3'-Me), 2.13–2.08 (2H, m, 3-H), 2.02–1.96 (2H, m, 2”-H). – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.80 (+), 146.01 (+), 143.07 (+), 138.48 (+), 128.69 (+), 128.69 (+), 128.26 (+), 127.13 (+), 126.80 (+), 123.56 (+), 118.93 (+), 118.12 (+), 114.98 (+), 56.36 (+), 48.53 (+), 42.35 (-), 35.27 (-), 31.43 (-), 21.24 (+), 18.28 (-). – MS (EI): *m/z* = 306.17 [M^+] (100). – $C_{20}H_{22}N_2O$: calcd. C 78.40, H 7.24, N 9.14; found C 78.32, H 7.15, N 9.09.

Microorganism and screening scale experiments

The microorganisms used were from the American Type Culture Collection (ATCC): *Mortierella isabelina* ATCC 38063.

Liquid soybean medium (30 mL) held in 125-mL Erlenmeyer flasks was inoculated with a spore suspension in a first fermentation stage. The stage I culture was incubated on a rotatory shaker at 250 rpm and 28 °C for 48 h. The second fermentation stage was initiated by transferring the inoculum from the stage I culture to a final content of 10% (10 mL of inoculum of stage I in 100 mL of medium). After 48 h of incubation, substrate (20 mg) dissolved in DMSO was added to the flask. Samples (5 mL of incubation medium) were withdrawn every 12, 24 and 48 h, partitioned with EtOAc (2 mL), and analyzed by TLC. Three experiments with two repetitions were carried out for the fungal agent. Blank assays without substrate and fungi were carried out.

Incubation of THQ with M. isabelina and product isolation

Mortierella isabelina was grown in shaked culture medium comprising (g per liter): dextrose (20), yeast extract (5), soy flour (5), NaCl (5), K_2HPO_4 (5). The culture was grown in 300-mL Erlenmeyer flaks each containing 50 mL medium for 48 h at 25 °C prior to the addition of the substrate. 200 mg of THQ solubilized in DMSO (200 μL) were evenly distributed in 10 flasks of *M. isabelina* and the fermentation was subsequently continued for 14 d.

The culture was separated by filtration into mycelium and culture filtrate. The latter was extracted with EtOAc (3 x 50 mL). The solvent was evaporated to give a dry residue of 230 mg. Chromatography of the crude residue on silica gel with a petroleum ether/ethyl acetate gradient afforded

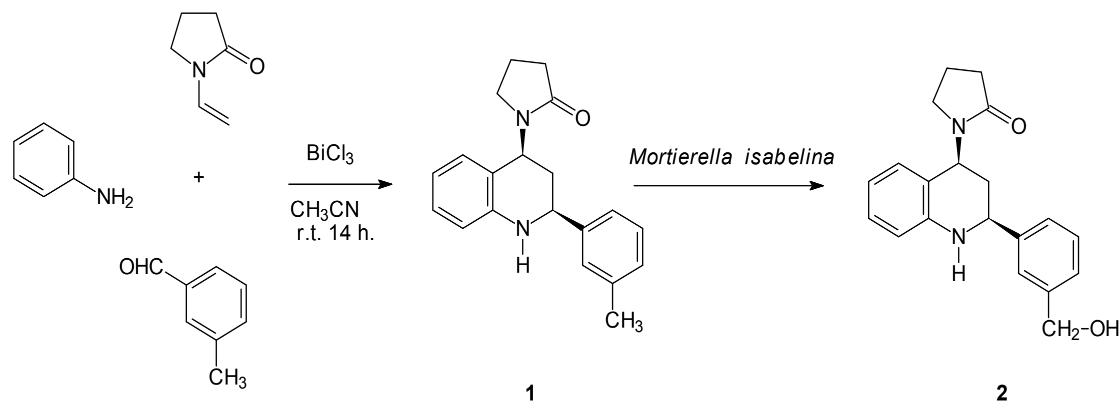

Fig. 1. Synthesis of tetrahydroquinoline (**1**) and biotransformation by *M. isabelina.*

30 fractions, which were pooled into 7 groups according to the TLC patterns. The fraction pools 15–22 (100 mg) yielding 63 mg of THQ were biotransformed to **2**: Colourless crystals. – IR (KBr): ν_{max} = 3550, 3350, 2951, 1670, 1607 cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46 (1H, br. s, 2'-H), 7.35 (1H, t, J = 8.0 Hz, 5'-H), 7.32 (1H, dd, J = 8.0 and 4.0 Hz, 4'-H), 7.32 (1H, dd, J = 8.0 and 4.0 Hz, 6'-H), 7.06 (1H, t, J = 8.0 Hz, 7-H), 6.87 (1H, d, J = 8.0 Hz, 8-H), 6.58 (1H, d, J = 8.0 Hz, 5-H), 6.73 (1H, t, J = 8.0 Hz, 6-H), 6.58 (1H, d, J = 8.0 Hz, 5-H), 5.72 (1H, t, J = 9.0 Hz, 4-H), 4.83 (1H, br.s, NH), 4.72 (2H, s, CH_2-OH), 4.60 (1H, t, J = 8.1 Hz, 2-H), 3.26–3.21 (2H, m, 1''-H), 2.53–2.43 (2H, m, 3''-H), 2.13–2.08 (2H, m, 3-H), 2.02–1.96 (2H, m, 2''-H).

Result and Discussion

Using the imino Diels-Alder reaction THQ (**1**) was obtained. In a typical aerobic fermentation, **1** was incubated with *M. isabelina* for 14 days and compound **2** was obtained. The synthetic routes used for the preparation of THQ and biotransformation with *M. isabelina* are shown in Fig. 1.

The ^{1}H NMR spectrum of THQ (**1**) showed a signal with a chemical shift of δ 2.38 ppm, corresponding to a singlet that integrates three protons corresponding to a methyl group. The structure of compound **2** was deduced mainly from the ^{1}H NMR spectrum which was similar to that of THQ. Instead of the singlet at δ 2.38 for THQ, a signal appeared at δ 4.72 ppm for two protons suggesting the presence of a methylene group, which indicates the incorporation of a hydroxy group by the microorganism used. The presence of the hydroxy group was verified by the IR spectrum showing the presence of a broad band at 3550 cm^{-1}. This alcoholic group is absent in THQ, but there was a weak band at 3350 cm^{-1} characteristic of an NH group.

Several studies reported the successful use of microorganisms for the bioconversion of synthetic compounds. 3α,4α-Dihydroxy-dihydro-β-agarofuran was synthesized and then biotransformated by *Rhizopus nigricans* (Alarcón and Aguilar, 2004). Some 4-chromenones were synthesized and then biotransformed by *Mortierella isabelina* (Holland *et al.*, 1995). Synthetic 3-hydroxydibenzo-α-pyrone was biotransformed into 3,8-dihydroxydibenzo-α-pyrone and aminoacyl conjugates by *Aspergillus niger* (Islam *et al.*, 2008). We have shown that the microbial hydroxylation of THQ (**1**) by *M. isabelina* gave a THQ hydroxylated in a specific position at higher yield than by chemical synthesis. This methodology may be a convenient new route for the synthesis of tetrahydroquinolines with several hydroxy groups and better solubility and biological activity than **1**.

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