cis-Dihydrodiol, arene oxide and phenol metabolites of dictamnine: key intermediates in the biodegradation and biosynthesis of furoquinoline alkaloids

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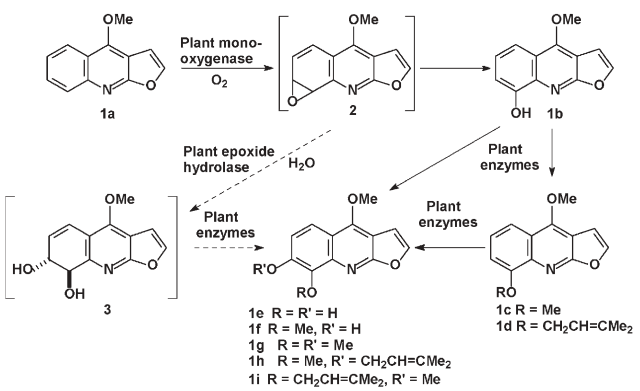
Biotransformation of the parent furoquinoline alkaloid dictamnine and its 4-chlorofuroquinoline precursor, using the B8/36 bacterial mutant strain of *Sphingomonas yanoikuyae*, yielded, *via* biphenyl dioxygenase-catalysed dihydroxylation, the first isolable alkaloid *cis*-dihydrodiol metabolites; these metabolites were used in the chemoenzymatic synthesis of postulated arene oxide and phenol intermediates, and a range of derived furoquinoline alkaloids.

Bacteria and fungi play an important role in the biotransformation and biodegradation of plant alkaloids in the soil.[1–3] Alkyl monohydroxylations, N-oxidations and dealkylations are among the most common types of microbial alkaloid oxidations to have been reported.[1–3] However, microbial aromatic hydroxylations of alkaloids are relatively rare, and the isolation of arene *cis*-dihydrodiol metabolites of alkaloids is, to our knowledge, unprecedented. In prokaryotes, more than three hundred *cis*-dihydrodiols have been isolated from dioxygenase-catalysed dihydroxylation of arene substrates. Both mutant and recombinant bacterial strains containing dioxygenases, *e.g.* toluene dioxygenase (TDO), but lacking in the corresponding diol dehydrogenase enzymes, have been used to produce substituted benzene *cis*-dihydrodiols which have been widely utilised in synthesis.[4–7]

The major objective of this study was to find the first evidence of *cis*-dihydrodiol intermediate formation in the bacterial biotransformation of an alkaloid. If the *cis*-dihydrodiol metabolite was present, a further target was to isolate and utilise it for the chemoenzymatic/biomimetic synthesis of other related alkaloids.

Earlier reports from these laboratories had shown that *cis*-dihydrodiol metabolites could be isolated *via* dioxygenase-catalysed asymmetric dihydroxylation of quinolines, using mutant strains of bacteria.[8,9] Furthermore, a relatively stable 7,8-arene oxide derivative of quinoline was chemically synthesised[10] and isolated as a eukaryotic metabolite from monooxygenase-catalysed epoxidation using liver microsomes.[11] Dioxygenase-catalysed asymmetric dihydroxylations of larger polycyclic azaarene substrates *e.g.* acridine[12] and benzo[*c*]phenanthridine[13] were also observed using a mutant strain (B8/36) of *Sphingomonas yanoikuyae* (a source of biphenyl dioxygenase, BPDO).

Dictamnine **1a** is the parent compound of the furoquinoline alkaloid series. It is widely distributed among plants of the *Rutaceae* family. To date, more than eighty furoquinoline alkaloids have been identified, the majority having hydrogen atoms on the benzo ring of dictamnine **1a** replaced by OH, OMe, OEt, OCH_2O or $OCH_2CH{=}CMe_2$ groups and their derivatives. Earlier biosynthetic labelling studies in *Skimmia japonica* and *Choisya ternata* plants had shown that dictamnine **1a** was biotransformed into other furoquinoline alkaloids, *e.g.* skimmianine **1g**, *via* the putative arene oxide **2** as the biosynthetic precursor (Scheme 1).[14] Although, in some cases, these plant biosynthetic sequences remain to be elucidated, the phenols, robustine **1b** and haplopine **1f**, and ethers, γ-fagarine **1c**, haplophydine **1d**, skimmianine **1g**, 7-isopentenyl-γ-fagarine **1h** and isohaplopine 3,3′-dimethylallyl ether **1i** are also among the range of quinoline alkaloids that could be derived from dictamnine **1a** *via* arene oxide intermediate **2**. In this context, it is evident that the enzyme-catalysed oxidation of dictamnine **1a** to yield arene oxide **2**, and its isomerisation to the corresponding phenol metabolite **1b** could lead to alkaloids **1c** and **1d**. Further aromatic hydroxylation of alkaloids **1b** or **1c**, or possibly epoxide hydrolase-catalysed hydrolysis of arene oxide **2** to yield *trans*-dihydrodiol **3** followed by dehydrogenation to yield catechol **1e**, could be involved in the biosynthesis of the 7,8-disubstituted furoquinoline alkaloids, **1f–1i**. This report is thus focused on the chemoenzymatic synthesis of the postulated arene oxide intermediate **2** of dictamnine **1a** and furoquinoline alkaloids derived from *cis*-dihydrodiol metabolite



Scheme 1

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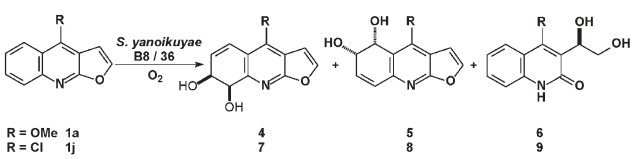
precursors **4**, **5**, **7** and **8** (Scheme 2), as several of these furoquinoline alkaloids have recently been reported to possess interesting biological activities.[15]

Earlier successful bacterial biotransformations of the tricyclic azaarene acridine,[12] to yield a *cis*-dihydrodiol metabolite using *S. yanoikuyae* B8/36, and of benzofuran[16] to furnish *cis*-dihydrodiols in both the carbocyclic and heterocyclic rings using *Pseudomonas putida* UV4 (a source of TDO), influenced our choice in favour of dictamnine **1a** and its precursor 4-chlorofuroquinoline **1j** as suitable substrates for the study with whole cells of *S. yanoikuyae* B8/36 (Scheme 2). Neither of these substrates has a substituent in its benzo ring.

Dictamnine **1a**, synthesised from 4-chlorofuroquinoline **1j** by the literature method,[17] was oxidised using *S. yanoikuyae* B8/36 to yield a mixture of bioproducts (**4–6**). These metabolites were separated and purified by PLC (7% MeOH in $CHCl_3$) to furnish *cis*-dihydrodiols **4** (R_f 0.37, 20–29% yield), **5** (R_f 0.32, 0–3% yield) and acyclic diol **6** (R_f 0.26, 0–1% yield). Under similar conditions, the chlorofuroquinoline **1j** yielded the corresponding *cis*-dihydrodiols **7** (R_f 0.6, 10% yield), **8** (R_f 0.5, 30% yield) and 2-quinolone **9** (R_f 0.4, 2% yield). Treatment of *cis*-dihydrodiols **4** ($[\alpha]_D$ +94, MeOH), **5** ($[\alpha]_D$ −110, MeOH), **7** ($[\alpha]_D$ +138, $CHCl_3$), and **8** ($[\alpha]_D$ +214, MeOH), with (*R*) and (*S*)-2-(1-methoxyethyl)-phenylboronic acid (MEPBA) yielded the corresponding diastereoisomeric boronates. ^{1}H-NMR analysis of the MEPBA diastereoisomers confirmed that all these *cis*-dihydrodiols were enantiopure (>98% ee). Using both the MEPBA method and circular dichroism spectral comparison, it was concluded that the absolute configurations of the *cis*-dihydrodiol metabolites **4** and **7** (7*S*,8*R*), **5** and **8** (5*R*,6*S*) were similar to those found for *cis*-dihydrodiol metabolites of quinoline and acridine substrates.[8,12] Catalytic hydrogenation of 5,6-*cis*-dihydrodiol **5** (H_2, 5% Pd/C) gave 5,6-dihydroxy-5,6,7,8-tetrahydrodictamnine ($[\alpha]_D$ +15, MeOH, 95% yield). The latter compound was also formed by (i) catalytic hydrogenation (H_2, 5% Pd/C) of chloro-*cis*-dihydrodiol **8** to give the corresponding tetrahydrodiol ($[\alpha]_D$ −55, MeOH, 95% yield), (ii) protection as an acetonide ($[\alpha]_D$ +129, $CHCl_3$, 98% yield), (iii) substitution of the acetonide chlorine atom by a methoxy group ($[\alpha]_D$ +151, $CHCl_3$, 85% yield) and (iv) deprotection to give 5,6-dihydroxy-5,6,7,8-tetrahydrodictamnine ($[\alpha]_D$ +16, MeOH, 75% yield), thus confirming the identical (5*R*,6*S*) absolute configurations of both *cis*-dihydrodiols **5** and **8** by stereochemical correlation.

The small quantities of acyclic diols **6** and **9** obtained from furoquinolines **1a** and **1j**, were reacted with either (*R*) and (*S*)-MEPBA or (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA chloride) to form the corresponding diastereoisomeric esters. ^{1}H-NMR analysis of the MEPBA and diMTPA esters showed the enantiopurity values to be variable (75% ee, $[\alpha]_D$ +9, MeOH for **6** and 18% ee, $[\alpha]_D$ −5, MeOH for **9**).

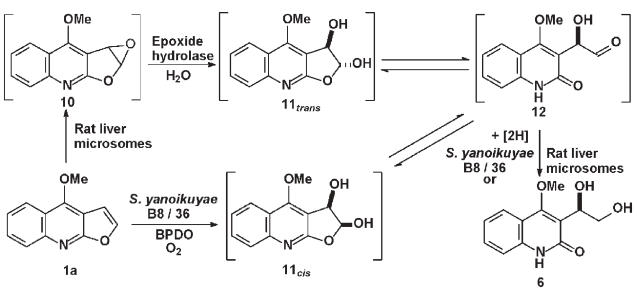


Scheme 2

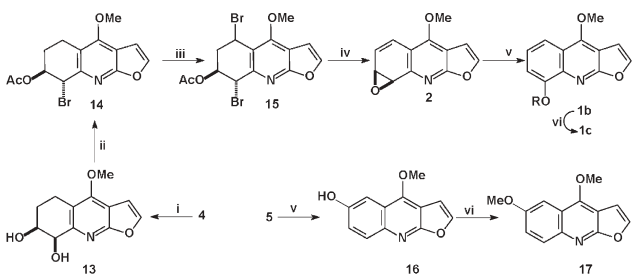
A tentative assignment of an (*R*) absolute configuration was made in each case.

The biotransformation of dictamnine **1a** using rat liver microsomal enzymes has also been reported to give the acyclic diol **6** as a bioproduct of unspecified stereochemistry (Scheme 3).[18] It was proposed, in the eukaryotic study, that the isolated 2-quinolone bioproduct **6** had resulted from the initial monooxygenase-catalysed epoxidation of dictamnine **1a** to yield the transient arene oxide **10**. The epoxide hydrolase-catalysed hydrolysis of arene oxide **10** to yield the *trans*-diol $\mathbf{11}_{trans}$, followed by spontaneous reversible ring opening to aldehyde **12**, and then enzyme-catalysed reduction could account for the formation of acyclic diol **6** (Scheme 3). Similarly the *cis*-dihydrodiol metabolite $\mathbf{11}_{cis}$, resulting from BPDO-catalysed oxidation of the furan ring in dictamnine **1a**, would also undergo ring opening to aldehyde **12** prior to being reduced enzymatically to diol **6** (Scheme 3). A similar metabolic sequence (furan → furan *cis*-diol ⇌ hydroxyaldehyde ⇌ furan *trans*-diol → acyclic diol) was observed during the dioxygenase-catalysed *cis*-dihydroxylation of benzofuran using *P. putida* UV4 to yield an equilibrating mixture of *cis*/*trans* diols and a derived acyclic diol [55% ee and of (*R*) configuration].[16]

The proposed intermediacy of arene oxide metabolites, during both plant biosynthesis (*e.g.* compound **2**, Scheme 1) and eukaryotic metabolism (*e.g.* compound **10**, Scheme 3) of dictamnine **1a**, prompted the synthesis of arene oxide **2** from the corresponding major *cis*-dihydrodiol **4** (Scheme 4). The synthetic sequence involved (i) catalytic hydrogenation of 7,8-*cis*-dihydrodiol **4** to give tetrahydrodiol **13**, ($[\alpha]_D$ −25, $CHCl_3$), (ii) bromoacetylation to produce the *trans*-bromoacetate **14** ($[\alpha]_D$ −38, $CHCl_3$), (iii) benzylic bromination to provide the unstable dibromoacetate **15** and (iv) dehydrobromination/cyclisation to yield the required enantiopure 7,8-arene oxide **2** ($[\alpha]_D$ +93, $CHCl_3$).



Scheme 3

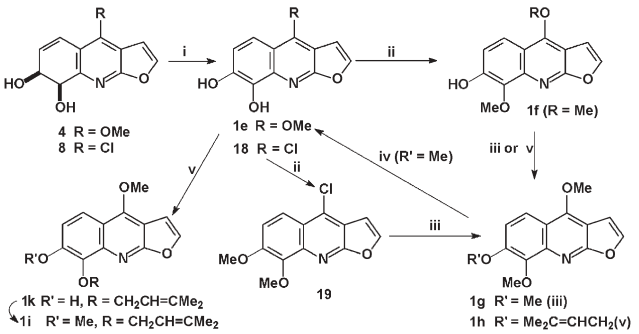


Scheme 4 *Reagents:* i H_2, Pd/C (96%); ii $AcOCMe_2COBr$, MeCN (90%); iii NBS, CCl_4 (68%); iv NaOMe (90%); v TFA (95–98%); vi CH_2N_2 (90%).

Thermal- or acid-catalysed isomerisation of arene oxide **2**, and dehydration (TFA) of *cis*-dihydrodiol **4** yielded the phenolic alkaloid 8-hydroxydictamnine (robustine, **1b**) exclusively, which was in turn methylated to give γ-fagarine **1c**. The alkaloids **1f–1i** could also be formed from dictamnine **1a** *via* further P-450-catalysed monohydroxylation of robustine **1b** or γ-fagarine **1c**. While no direct evidence was obtained for the formation of *trans*-dihydrodiol **3** from acid treatment of arene oxide **2**, the possibility of its involvement during the biosynthetic pathway in plants, where both P-450 monooxygenase and epoxide hydrolase are likely to be present, cannot be excluded. It is noteworthy that during comprehensive studies of the analogous 7,8-oxide of quinoline under carefully controlled conditions, while 8-hydroxyquinoline was the sole product under acid conditions (pH $<$ 7.0), the 7,8-*trans*-dihydrodiol derivative of quinoline was the only product under basic conditions (pH $>$ 12).[19] Acid-catalysed dehydration of 5,6-*cis*-dihydrodiol **5** also yielded the phenol derivative 6-hydroxydictamnine **16**, a possible eukaryotic metabolite of dictamnine **1a**.[18] Although 6-hydroxydictamnine **16** has not yet been isolated as a plant alkaloid, it is assumed to be an intermediate during the biosynthesis of pteleine **17** which was obtained after methylation of phenol **16** (Scheme 4).

A small sample of *cis*-dihydrodiol **4**, was used as a substrate with whole cells of the recombinant bacterial strain *Escherichia coli* nar B (a source of naphthalene *cis*-diol dehydrogenase, NDD),[20] and gave catechol **1e** but only in very poor yield (<5%). Surprisingly, the 7,8-*cis*-dihydrodiol of chlorofuroquinoline, **7**, proved to be a much better substrate for *E. coli* nar B; it formed catechol **18** in ~40% yield and this provided an indirect route to skimmianine **1g** (Scheme 5). Thus, catechol **18** was methylated (CH_2N_2) to give dimethoxy derivative **19** (95% yield) which allowed substitution of the chlorine atom by the methoxy group (NaOMe) and yielded skimmianine **1g** (20% yield). A further supply of catechol **1e** was then synthesised from skimmianine **1g** by selective demethylation using BBr_3 (85% yield). Partial methylation of catechol **1e** by reacting with CH_2N_2 (60 s) occurred mainly at the OH group on C-8 to give haplopine **1f** (55% yield); further methylation (CH_2N_2) yielded skimmianine **1g** (95% yield from catechol **1e**). Prenylation of haplopine **1f** with 1-chloro-3-methyl-but-2-ene in the presence of K_2CO_3 gave the alkaloid 7-isopentenyl-γ-fagarine **1h** (85% yield). The reverse sequence involving initial prenylation of catechol **1e** to yield phenol **1k** followed by methylation (CH_2N_2) gave the isomeric alkaloid isohaplopine 3,3′-dimethylallyl ether **1i** (63% yield from catechol **1e**).

In conclusion, the isolation of *cis*-dihydrodiol metabolites of an alkaloid, dictamnine **1a**, using dioxygenase enzymes, has been accomplished. Based on the above observations, the formation of arene *cis*-diol metabolites may be of considerable significance in the general context of bacterial biodegradation of plant alkaloids in the environment. *cis*-Dihydrodiol **4** proved to be a remarkably stable precursor of the corresponding arene oxide **2**. Both arene oxide **2** and *cis*-dihydrodiols **4**, **5**, and **7** yielded phenolic derivatives from which a range of furoquinoline alkaloids were synthesised *via* biomimetic routes.



Scheme 5 *Reagents:* i *E. coli* nar B; ii CH_2N_2; iii NaOMe; iv BBr_3; v $ClCH_2CH{=}CMe_2$, K_2CO_3.

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Notes and references

1 D. A. Rathbone, D. L. Lister and N. C. Bruce, Biotransformations of Alkaloids, in *The Alkaloids: Chemistry and Biology*, ed. G. A. Cordell, Academic Press, San Diego, 2002, vol. 58, 1.
2 W. R. Abraham and G. Spassov, *Heterocycles*, 2002, **56**, 711.
3 D. A. Rathbone and N. C. Bruce, *Curr. Opin. Microbiol.*, 2002, **5**, 274.
4 D. R. Boyd and G. N. Sheldrake, *Nat. Prod. Rep.*, 1998, **15**, 309.
5 T. Hudlicky, D. Gonzalez and D. T. Gibson, *Aldrichimica Acta*, 1999, **32**, 35.
6 D. R. Boyd, N. D. Sharma and C. C. R. Allen, *Curr. Opin. Biotechnol.*, 2001, **12**, 564.
7 R. A. Johnson, *Org. React.*, 2004, **63**, 117.
8 D. R. Boyd, N. D. Sharma, M. R. J. Dorrity, M. V. Hand, R. A. S. McMordie, J. F. Malone, H. P. Porter, H. Dalton, J. Chima and G. N. Sheldrake, *J. Chem. Soc., Perkin Trans 1*, 1993, 1065.
9 D. R. Boyd, N. D. Sharma, L. V. Modyanova, J. G. Carroll, J. F. Malone, C. C. R. Allen, J. T. G. Hamilton, D. T. Gibson, R. E. Parales and H. Dalton, *Can. J. Chem.*, 2002, **80**, 589.
10 S. K. Agarwal, D. R. Boyd, R. J. H. Davies, L. Hamilton, D. M. Jerina, J. J. McCullough and H. P. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1969.
11 S. K. Agarwal, D. R. Boyd, H. P. Porter, W. B. Jennings, S. J. Grossman and D. M. Jerina, *Tetrahedron Lett.*, 1986, **27**, 4253.
12 D. R. Boyd, N. D. Sharma, J. G. Carroll, C. C. R. Allen, D. A. Clarke and D. T. Gibson, *Chem. Commun.*, 1999, 1201.
13 D. R. Boyd, N. D. Sharma, F. Hempenstall, M. A. Kennedy, J. F. Malone, C. C. R. Allen, S. M. Resnick and D. T. Gibson, *J. Org. Chem.*, 1999, **64**, 4005.
14 M. F. Grundon, D. M. Harrison and C. G. Sypropoulos, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2181.
15 J. P. Michael, *Nat. Prod. Rep.*, 2003, **20**, 476.
16 D. R. Boyd, N. D. Sharma, I. N. Brannigan, S. A. Haughey, J. F. Malone, D. A. Clarke and H. Dalton, *Chem. Commun.*, 1996, 2361.
17 M. F. Grundon and N. J. McCorkindale, *J. Chem. Soc.*, 1957, 2177.
18 B. Klier and O. Schimmer, *Mutagenesis*, 1999, **14**, 181.
19 D. R. Bushman, J. M. Sayer, D. R. Boyd and D. M. Jerina, *J. Am. Chem. Soc.*, 1989, **111**, 2688.
20 D. R. Boyd, N. D. Sharma, V. Ljubez, B. E. Byrne, S. D. Shepherd, C. C. R. Allen, L. A. Kulakov, M. J. Larkin and H. Dalton, *Chem. Commun.*, 2002, 1914.