Journal of Molecular Catalysis B: Enzymatic 117 (2015) 1–6

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Fungal metabolism of naphthoflavones

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ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 1 April 2015
Accepted 10 April 2015
Available online 20 April 2015

Keywords:
Naphthoflavones
Biotransformation
Hydroxylation
Demethylation
Fungi

ABSTRACT

Naphthoflavones (benzoflavones) are synthetic flavonoids commonly used in drug metabolism studies as selective activators or inhibitors of cytochrome P-450 enzymes. Nowadays they are also used as a component of food supplements for body builders. There is no data regarding naphthoflavone microbial metabolism. In the present studies sixty-three fungal strains have been screened for their ability to transform α-naphthoflavone (7,8-benzoflavone) or β-naphthoflavone (5,6-benzoflavone). Five strains belonging to the genera *Penicillium*, *Cladosporium*, *Aspergillus* and *Verticillium* transformed α-naphthoflavone and β-naphthoflavone to the corresponding 4′-hydroxy derivatives. These selected fungi have been used in a further study on biotransformation of naphthoflavones with a differently substituted B-ring. Only 4′-methoxy derivatives have been transformed to the related 4′-hydroxy products. Selected strains are good biocatalysts to obtain 4′-hydroxy naphthoflavones in the one step reaction.

1. Introduction

Naphthoflavones are synthetic derivatives of flavones, a group of naturally occurring flavonoids widely distributed in the plant world. At the beginning of the 1970s α-naphthoflavone (α-NF, 7,8-benzoflavone, **1**) was found to be in the spotlight of researchers because of its effect on the metabolism of carcinogens [1]. The subsequent studies showed that this compound inhibits the activation of procancerogenic polycyclic hydrocarbons and activates aflatoxin B1 to mutagenic metabolites *in vitro* [2,3]. Interestingly, β-naphthoflavone (β-NF, 5,6-benzoflavone, **2**), an isomer of α-NF (**1**), also affects the activity of microsomal polycyclic hydrocarbon hydroxylases, however, it acts as an inducer of these enzymes activities *in vitro* [4,5]. A few years later Kellis and coworkers discovered that α-NF (**1**) is a potent inhibitor of aromatase, the enzyme responsible for transformations of androgens into estrogens [6]. They also showed that β-NF (**2**) has no inhibitory activity. Nowadays the properties of these two naphthoflavones, that rely on the selective modulation of the activity of particular cytochrome P-450 monooxigenases, are widely applied in drug metabolism studies [7,8].

The biological activity of naphtoflavones was tested in parallel with their metabolism (Table 1). The much greater interest in α-NF (**1**) compared to β-NF (**2**) may be associated with the fact that some metabolites of α-NF (**1**) have a higher inhibitory potency [9,10]. Further studies on the metabolism of naphtoflavones seem to be mandatory especially due to the action of aromatase-inhibiting α-NF (**1**) that is used as a component of dietary supplements sold for bodybuilding. In a consequence humans may be exposed to gut microbial metabolites of α-NF (**1**) and such may differ from those already identified.

For years whole cell microorganism biotransformations have been used to mimic mammalian metabolism or to obtain human particular metabolites at reasonable quantities using simple methods [11]. To the best of our knowledge there is no data concerning naphthoflavones microbial metabolism, therefore we decided to study this phenomenon.

2. Experimental

2.1. Materials

α-NF (**1**), β-NF (**2**), 1-hydroxy-2-acetonaphthone, 2-hydroxy-1-acetonaphthone, 2-methoxybenzaldehyde, 3-methoxybenzaldehyde, 4-methoxybenzaldehyde, 4-methylbenzaldehyde and 4-fluorbenzaldehyde were purchased from Sigma Aldrich. Ten naphthoflavone derivatives were obtained from corresponding hydroxy-acetonaphthones and benzaldehydes as starting materials. Two stage synthesis was used in which first step was base aldol condensation of hydroxy-acetonaphthones and benzaldehydes to afford chalcones. Second step was oxidative-cyclisation of chalcones with iodine in DMSO to afford flavones (Scheme 1) [24].

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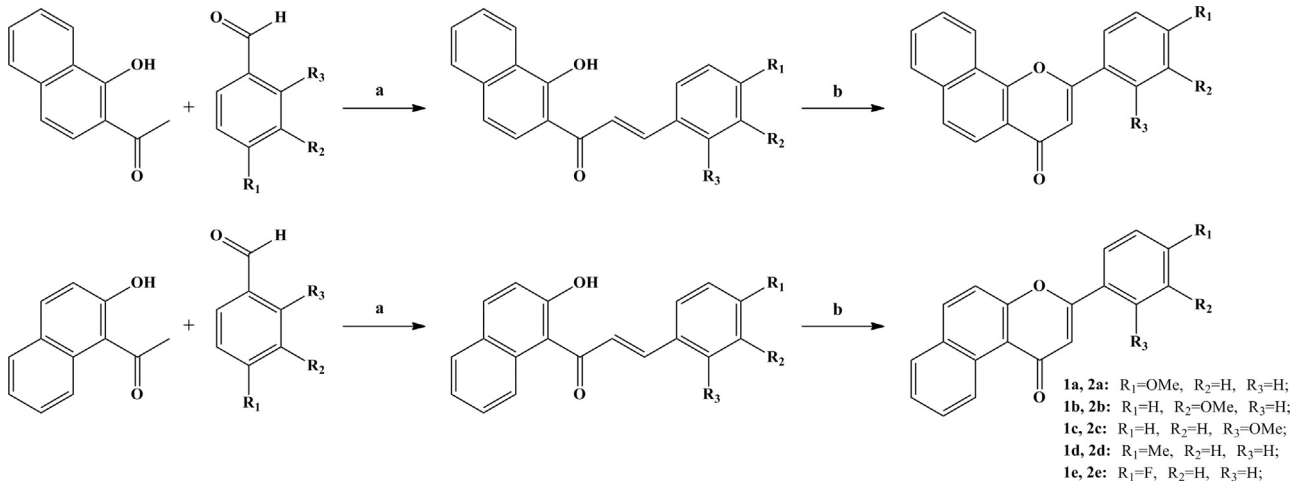
http://dx.doi.org/10.1016/j.molcatb.2015.04.007
1381-1177/

Table 1
Comparative data from different studies on α-NF (**1**) and β-NF (**2**) metabolism.

	Metabolites of	
	α-NF (**1**)	β-NF (**2**)
Long-Evans rat liver microsomes and reconstituted Cytochrome P-450 System [12]	5,6-oxide, 5,6-dihydrodiol, 7,8-dihydrodiol, 6-hydroxy, 7-hydroxy, 9-hydroxy and one unknown compound	5,6-dihydrodiol, 7,8-dihydrodiol, 8-hydroxy, 5-hydroxy and five unknown compounds
Rat liver microsomes and reconstituted selection of Cytochrome P-450 System [13]	5,6-oxide, 5,6-dihydrodiol, 7,8-dihydrodiol, 6-hydroxy and one unknown metabolite	
Charles River CD rat liver microsomes [14]	5,6-oxide, 5,6-dihydrodiol, 6-hydroxy, 9-hydroxy	
Charles River CD rat, mouse, rabbit and Syrian golden hamster liver microsomes [15]	5,6-oxide, 5,6-dihydrodiol, 7,8-dihydrodiol, 6-hydroxy, 7-hydroxy, 9-hydroxy	
Sprague Dawley rat liver microsomes [16]	5,6-oxide, 5,6-dihydrodiol, 9,10-dihydrodiol, 6-hydroxy	
Hepatic microsomes from the marine fish *Stenotomus versicolor* [17]	Unidentified dihydrodiol	
Liver microsomes [18]	5,6-oxide, 5,6-dihydrodiol, 9,10-dihydrodiol, 5-hydroxy	
Syrian golden hamster liver microsomes [19]	6-hydroxy, 7-hydroxy and six other metabolites	
Syrian golden hamster hepatocytes [20]	5,6-dihydrodiol, 7,8-dihydrodiol, 6-hydroxy, 7-hydroxy	
Syrian golden hamster liver and kidney microsomes [21]	5,6-dihydrodiol, two isomeric hydroxy derivatives	
Human liver microsomes [22]	5,6-oxide, 5,6-dihydrodiol, 7,8-dihydrodiol, 6-hydroxy, 7-hydroxy	
Recombinant human CYP3A4 and NADPH:CYP reductase with cytochrome b5 [23]	5,6-oxide, 7,8-dihydrodiol	



Scheme 1. Synthesis of naphthoflavone derivatives (a) KOH, EtOH; (b) I_2, DMSO.

The structures of the obtained compounds were confirmed by NMR spectra analysis. Spectroscopic data of methoxy-derivatives (**1a–c**, **2a–c**) are with agreement with literature data [24]. Data of other compounds (**1d–e**, **2d–e**) are as follows:

*2.1.1. 4′-Methyl-α-naphthoflavone (**1d**)*

^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.39 (3H, s, H-Me), 7.11 (1H, s, H-3), 7.40 (2H, m, H-3′, H-5′), 7.80 (2H, m, H-8, H-9), 7.97 (1H, m, H-5), 7.90 (1H, m, H-6), 8.08 (2H, m, H-2′, H-6′), 8.10 (1H, m, H-7), 8.63 (1H, m, H-10). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 21.05 (C-Me), 107.45 (C-3), 119.59 (C-13), 119.97 (C-5), 122.23 (C-10), 123.47 (C-11), 125.29 (C-6), 126.24 (C-2′, C-6′), 127.66 (C-9), 128.22 (C-7), 128.36 (C-1′), 129.48 (C-8), 129.79 (C-3′, C-5′), 141.91 (C-4′), 135.40 (C-14), 152.65 (C-12), 162.04 (C-2), 176.76 (C-4).

*2.1.2. 4′-Fluoro-α-naphthoflavone (**1e**)*

^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.21 (1H, s, H-3), 7.47 (2H, m, H-3′, H-5′), 7.83 (2H, m, H-8, H-9), 7.95 (1H, m, H-6), 8.00 (1H, m, H-5), 8.12 (1H, m, H-7), 8.33 (2H, m, H-2′, H-6′), 8.69 (1H, m, H-10); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 108.07 (C-3), 116.29 (d, J = 22.00 Hz, C-3′, C-5′), 119.53 (C-13), 119.93 (C-5), 122.29 (C-10), 123.43 (C-11), 125.41 (C-6), 127.67 (C-9), 127.77 (d, J = 2.90 Hz, C-1′), 128.22 (C-7), 129.07 (d, J = 9.00 Hz, C-2′, C-6′), 129.55 (C-8), 135.44 (C-14), 152.74 (C-12), 161.05 (C-2), 164.09 (d, J = 250.3 Hz, C-4′), 176.74 (C-4).

*2.1.3. 4′-Methyl-β-naphthoflavone (**2d**)*

^{1}H NMR (600 MHz, DMSO-d_6) δ: 2.41 (3H, s, H-Me), 7.16 (1H, s, H-3), 7.42 (2H, m, H-3′, H-5′), 7.69 (1H, m, H-7), 7.78 (1H, m, H-6), 7.90 (1H, d, J = 9.0 Hz, H-10), 7.97 (1H, m, H-8), 8.06 (2H, m, H-2′, H-6′), 8.36 (1H, d, J = 9.0 Hz, H-9), 9.97 (1H, m, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 21.05 (C-Me), 109.12 (C-3), 116.18 (C-12), 118.16 (C-10), 126.08 (C-5), 126.11 (C-2′, C-6′), 126.55 (C-7), 127.51 (C-1′), 128.57 (C-8), 129.01 (C-6), 130.36 (C-13), 129.69 (C-14), 129.75 (C-3′, C-5′), 135.69 (C-9), 141.85 (C-4′), 156.94 (C-11), 160.39 (C-2), 179.18 (C-4).

*2.1.4. 4′-Fluoro-β-naphthoflavone (**2e**)*

^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.20 (1H, s, H-3), 7.45 (2H, m, H-3′, H-5′), 7.68 (1H, m, H-7), 7.78 (1H, m, H-6), 7.89 (1H, m, H-10), 8.10 (1H, m, H-6″), 8.23 (2H, m, H-2′, H-6′), 8.36 (1H, m, H-9), 9.95 (1H, m, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 109.65 (C-3),