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Dioxygenase-catalysed formation of dihydrodiol metabolites of *N*-methyl-2-pyridone

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Abstract

IPTG-induced cells of a recombinant strain of *Escherichia coli* JM109(DE3)pDTG141 expressing naphthalene dioxygenase from *Pseudomonas* sp. NCIB 9816-4 oxidized specifically *N*-methyl-2-pyridone to give the corresponding *cis*-5,6-dihydro-5,6-dihydroxy derivative as a major product. A small amount of the *cis*-3,4-dihydro-3,4-dihydroxy isomer was simultaneously formed.

Keywords: dihydroxylation; biocatalysis; heterocyclic nitrogen compounds.

The microbial metabolism of pyridine derivatives (unlike that of benzene) does not involve a *cis*-dihydroxylation reaction as the initial step of their biodegradation.[1,2] Instead, the metabolism of such heterocycles usually starts with hydroxylation in an adjacent position to the nitrogen atom, leading to the formation of monohydroxy derivatives. While working with whole microorganisms which are known to contain dioxygenase enzymes (*Pseudomonas* wild strains or mutants for example),[3] only monohydroxylation in the 2(6)-position of the pyridine ring or *N*-oxidation are observed, masking any possible dihydroxylation reaction.

However, a dihydroxylation reaction (and especially a stereospecific one) should be quite a useful tool for the functionalization of pyridine derivatives to the corresponding dihydropyridines, in view of their potential synthetic use as precursors of azasugars,[4] alkaloids,[5] unnatural amino acids,[6] etc.

We have thus undertaken a systematic investigation of the biotransformation of several substituted pyridine derivatives by available recombinant strains of *Escherichia coli* expressing various dioxygenases from *Pseudomonas* species.

As shown in Scheme 1, *N*-methyl substituted 2-pyridone (**1**) was specifically and completely transformed[7] by *Escherichia coli* JM109(DE3)(pDTG141), a recombinant strain which expresses naphthalene dioxygenase (NDO) from *Pseudomonas* sp. NCIB 9816-4,[8,9] to give in good yield (about 50%)

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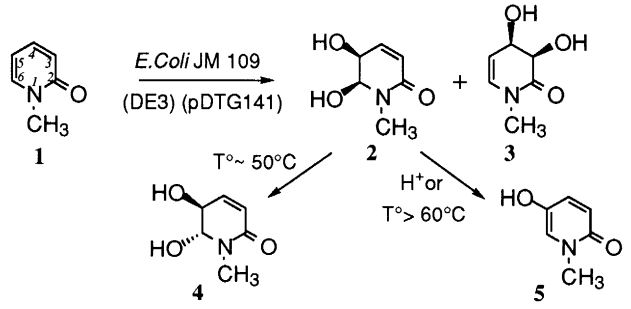
one major dihydroxylated product, isolated by silica gel chromatography, and identified as the *cis*-5,6-dihydro-5,6-dihydroxy-1-methyl-2-pyridone (**2**).



Scheme 1.

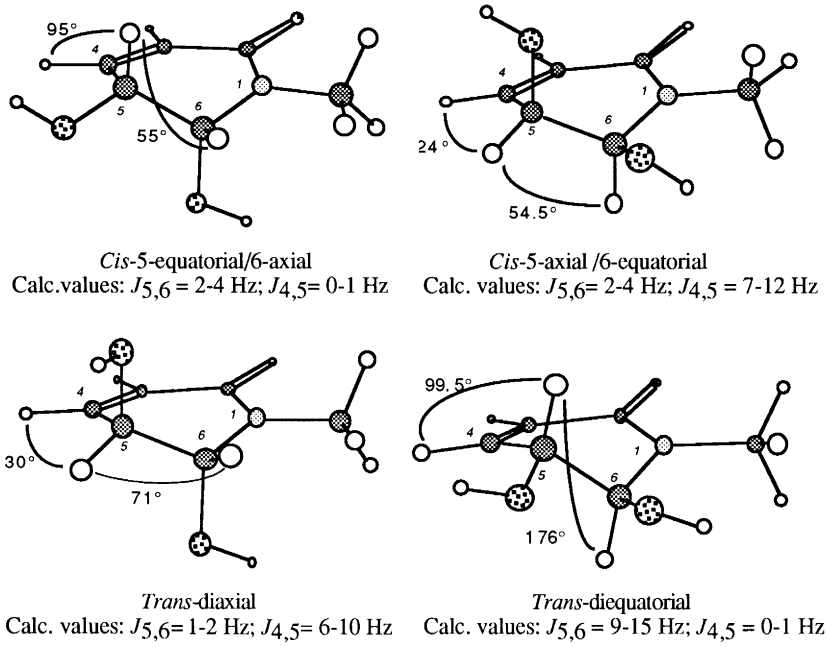
In addition, a minor metabolite, identified as the *cis*-3,4-dihydro-3,4-dihydroxy isomer **3** was also isolated in minute amount (about 3%). Moreover, depending on the work-up conditions, a variable amount of *trans*-5,6-dihydro-5,6-dihydroxy-1-methyl-2-pyridone **4** (2–10%) could be isolated. The structures of all three compounds were established by NMR (^{1}H, ^{13}C, 2D COSY, HMQC, HMBC, NOESY) and MS studies.[10]

When analyzed by GC/EI-MS, pure dihydroxy isomers **2** and **4** exhibited molecular ions at *m/z* 143 (corresponding to dihydro dihydroxy-derivatives) and major fragment ions at *m/z* 115, 86 and 85.[11] A small molecular ion at *m/z* 143 was also found for isomer **3**, together with a base peak corresponding to a dehydration fragment ion at *m/z* 125**.** In NMR spectra, characteristic coupling constants were found for the vinylic protons in 3,4-position (**2**, *J*=10 Hz; **4**, *J*=9.6 Hz) compared to those in 5,6-position (**3**, *J*=7.8 Hz), clearly demontrating 5,6- or 3,4-dihydroxylation patterns.[4a] Moreover, the HMBC spectrum of **2** exhibited ^{3}J-coupling between the *N*-methyl protons and the nearest CH(OH) carbon, without any significant coupling with a double-bonded carbon atom. The reverse situation was found in the HMBC spectrum of **3**. The assignment of *cis*- and *trans*-relative configurations of **2** and **4** was based upon the measured ^{3}J-coupling constants between H-5 and H-6 vicinal protons, correlated with dihedral angle values calculated from molecular modelisation after energy minimization[12] (Fig. 1). As a result, the vicinal coupling constants for the *cis*-isomer **2** are larger ($J_{5,6}$=4.4 Hz) compared to the *trans*-isomer **4** ($J_{5,6}$=2.0 Hz), and they are in agreement with the calculated values of dihedral angles in the model (55 and 71°, respectively). On the other hand, the observed H-4/H-5 coupling constants similarly fit with the calculated dihedral angles: 2.0 Hz for the *cis*-isomer (95°), compared to 5.2 Hz for the *trans*-isomer (30°).

In case of the 3,4-dihydroxy isomer (**3**), the characteristic H-3/H-4 and H-4/H-5 coupling constants (4.7 and 6.2 Hz respectively) were in full agreement with the calculated corresponding dihedral angles (51 and 27°) for a *cis*-compound (Fig. 2).

When kept at room temperature, *cis*-dihydrodiols **2** and **3** were shown to be relatively stable. However, even mild heating of **2** (~50°C) led to isomerisation to the corresponding *trans*-isomer **4.** The formation of *trans*-dihydrodiols along with the *cis*-isomers has been previously described in the dihydroxylation of thiophene,[13] benzothiophene,[13,14] and benzofuran[13] by the toluene dioxygenase of *P. putida* UV4 and attributed to the properties of the resulting *cis*-hemi(thio)acetal ring, which equilibrates with its anomer (*trans*-isomer) via an open chain aldehyde intermediate.[15] The slight formation of the *trans*-dihydro-diol **4** which we observed could be attributed to a similar isomerisation, during incubation and work-up of the *cis*-hemiaminal **2** (major product) initially formed in the NDO-catalysed dihydroxylation reaction.

However, no mutarotation was observed at room temperature, indicating a higher stability of the



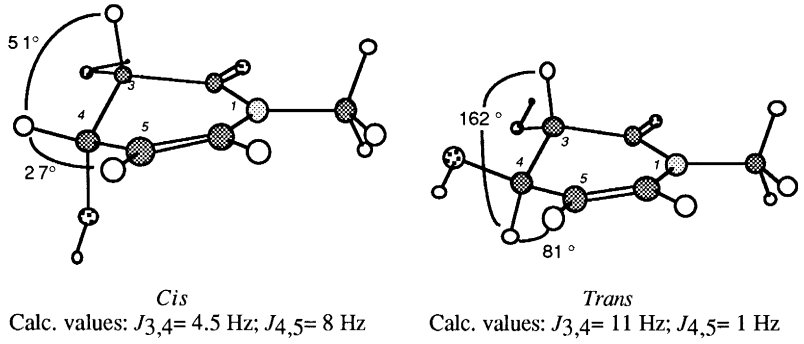
Cis-5-equatorial/6-axial
Calc.values: $J_{5,6}$ = 2-4 Hz; $J_{4,5}$= 0-1 Hz

Cis-5-axial /6-equatorial
Calc. values: $J_{5,6}$= 2-4 Hz; $J_{4,5}$ = 7-12 Hz

Trans-diaxial
Calc. values: $J_{5,6}$= 1-2 Hz; $J_{4,5}$= 6-10 Hz

Trans-diequatorial
Calc. values: $J_{5,6}$ = 9-15 Hz; $J_{4,5}$ = 0-1 Hz

Fig. 1. Energy minimized conformations of *cis*-5,6-dihydroxylated 1-methyl-2-pyridone (**2**) and its *trans*-isomer **4** and vicinal coupling constants calculated from the corresponding dihedral angles H-5/H-6 and H-4/H-5



Cis
Calc. values: $J_{3,4}$= 4.5 Hz; $J_{4,5}$= 8 Hz

Trans
Calc. values: $J_{3,4}$= 11 Hz; $J_{4,5}$= 1 Hz

Fig. 2. Energy minimized conformations of *cis*- and *trans*-3,4-dihydro-3,4-dihydroxy-1-methyl-2-pyridone and vicinal coupling constants calculated from the dihedral angles H-3/H-4 and H-4/H-5

hemiaminal compounds. As expected, the *cis*-3,4-dihydro dihydroxy-derivative **3** did not suffer such an isomerisation reaction.

Heating above 60°C or a mild acidic treatment converted both 5,6-dihydroxy isomers **2** and **4** into a dehydration product, 5-hydroxy-2-pyridone **5**.[16] Consequently, all attempts to prepare mono- (or di-)ester derivatives with activated acylating reagents were unsuccessful due to the easy aromatization of the diols in the reaction conditions. Such features prevented the use of diastereomeric esters of optically active acids (such as MTPA or camphanic acid) to estimate the optical purity of the diols and eventually determine their absolute configuration. It has been shown[15] that the dihydroxylation of aromatic compounds by toluene dioxygenase or naphthalene dioxygenase generally yields enantiopure dihydro dihydroxylated products with a single absolute configuration. However, in a few examples, the formation of *cis*-dihydrodiols of lower enantiomeric excess has been described.[15]

No reaction was observed with unsubstituted 2-pyridone or *N*-methyl-4-pyridone. In control experi-

ments with *E. coli* JM109(DE3)(pT7-5), a strain which does not contain the structural genes for NDO, no oxidation of 1-methyl-2-pyridone was observed. Until now, the NDO system had not been reported to catalyse the *cis*-dihydroxylation of monocyclic arenes or heteroarenes at any bond.[15,17] It is also remarkable that another recombinant strain, *E. coli* JM109(pDTG601) expressing toluene dioxygenase from *P. putida* F1, is not able to oxidize 1-methyl-2-pyridone.

The reported results appear to be an unprecedented example of dihydrodiol formation from a mono-heterocyclic nitrogen compound, resulting from a dioxygenase-catalysed oxidation. To our knowledge, there is only one reported example of a comparable dioxygenase-catalysed *cis*-dihydroxylation in the pyridine ring of a bicyclic heteroarene compound: the oxidation of 2-chloroquinoline by toluene dioxygenase of *P. putida* UV-4 has been recently described to give, as a minor product, a *cis*-3,4-dihydro-3,4-dihydroxy-2-quinolone,[18] which probably derives from an intermediate 2-quinolone.[15,18]

Work is in progress to determine the absolute configuration of the dihydroxylated products, and to extend the scope of this reaction to a series of unsubstituted and substituted monocyclic and bicyclic heteroaromatic systems.

Acknowledgements

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7. Typical experimental procedure: IPTG-induced cells of *E. coli* JM109(DE3)pDTG141, grown at 37°C in 1 L of minimal medium (see Ref. 8), were harvested at the end of exponential growth, washed and resuspended in 0.3 vol. of 0.05 M Na/K phosphate buffer pH 7.2 containing 0.2% glucose. After addition of *N*-methyl-2-pyridone (60 mg), incubation was continued at 30°C for 20–22 h and the lyophilized supernatant was repeatedly extracted with ethanol and purified by column chromatography and preparative TLC on silica gel to give the dihydro dihydroxy derivatives.
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10. Compound **2**: $[\alpha]_D$ +41 (*c* 0.345, MeOH). ^{1}H NMR (CD_6-acetone), δ=6.37 (1H, dt, $J_{4,3}$=10, $J_{4,5}$=2, $J_{4,6}$=1.7, H-4), 5.70 (1H, dd, $J_{3,4}$=10, $J_{3,5}$=2.0, H-3), 4.92 (1H, dd, $J_{6,5}$=4.4, $J_{6,4}$=1.7, H-6), 4.57 (1H, br.dd, $J_{5,6}$=4.4, $J_{5,4}$=$J_{5,3}$=2, H-5), 2.94 (3H,

s, CH_3). ^{13}C NMR (CD_3OD), δ=168.1 (CO), 146.7 and 125.1 (−CH=), 86.7 and 69.6 (CHOH), 35.0 (CH_3). EI-MS, see Ref. 11. Compound **3**: $[\alpha]_D$ +25 (*c* 0.16, MeOH). ^1H NMR (CD_3OD), δ=6.28 (1H, dd, $J_{6,5}$=7.8, $J_{6,4}$=0.8, H-6), 5.38 (1H, dd, $J_{5,6}$=7.8, $J_{5,4}$=6.2, H-5), 4.26 (1H, d, $J_{3,4}$=4.7, H-3), 4.17 (1H, ddd, $J_{4,3}$=4.7, $J_{4,5}$=6.1, $J_{4,6}$=0.8, H-4), 3.06 (3H, s, CH_3). ^{13}C NMR (CD_3OD), δ=172.1 (CO), 134.0 and 106.8 (−CH=), 72.1 and 65.6 (CHOH), 34.2 (CH_3). EI-MS: *m/z* 143 (4), 125 (100), 97 (30), 96 (30), 82 (8), 68 (36), 55 (23). Compound **4**: $[\alpha]_D$ +207 (*c* 0.285, MeOH). ^1H NMR (CD_6-acetone), δ=6.57 (1H, ddd, $J_{4,3}$=9.6, $J_{4,5}$=5.2, $J_{4,6}$=1.5, H-4), 5.86 (1H, d, $J_{3,4}$=9.6, H-3), 4.90 (1H, br.t, H-6), 4.10 (1H, dd, $J_{5,6}$=2, $J_{5,4}$=5.2, H-5), 2.96 (3H, s, CH_3). EI-MS, see Ref. 11.

11. Both pure 5,6-dihydroxy isomers **2** and **4** exhibited two distinct chromatographic peaks, with retention time at 7.5 and 9.4 min, in a constant 1:1 ratio, with very small differences in their fragmentation pattern (M^+ at *m/z* 143; base peak at *m/z* 86 or 85, respectively). This chromatographic splitting and the resulting fragmentation pattern probably originates from the isomerisation of the remaining double bond under the GC conditions.
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16. Compound **5**: ^1H NMR (CD_3OD), δ=7.28 (1H, dd, $J_{4,3}$=9.6, $J_{4,6}$=3, H-4), 7.05 (1H, d, $J_{6,4}$=3, H-6), 6.46 (1H, d, $J_{3,4}$=9.6, H-3), 3.15 (3H, s, CH_3). EI-MS: *m/z* 125 (100), 97 (35), 96 (16), 82 (8), 68 (29), 55 (16).
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