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Naphthalene-dioxygenase-catalysed *cis*-dihydroxylation of azaarene derivatives Part 1: 2-Pyridones and 2-quinolones

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Abstract

The scope of the biotransformation of 2-pyridone- and 2-quinolone-derived compounds by recombinant whole-cells of *E. coli* JM109(DE3)(pDTG141) expressing the naphthalene-dioxygenase system from *Pseudomonas* sp. NCIB 9816-4 was explored, using a series of *N*- and *C*-substituted derivatives. Among them, only the *N*-methyl substituted compounds were good substrates for a regio- and stereoselective dihydroxylation reaction leading to *cis*-dihydroxydihydro pyridone derivatives, corresponding to the general pattern expected for this enzyme. In the absence of dihydroxylation reactions, *N*-dealkylations and monohydroxylations on external methyl groups were observed.

Keywords: *N*-Methyl-2-pyridone; *N*-Methyl-2-quinolone; *Pseudomonas* sp.; Dihydroxylation; Naphthalene-dioxygenase

1. Introduction

The bacterial metabolism of aromatic compounds often proceeds via an initial dioxygenase-catalysed dihydroxylation to yield *cis*-dihydrodiol derivatives. A large range of mono- and polycyclic substrates has been reported to form the corresponding enantiopure *cis*-dihydrodiol metabolites [1–3]. Multicomponent bacterial dioxygenases, mainly toluene dioxygenase (TDO) and naphthalene-dioxygenase (NDO), have been frequently utilized to produce hundreds of such *cis*-1,2-dihydrodiols, several of which have been used as chiral synthons for the synthesis of biologically active products and value-added chemicals [4].

However, to date, only a few data have been reported about the *cis*-dihydroxylation of compounds derived from heterocyclic arenes [1,5,6], including polycyclic azaarenes [5,7], quinoline [8,9], isoquinoline [9], quinoxaline [9], quinazoline [9], benzothiophene [6,10] and benzofuran [6,11]. Generally, dihydroxylation of such substrates occurs in the carbocyclic rings and only scarce examples of dihydroxylation in the heteroarene portion of the substrate or dihydrodiol metabolite formation from monocyclic heteroarenes had been described in Refs. [6,9]. However, in the recent years, direct evidence for the *cis*-dihydroxylation of a 2-pyridone ring by NDO has been reported in our laboratory and a major dihydroxylated metabolite, *N*-methyl-*cis*-5,6-dihydroxy-5,6-dihydro-2-pyridone was obtained from the corresponding *N*-methyl-2-pyridone substrate, together with small amounts of its 3,4-dihydroxy-dihydro isomer [12,13].

The present study was initiated to extend this observation by investigating the effect of new substituents on 2-pyridone and 2-quinolone rings (see Scheme 1) in the NDO-catalysed reaction and to elaborate new methods to directly and definitively determine the absolute configuration and enantiomeric

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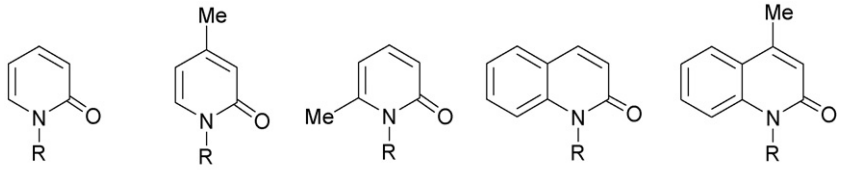

1a, R = Me
1b, R = CH_2-Phe
1c, R = CH_2-CH=CH_2

2a, R = Me
2b, R = CH_2-Phe

3a, R = Me
3b, R = CH_2-Phe

4a, R = Me
4b, R = CH_2-Phe

5a , R = Me

Scheme 1.

purity of the dihydrodiols, respectively, formed from *N*-methyl-2-pyridone and *N*-methyl-2-quinolone by this bacterial dioxygenase.

2. Experimental

2.1. Substrates and chemicals

Substrate **1a** was from commercial source. 4-Bromophenyl boronic acid was from Aldrich and 4-bromobenzoyl chloride from Fluka. (*R*)-Phenylglycine methyl ester hydrochloride was from Aldrich, (*S*)-phenylglycine methyl ester hydrochloride, and (*S*)-phenylglycine *t*-butyl ester hydrochloride were from Novabiochem. (*R*)-Phenylglycine *t*-butyl ester hydrochloride was from Bachem. Substrates **1b**, **1c**, **2a**, **2b**, **3a**, **3b**, **4a**, **4b** and **5a** were, respectively, prepared from 2-hydroxypyridine (**1b**, **1c**), 2-hydroxy-4-methyl pyridine (**2a**, **2b**), 2-hydroxy-6-methyl pyridine (**3a**, **3b**), 2-hydroxyquinoline (**4a**, **4b**) and 2-hydroxy-4-methylquinoline (**5a**), by literature procedures [14] and fully characterised by ESI-MS and NMR. Detailed complementary physical data of some compounds are given below.

N-Methyl-4-methyl-2-pyridone **2a**: $(M+H^+)$ 124; δ_H 250 MHz: 7.43 (1H, d, $J_{6,5}=6.9$, 6-H), 6.13 (1H, br.s, 3-H), 5.94 (1H, dd, $J_{5,6}=6.9$, $J_{5,3}=1.9$, 5-H), 3.39 (3H, s, N–CH_3), 2.07 (3H, s, 4-CH_3). δ_C 62.9 MHz: 162.5 (C2), 118.5 (C3), 151.1 (C4), 107.4 (C5), 138.7 (C6), 36.3 (CH_3–N), 20.6 (CH_3–C).

N-Benzyl-4-methyl-2-pyridone **2b**: $(M+H^+)$ 200; δ_H 250 MHz: 7.54 (1H, d, $J_{6,5}=7.0$, 6-H), 7.25–7.35 (5H, m, ArH), 6.26 (1H, br.s, 3-H), 6.05 (1H, dd, $J_{3,5}=1.9$, $J_{5,6}=7.0$, 5-H), 5.13(2H, s, N–CH_2), 2.15 (3H, s, CH_3). δ_C 62.9 MHz: 162.4 (C2), 119.3 (C3), 151.3 (C4), 108.1 (C5), 137.8 (C6), 51.3 (CH_2–N), 128–129 (Ar–C), 138.5 (Ar–C).

N-Methyl-6-methyl-2-pyridone **3a**: $(M+H^+)$ 124; δ_H 250 MHz: 7.25 (1H, dd, $J_{4,3}=9.1$, $J_{4,5}=6.8$, 4-H), 6.27 (1H, d, $J_{3,4}=9.1$, 3-H), 6.10 (1H, d, $J_{5,4}=6.8$, 5-H), 3.48 (3H, s, N–CH_3), 2.38 (3H, s, 6-CH_3). δ_C 62.9 MHz: 163.9 (C2), 116.8 (C3), 139.4 (C4), 106.4 (C5), 148.2 (C6), 30.9 (CH_3–N), 20.6 (CH_3–C).

N-Benzyl-6-methyl-2-pyridone **3b**: $(M+H^+)$ 200; δ_H 250 MHz: 7.33 (1H, m, 4-H), 7.22–7.36 (5H, m, ArH), 6.36 (1H, d, $J_{3,4}=9.1$, 3-H), 6.06 (1H, d, $J_{5,4}=6.7$, 5-H), 5.36 (2H, s, N–CH_2), 2.27 (3H, s, C–CH_3). δ_C 62.9 MHz: 163.8 (C2), 118.1 (C3), 140.3 (C4), 106.7 (C5), 148.2 (C6), 47.4 (–CH_2–N), 127–129 (Ar–C), 138.4 (Ar–C).

2.2. Analytical procedures

^{1}H NMR and ^{13}C NMR spectra were recorded in d_6-acetone (unless stated otherwise) on Bruker ARX250 (250.13 MHz Larmor frequency for ^{1}H) or Bruker Avance500 (500.13 MHz Larmor frequency for ^{1}H) spectrometers. Chemical shifts (δ) are reported in ppm and coupling constants are given in Hz. ^{1}H signals were assigned using 2D homonuclear ^{1}H–^{1}H COSY and TOCSY experiments. The TOCSY and the COSY spectra were used to distinguish the spin groups and to confirm the assignments of the hydrogen resonances within each spin group. The $^3J_{1H,1H}$ coupling constants were determinated by homodecoupling experiments of 1D ^{1}H spectra. ^{13}C signals were assigned using 2D heteronuclear ^{1}H–^{13}C HSQC and HMBC experiments. Optical rotation measurements ($[\alpha_D]$) were carried out with a Perkin-Elmer 241 polarimeter at ambient temperature (ca 20 °C).

1,2-Dihydroxy-1,2-dihydronaphthalene determinations were performed at 40 °C with UV detection at 254 nm on a Lichrospher 100 RP18e column (Hewlett Packard, 4.6 mm × 250 mm, 5 μm), equilibrated with a water–acetonitrile mobile phase (60:40) and eluted with an acetonitrile gradient containing formic acid (999:1) at a 0.5 mL/min flow rate.

HPLC-MS analyses were performed on a Thermo Finnigan LCQ Advantage Instrument (electrospray positive mode) equipped for HPLC with a Polarity dC18 column (Waters, 4.6 mm × 250 mm, 5 μm) equilibrated with the appropriate water–acetonitrile mobile phase. Products were eluted with an acetonitrile gradient containing formic acid (999:1) at 40 °C and 0.4 mL/min flow rate.

Flash chromatography was performed on Kieselgel Si60 (Merck, 40–63 μm) columns equilibrated with CH_2Cl_2 and eluted with CH_2Cl_2–acetone–methanol in the appropriate ratios.

2.3. Microorganism and culture media

E. coli JM109(DE3)(pDTG141) was kindly provided by D.T. Gibson (Iowa University). This strain which contains the cloned nahAaAbAcAd genes encoding for the NDO components from *Pseudomonas* sp. strain NCIB 9816-4 [15] was used for all biotransformations. Cells were grown at 37 °C and 350 rpm stirring in a 6 L fermentor containing mineral salt broth [16] supplemented with yeast extract (Difco, 4 g L^{-1}), thiamine (1 mM), ampicillin (sodium salt, 100 mg L^{-1}) and glucose (0.36%). During the growth, pH was maintained at 7.2 by addition of ammonia