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Biphenyl dioxygenase-catalysed cis-dihydroxylation of tricyclic azaarenes: chemoenzymatic synthesis of arene oxide metabolites and furoquinoline alkaloids†

Derek R. Boyd,*a Narain D. Sharma,a Jonathan G. Carroll,a Pui L. Loke,a Colin R. O’Dowda and Christopher C. R. Allenb

Biotransformation of acridine, dictamine and 4-chlorofuro[2,3-b]quinoline, using whole cells of Sphingomonas yanoikuyae B8/36, yielded five enantiopure cyclic cis-dihydrodiols, from biphenyl dioxygenase-catalysed dihydroxylation of the carbocyclic rings. cis-Dihydroxylation of the furan ring in dictamine and 4-chlorofuro[2,3-b]quinoline, followed by ring opening and reduction, yielded two exocyclic diols. The structures and absolute configurations of metabolites have been determined by spectroscopy and stereochemical correlation methods. Enantiopure arene oxide metabolites of acridine and dictamine have been synthesised, from the corresponding cis-dihydrodiols. The achiral furoquinoline alkaloids robustine, γ-fagarine, haplopine, isohaplopine-3,3′-dimethylallyl ether and pteleine have been obtained, from either cis-dihydrodiol, catechol or arene oxide metabolites of dictamine.

Further metabolism of the benzofuran 2,3-cis-diols involved spontaneous ring opening and enzyme-catalysed carbonyl reduction to give exocyclic phenolic diol products (Scheme 1b).2e

Dihydroxylation of the 3,4-bond in the electron-deficient pyridine ring of the quinoline substrates was found to yield only minor metabolites in comparison with its carbocyclic 5,6- and 7,8-bonds. However, when benzo[b]thiophene and benzo[b]furan substrates, containing electron-rich heterocyclic rings, were used as substrates, dihydroxylation of the 2,3-bond revealed a more favourable metabolic route (Schemes 1a and 1b).

The steric dimensions of the active site in TDO, expressed in P. putida UV4, limited the acceptable size of substrates to mono- or bi-cyclic arenes (Schemes 1a and 1b). However, the biphenyl dioxygenase (BPDO) enzyme, present in the B8/36 mutant strain of Sphingomonas yanoikuyae, has a larger active site and was able to accept tri-, and tetra-cyclic arenes (e.g. benzof[b]quinoline, benzo[b]quinoine, phenanthridine,3a benzo[c]phenanthridine,3b Scheme 2) as substrates. It is noteworthy that in these examples a marked regioselective preference for cis-dihydroxylation was found at a bond within the bay-region.

As part of an earlier programme1b,c to investigate the ability of BPDO to catalyse the cis-tetrahydroxylation of larger polycyclic aromatic rings, it was found that bis-cis-dihydrodiols were formed as further metabolites of the initial cis-dihydrodiols derived from larger carbocyclic (e.g. anthracene, chrysene, benzo[a]anthracene) and heterocyclic (e.g. acridine, phenazine, benzof[b]naphth[a]2,1-d-thiophene) substrates. The
similarity in size and shape of the linear tricyclic arenes, anthracene and acridine, and their acceptability as substrates for the BPDO enzyme, prompted this comparative biotransformation study of acridine with furo[2,3-b]quinoline substrates. Following our earlier reports on the isolation and synthesis of quinoline alkaloids, from plants of the Rutaceae family, e.g. Choisya ternata, and Skimmia japonica, linear furoquinolines (4-chlorofuro[2,3-b]quinoline and dictamnine) were briefly examined as potential substrates, using whole cells of S. yanoikuyae B8/36 expressing BPDO enzyme.

In our preliminary studies of the biotransformations of acridine and dictamnine, using S. yanoikuyae B8/36, we had reported the presence of the corresponding cis-dihydrodiol metabolites. This comprehensive study now provides full structural and stereochemical characterization of all new bacterial metabolites and shows how they can be utilized in
the chemoenzymatic synthesis of a wider range of animal and plant metabolites, e.g. arene oxides and furoquinoline alkaloids.

Results and discussion

(i) Biotransformation of acridine 1

The mammalian metabolism and mutagenicity of acridine 1 have been studied over many years using dog, rabbit and rat liver cells. The major metabolites were found to be 2-hydroxyacridine, 9-acridone, 2-hydroxy-9-acridone and trans-1,2-dihydroxy-1,2-dihydroacridine 3 (Scheme 3). It is probable that trans-dihydrodiol 3 and 2-hydroxyacridine were derived from the undetected acridine 1,2-oxide 2. The identification of these mammalian metabolites of acridine 1, which could be accounted for, mainly, by monoxygenase-catalysed oxidation, prompted the preliminary study and current study of its dioxygenase-catalysed metabolism.

Biotransformation of acridine 1, using S. yanoikuyae B8/36 under similar conditions to those used for other azaarene substrates, followed by extraction (EtOAc) and column chromatography, yielded cis-dihydrodiol 4 ([α]D +71) in acceptable yield (42%). The structure of cis-diol 4 was determined by NMR, MS and elemental microanalysis. The enantiomeric excess value (ee) was estimated as >98% by reaction with (R)-(+)- and (S)-(−)-2-(1-methoxyethyl)phenylboronic acid (MPBA) and 1H-NMR analysis of the resulting boronates.

The absolute configuration of cis-dihydrodiol 4 was initially assigned as (1R,2S), based on the well established 1H-NMR pattern previously observed for MPBA derivatives from other polycyclic arene cis-dihydrodiol metabolites (e.g. from naphthalene, anthracene, phenanthrene and their aza-analogues). A large chemical shift value (δH 3.18) for the MeO group protons, using the (R)-(+)-MPBA compared with the value obtained using (S)-(−)-MPBA (δH 3.11), was again assumed to be consistent with a benzylic (R) and an allylic (S) configuration for cis-dihydrodiol 4. The reliability of the MPBA method for the linear azaarene cis-dihydrodiol 4 was confirmed by an unequivocal stereochi- mical correlation sequence similar to that used for other polycyclic arene cis-dihydrodiols (Scheme 4). The sequence involved a catalytic hydrogenation (H2,Pd/C) to yield cis-tetrahydrodiol 5 followed by bis-acetylation (Ac2O, pyridine) to give cis-diacetate 6. In the final step, an oxidative ring opening reaction (RuO2/NaO4) gave a mixture of dicarboxylic acid products (7/8). It was assumed that the bicyclic dicarboxylic acid 7 was formed initially and then a part of it degraded to acyclic dicarboxylic acid 8 via a further oxidative ring opening reaction. The mixture of dicarboxylic acids 7 and 8 was methylated (CH3N2) to yield dimethylesters 9 and 10 which were separated by column chromatography. The minor component, dimethyl(2,3-diacetoxy)adipate 10 ([α]D −14) was of established configuration and thus the (1R,2S) configuration was unequivocally assigned to (+)-cis-dihydrodiol 4.

It has been proposed that the mutagenicity/carcinogenicity associated with some larger PAHs and APAHs results from: (i) a monoxygenase-catalysed epoxidation of a carbocyclic ring to yield an arene oxide (cf. compound 2), (ii) an epoxide hydrolyse-catalysed hydrolysis of the arene oxide to yield a trans-dihydrodiol (cf. compound 3), (iii) a monoxygenase-catalysed epoxidation of the alkene bond in the trans-dihydrodiol to yield diastereoismeric trans-diol epoxides and (iv) nucleophilic attack of DNA on the epoxide ring within a bay region to yield a covalent adduct. Although the corresponding acridine trans-diol epoxides from metabolite 3 could, in principle, also be mutagens, their synthesis and mutagenicity has not yet been reported.

(ii) Biotransformation of furoquinolines 11–13

In common with acridine 1, the mammalian metabolism and mutagenicity of dictamine 12 and other furoquinoline alkaloids, e.g. γ-fagarine, had been reported earlier. In a more recent study, from these laboratories, the furoquinoline
alkaloid skimmianine 13 was found to be the major compound present in *C. ternata*, and was thus available as a potential substrate for the current biotransformation studies. However, dictamnine 12, another furoquinoline alkaloid required as a potential substrate, was not isolated from *C. ternata*. Thus, a five-step chemical synthesis of dictamnine 12 was carried out, starting from aniline and using the literature procedure which involved 4-chlorofuroquinoline 11 as precursor. Furoquinolines 11–13 were thus also available as possible substrates for BPDO.

Furoquinolines 11–13 were added, individually, as substrates to *S. yanoikuyae* B8/36, under the conditions used previously for the successful biotransformation of acridine 1. The results, shown in Scheme 5, indicate that while 4-chlorofuro[2,3-b]quinoline 11 and dictamnine 12 each yielded a mixture of two *cis*-dihydrodiol products 14–17, resulting from BPDO-catalysed *cis*-dihydroxylation of the 5,6 and 7,8 bonds of the carbocyclic ring, skimmianine 13 was not an acceptable substrate. The mixtures of metabolites 14/15 and 16/17 were separated into individual *cis*-dihydrodiols by PLC purification. The structures of diol metabolites 14–17 were established by analyses of NMR and MS data while the *ee* values (>98%) and absolute configurations were again determined by formation of the corresponding diastereomeric MPBA esters and their analysis by *1H*-NMR spectroscopy. As found for the acridine *cis*-dihydrodiol 4, the larger chemical shift values (δH) for the exocyclic MeO group of *cis*-dihydrodiols 14–17, (δH 3.23–3.25) using the (*R*)-(+)-MPBA compared with (S)-(−)-MPBA 14–17, (δH 3.19–3.20) were consistent with benzylic (*R*) and allylic (S) configurations in each case.

While *cis*-dihydroxylation had occurred exclusively at the 1,2 bond of acridine 1, similar regioselectivity for the equivalent

Scheme 4

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Reagents: i S. yanoikuyae B8/36; ii H2/Pd/C, MeOH; iii Ac2O/pyridine; iv RuO2/NaIO4; v CH3N2
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Scheme 5

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Reagents and conditions: i S. yanoikuyae B8 /36; ii liver microsomes
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(5,6) bond in furoquinolines 11 and 12 was not found. A modest preference (38% yield) was observed for BPDO-catalysed cis-dihydroxylation at the 7,8-bond to form cis-diol 15 compared with the 5,6-bond (10% yield) to give cis-diol 14, when 4-chlorofuro[2,3-b]quinoline 11 was the substrate. A stronger preference for oxidation of the 7,8-bond was found with dictamine 12 as the substrate, which resulted in cis-diol 17 being the major metabolite (20–30% yield) relative to cis-diol 16 (1–3% yield). The combined isolated yields (21–33%) of dictamine cis-dihydrodiols (16 and 17) were slightly lower than 4-chlorofuroquinoline cis-dihydrodiols (14 and 15, 48%); no cis-dihydrodiol metabolites were detected from skimmianine 13 as substrate. These observations suggest that the presence of substituents at C-4, C-7 and C-8 and the overall steric requirements of the substrate within the active site of the BPDO enzyme are important factors. Based on isolated yields, it appears that cis-dihydroxylation occurred preferentially at the less sterically hindered 7,8-bond and that the best yields resulted from the use of the smaller substrates (11 and 12). As the largest substrate, skimmianine 13, did not yield cis-diol metabolites, this is consistent with its failure to be accommodated within the BPDO active site. However, alternative factors, including aqueous solubility, toxicity and further metabolism, could influence the isolated yields of bioproducts.

The most polar metabolites, formed from 4-chlorofuro[2,3-b]quinoline 11 and dictamine 12, were found to be exocyclic diols (compounds 20 and 23) but were isolated in very low yields (1–2%, Scheme 5). While the structures of optically active diols 20 and 23 were assigned by NMR and MS spectroscopic analysis, their ee values and absolute configurations were not determined. It was assumed, that the exocyclic diols 20 and 23, resulted from: (a) BPDO-catalysed cis-dihydroxylation at the 2,3-bond to give transient intermediates 18 and 21, (b) reversible ring opening of these hemiacetals (cf. mutarotation) to yield the undetected aldehydes 19 and 22, (c) epimerization, following reversible ring closure, to yield a mixture of the corresponding cis- and trans-dihydrodiols, and (d) carbonyl reductase-catalysed (CRED) reduction of the aldehyde group in intermediates 19 and 22. A similar sequence of TDO-catalysed cis-dihydroxylation of the furan ring of benzo[b]furans, spontaneous equilibration via a reversible ring opening process to yield the corresponding phenolic aldehydes and CRED-catalysed reduction of the resulting aldehyde group was earlier assumed to account for the isolation of the exocyclic diols shown in Scheme 1 (b).20

The origin of mutagenicity associated with dictamine 13 has not yet been rigorously established.24–26 However, it has been proposed that, in common with other naturally occurring mutagenic furans, e.g. aflatoxin B1 and 8-methoxypsoralen, the corresponding transient furan epoxides,27 formed as initial mammalian metabolites via monooxygenase-catalysed epoxidation, e.g. arene oxide 24 (Scheme 5) may be responsible for their mutagenicity. It has been proposed that the mutagenicity results from the ability of furan epoxides to form covalently bound adducts following nucleophilic ring-opening reactions with DNA.27–29

(iii) Application of acridine cis-dihydrodiol 4 in the synthesis of arene oxide 2
As part of an earlier study of the mammalian metabolism and mutagenicity/carcinogenicity of PAHs and APAHs, (1R,2S)-arene oxide 2 was obtained via an eight stage chemical synthesis, involving a chemical resolution of MTPA esters, with an overall yield of ca. 13%.9 Alkaline hydrolysis (KOH, t-BuOH) of (1R,2S)-arene oxide 2 gave the mammalian metabolite (1R,2R)-trans-1,2-dihydroacridine-1,2-diol 3.9 In the current study, the possibility of a much shorter synthesis of acridine 1,2-oxide 2 was examined (Scheme 6), using the readily available bacterial metabolite, (1R,2S)-cis-1,2-dihydroacridine-1,2-diol 4. Treatment of diol 4 with 1-bromocarbonyl-1-methylmethanethiol, in acetonitrile solution, gave a mixture of bromoacetates 25/26 whose structures were determined from 1H-NMR and MS data. Due to their instability, during attempted separation, the mixture of bromoacetates 25 and 26 in EtO solution was reacted directly with NaOMe. Using this two step method, the relatively stable (1R,2S)-arene oxide 2 was synthesised from cis-dihydrodiol 4 in 66% yield. Despite the stability of arene oxide 2, it was not detected during mammalian metabolism, probably due to its further metabolism via a rapid epoxide hydrolase-catalysed conversion to the corresponding trans-dihydrodiol 3.30,31 A preliminary study26 later showed that when the stable acridine cis-dihydrodiol 4 was used as a substrate for S. yanoikuyae B8/36, it was also further metabolised and formed a bis-cis-dihydrodiol bioproduct.

(iv) Application of dictamine cis-dihydrodiols 16 and 17 as precursors in the synthesis of furoquinoline alkaloids
The potential of dictamine cis-dihydrodiol metabolites 16 and 17 in the biomimetic synthesis of furoquinoline alkaloids, including the proposed arene oxide intermediate 27, was of biosynthetic interest (Schemes 7 and 8). Possible biosynthetic pathways to furoquinoline alkaloids occurring in Rutaceaeaeous plants, e.g. Skimmia japonica and Choisya ternata, have been studied using 13C-labelled precursors.10c These labelling studies showed that enzyme-catalysed hydroxylation could
occur on the benzene ring of dictamnine 12 to yield a wider range of furoquinoline alkaloids e.g, skimmianine 13 and possibly also robustine 30 and γ-fagarine 31 (Scheme 7). It was proposed that skimmianine 13 could be formed via a monoxygenase-catalysed epoxidation of dictamnine 12, to yield the transient arene oxide 27, followed by epoxide hydrolase-catalysed hydrolysis to yield trans-dihydrodiol 28. The possibility of an alternative dioxygenase-catalysed cis-dihydroxylation of dictamnine 12 to yield cis-dihydrodiol 17 was also discussed. The enzyme-catalysed oxidations of trans- and cis-dihydrodiols, to yield catechols followed by O-methylation, are well established metabolic steps and, when allied to the earlier labelling studies, either type of enzymatic oxidation could account for the formation of catechol 29 and skimmianine 13. To date, none of the potential biosynthetic intermediates 17, 27–29 have been detected by the labelling studies using Choisya ternata or found among the furoquinoline alkaloids recently isolated from this or other plants in the Rutaceae family.

As expected, the B8/36 mutant strain of S. yanoikuyae did not yield catechol metabolites e.g. compound 29 from dictamnine 12 (Scheme 7). The biphenyl cis-diol dehydrogenase (DD) activity required to catalyse the dehydrogenation of cis-dihydrodiols to yield catechols, was blocked in the B8/36 strain. However, when the wild type strain of S. yanoikuyae (B1), expressing both BPDO and DD enzymes, was used with dictamnine 12, the only metabolite identified and isolated was cis-dihydrodiol 16, albeit in low yield (8%). This observation is consistent with both cis-dihydrodiols 16 and 17 being formed but the major metabolite (17) being further metabolized preferentially.

The E. coli narB recombinant strain, expressing naphthalene DD, has been used successfully to produce catechols in good yields from the corresponding monocyclic arene cis-dihydrodiols. Using E. coli narB and cis-dihydrodiol 15 as substrate, catechol 35 was detected by 1H-NMR spectroscopy but in low yield. Surprisingly, the required catechol metabolite 29, derived from dictamnine cis-dihydrodiol 17, could not be obtained using this method. However, it was possible to obtain catechol 29 in good yield (85%) using boron tribromide for the selective O-demethylation of skimmianine 13, isolated earlier from Choisya ternata (Scheme 8).

Convincing evidence of monoxygenase-catalysed epoxidation of dimethylallyl groups, and hydrolysis of the resulting epoxides to yield vicinal diols, is available from biosynthetic studies of quinoline alkaloids from plants of the Rutaceae family. Furthermore, monoxygenase-catalysed epoxidation of azaarenes, to yield the corresponding arene oxides, using liver microsomes with inhibition of epoxide hydrolase activity, provides a precedent for the formation of the elusive dictamnine arene oxide 27 and trans-dihydrodiol 28 metabolites. While the dioxygenase-catalysed cis-dihydroxylation of polycyclic azaarenes in bacteria, e.g. S. yanoikuyae B8/36, is well established (Schemes 1, 2 and 4), there appears to be little evidence of this pathway occurring in plants. Consequently, the monoxygenase-catalysed epoxidation sequence, shown in Scheme 7, is currently favoured over the dioxygenase pathway.

Scheme 7

Reagents and conditions: i NaOMe/MeOH; ii Skimmia japonica or Choisya ternata

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as an initial step, can readily account for the formation of both monophenols (e.g. robustine 30), catechols (e.g. 7,8-dihydroxydictammine 29), and their methylated derivatives (e.g. γ-fagarine 31 and skimmianine 13).

Our attempt to synthesise the proposed dictamnine arene oxide metabolite 27 from cis-dihydrodiol 17, via a two-step process similar to that used earlier for acridine arene oxide 2 (Scheme 6), was unsuccessful. This was due to compound 17 being less stable under the reaction conditions and more readily dehydrated under acid conditions to yield phenols (e.g. robustine 30). An alternative approach (Scheme 8) was adopted involving the catalytic hydrogenation (H2, Pd–C) of compound 17 to yield the stable cis-tetrahydrodiol 32 (76% yield). Treatment of diol 32 with 1-bromocarbonyl-1-methylethyl acetate gave trans-bromoacetate 33 in good yield (90%). Benzylic bromination of bromoacetate 33 (NBS, CCl4) gave an inseparable mixture of diastereoisomers 34 which was immediately treated with sodium methoxide in THF, to yield the proposed dictamnine arene oxide metabolite 27 (60% yield from compound 33). Initial attempts to purify this elusive arene oxide by PLC resulted in its aromatization to give the furoquinoline alkaloid robustine 30. Purification of (7S,8R)-dictammine oxide 27 was achieved by careful crystallization. A sample of oxide 27 was found to survive in CDCl3 solution without decomposition, at ambient temperature over a 24 h period.

In the final phase of this study, cis-dihydrodiols 16 and 17, arene oxide 27 and catechol 29, as confirmed or proposed metabolites of dictamnine 12, were utilized as synthetic precursors of other furoquinoline alkaloids, using biomimetic methods (Scheme 8). While robustine 30 was obtained by isomerisation of arene oxide 27 under acidic conditions, the acid-catalysed dehydration of cis-dihydrodiol 17 was the preferred route. Methylation of robustine 30 with diazomethane yielded the alkaloid γ-fagarine 31. Under similar conditions, methylation of catechol 29 occurred mainly at C-8, to yield the alkaloid haplopine 36. Treatment of catechol 29 in acetone with 1-chloro-3-methylbut-2-ene in presence of K2CO3 resulted in the preferential prenylation at C-8 to yield phenol 37, which on methylation yielded the alkaloid isohaplopine-3,3′-dimethylallylether 38. Acid-catalysed dehydration of cis-dihydrodiol 16, to form phenol 39, followed by methylation, yielded the furoquinoline alkaloid pteleine 40.

**Conclusion**

The bacterial cis-dihydroxylation of acridine 1 and furoquinolines 11 and 12, catalysed by BPDO, yielded five carbocyclic cis-dihydrodiols, [4, 14–17] and two exocyclic diols (20 and 23), derived from the transient heterocyclic diols 18 and 21. The structures and absolute configurations of most of the isolated metabolites were established by spectroscopic analysis and
stereochemical correlation methods. cis-Dihydriodiol 4 and 17 were used in the synthesis of the corresponding arene oxides, 2 and 27, which had been proposed as intermediates in mammalian metabolism of acridine 1 and dictamine 12, cis-Dihydriodiol 16 and 17 and arene oxide 27, as derivatives of dictamine 12, have been used in the synthesis of a wide range of furoquinoline alkaloids including robustine 30, γ-fagarine 31, haplopine 36, isohaplopine-3,3’-dimethylallylether 38 and pteleone 40.

Experimental

1H and 13C NMR spectra were recorded on Bruker Avance 400, DPX-300 and DRX-500 instruments. Chemical shifts (δ) are reported in ppm relative to SiMe4 and coupling constants (J) are given in Hz. Mass spectra were run at 70 eV, on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate molecular weights were determined by peak matching techniques, with perfluorokerosene as the standard. CD spectra were recorded in spectroscopic grade acetonitrile using the matching method, with perfluorokerosene as the standard. CD spectra were recorded in spectroscopic grade acetonitrile using the matching method, with perfluorokerosene as the standard.

To a solution of 4-chlorofuro[2,3-b]quinoline in dry methanol (120 cm³) was added sodium iodide (3.0 mmol) and the aqueous mixture extracted with chloroform (3 cm³). The combined ether extract was dried (Na2SO4) and the filtrate evaporated under reduced pressure to yield (1R,2S)-1,2,3,4-Tetrahydrodioxypiperazine 4. cis-Dihydriodiol metabolite 4 (0.5 g, 23.3 mmol) was catalytically hydrogenated (H2, 10% Pd/C, 50 mg) and the aqueous mixture extracted with Et2O (3 cm³). The combined ether extract was dried (Na2SO4) and the residue treated with water and the aqueous mixture extracted with EtOAc (3 cm³). Merck Kieselgel type 60F254 analytical plates were employed for TLC. Authentic samples of 4-chlorofuro[2,3-b]quinoline and skimmianine 13 were available from earlier studies. 4-Chlorofuro[2,3-b]quinoline diacetate 6.

Sphingomonas yanoikuyae B8/36 was grown on minimal salts medium with 0.5% sodium succinate and 0.5 g L⁻¹ of yeast extract. Biophenyl dioxygenase (BPDO) was induced, during the exponential phase of growth, by the addition of m-xylene (1 cm³ L⁻¹) every 0.5 h for 7 h. Substrate concentration was 0.5 mg cm⁻³.

Synthesis of dictamine 12

To a solution of 4-chlorofuro[2,3-b]quinoline 11 (3 g, 14.7 mmol) in dry methanol (120 cm³) was added sodium methoxide (4.05 g, 75 mmol) and the mixture refluxed (2 h) under nitrogen. After removal of methanol from the reaction mixture, under reduced pressure, the brown residue obtained was treated with ice cold water and the aqueous mixture extracted with chloroform (3 × 100 cm³). The combined organic extract was dried (Na2SO4), concentrated under reduced pressure, and the crude product purified by flash chromatography (1 : 1, EtOAc : hexane) to afford dictamine 12 as a yellow solid (1.65 g, 56%); δH (1 H, m, OH), 4.35 (1 H, m, OH); 4.42 (1 H, dd, J12, 4.7, J12, 4.8, 2.4-H), 4.86 (1 H, d, J12, 4.7, 1-H), 5.64 (1 H, d, J12, 4.8, 10.1, 3-H), 6.77 (1 H, d, J12, 10.1, 4-H), 7.53 (1 H, dd, J12, 8.1, J6, 6.9, 7.4-H), 7.18 (1 H, dd, J6, 6.9, 8.5, 6.5-H), 7.90 (1 H, d, J6, 6.9, 8.5, 5-H), 8.28 (1 H, s, 9-H); δC (125 MHz, CDCl3), 66.6, 70.2, 126.3, 127.7, 127.9, 129.0, 129.1, 130.8, 131.2, 132.3, 136.3, 147.1, 153.0; m/z (EI): 213 (M⁺, 56%), 195 (23), 184 (100); ν 3369 cm⁻¹ (-OH); ECD: [α]D +15 (nm 311 (Δ Δ 0.306), 257 (Δ Δ 6.444), 243 (Δ Δ 6.241), 215 (Δ Δ 6.467), 199.40 (Δ Δ 0.573).

(1R,2S)-1,2,3,4-Tetrahydrodioxypiperazine 5.

cis-Dihydriodiol metabolite 5 (0.18 g, 0.84 mmol) was acetylated with Ac2O (1.5 cm³) in dry pyridine (0.7 cm³) solution by stirring the reaction mixture for 4 days at ambient temperature (10 °C). The catalyst was filtered off and the filtrate evaporated under reduced pressure to yield (1R,2S)-1,2,3,4-tetrahydroacridine-1,2-diol 5 as a semi-solid (0.420 g, 83%); [α]D +61 (c 1.00, MeOH); (Found: M⁺ 215.0937. C13H11NO2 requires 215.0946); δH (500 MHz, CDCl3), 2.12 (1 H, m, 3a-H), 2.32 (1 H, m, 3b-H), 3.11 (1 H, m, 4a-H), 3.36 (1 H, m, 4b-H), 4.28 (1 H, m, 5a-H, 2.35-2.55), 4.93 (1 H, d, J1, 3.5, 1.5-H), 7.19 (1 H, dd, J6, 8.0, J7, 7.1, 7-H), 7.86 (1 H, dd, J6, 7.1, J8, 8.4, 6-H), 7.79 (1 H, d, J6, 8.0, 8-H), 8.00 (1 H, d, J6, 8.4, 5-H), 8.30 (1 H, s, 9-H); δC (125 MHz, CDCl3), 26.3, 29.5, 68.9, 70.2, 126.0, 127.2, 127.6, 128.4, 129.8, 137.0, 157.4, 162.6, 171.9; m/z (EI): 215 (M⁺, 71%), 198 (16), 186 (74), 168 (75), 143 (100); ν 3435 cm⁻¹ (OH).

(1R,2S)-1,2-Diacetoxy-1,2,3,4-tetrahydroacridine 6.

cis-Tetrahydrodioxypiperazine 5 (0.18 g, 0.84 mmol) was acetylated with Ac2O (1.5 cm³) in dry pyridine (0.7 cm³) solution by stirring the mixture overnight at room temperature. Excess of pyridine was removed under high vacuum, the residue treated with water (10 cm³), and the aqueous mixture extracted with Et2O (3 × 15 cm³). The combined ether extract was dried (Na2SO4) and concentrated to yield (1R,2S)-1,2-diacetoxy-1,2,3,4-tetrahydroacridine 6 as a white crystalline solid (0.22 g, 88%); m.p. 84–85 °C (EtOH/hexane); [α]D +57 (c 0.85, MeOH); (Found: M⁺ 299.1150. C14H11NO2 requires 299.1158); δH (500 MHz, CDCl3), 2.05 (3 H, s, Me), 7.08 (1 H, d, J6, 8.2, 2.8, 3-H), 7.45 (1 H, dd, J6, 9.1, J5, 7.6, 7.0-H), 7.62 (1 H, d, J6, 2.8, 2.8, 7-H), 7.69 (1 H, dd, J7, 8.4, J6, 7.0, J 7.0, 7-H), 8.0 (1 H, d, J6, 8.4, 8-H), 8.26 (1 H, d, J5, 9.1, 5-H).

(a) Biotransformation of acridine 1

(1R,2S)-1,2-Dihydroacridine-1,2-diol 4. Biotransformation of acridine 1 (15 g) using S. yanoikuyae B8/36 followed by extraction with ETOAc yielded (1R,2S)-1,2-dihydroacridine-1,2-

Oxidative degradation of diacetate 6. Ruthenium(ii) oxide hydrate (2 mg) was added to a biphasic mixture of the diacetate 6 (0.1 g, 0.33 mmol) and NaNO3 (3.4 g, 16 mmol) in a mixture of CCl4 (2 cm³), MeCN (2 cm³) and water (3 cm³). After stirring the reaction mixture for 4 days at ambient tempera-

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turation, dilute HCl (20 cm³, 1.5 M) was added and the reaction mixture extracted with EtOAc (3 × 30 cm³). The combined extract was washed with water, dried (Na₂SO₄), and concentrated under reduced pressure to yield a crude mixture of two dicarboxylic acids. The mixture was dissolved in MeOH (5 cm³) and treated with 0.5 h with excess of freshly prepared ethereal diazomethane solution. The solvents were evaporated, in a fume hood under a stream of nitrogen, and the crude methyl esters separated by column chromatography (25% → 75% EtO/O/hexane) to yield dimethyl [5R,6S]-5,6-diacetoxy-5,6,7,8-tetrahydro-2,3-quinolinedicarboxylate 9, the more polar major compound (21 mg, 17%), and (2S,3S)-dimethyl-(2,3-diacetoxy)-adipate 10 (9 mg, 9.3%) as the less polar minor compound.

**Dimethyl [5R,6S]-5,6-diacetoxy-5,6,7,8-tetrahydro-2,3-quinolinedicarboxylate 9.** White crystalline solid; m.p. 115–117 ºC (from CHCl₃); [α]D0 = −53 (c 0.66, MeOH); (Found: C, 55.7; H, 5.1; N, 3.9. C₁₂H₁₃NO₄ requires C, 55.9; H, 5.2; N, 3.8%). (Found: M⁺ 365.1105. C₁₂H₁₃NO₄ requires 365.1111.) δH (500 MHz, CDCl₃) 2.04 (3 H, s, OCOME), 2.15 (3 H, s, OCOME), 2.16 (1 H, m, J₆,₇ 3.1, 7a-H), 2.35 (1 H, m, 7b-H), 3.11 (1 H, m, 8a-H), 3.25 (1 H, m, 8b-H), 3.92 (3 H, s, COMe), 3.99 (3 H, s, COMe), 5.42 (1 H, m, J₅,₆ 3.4, J₆,₇ 3.1, 6-H), 6.13 (1 H, d, J₆,₇ 4.5, 5-H), 8.11 (1 H, s, 4-H); δC (125 MHz, CDCl₃) 58.8, 64.5, 71.1, 90.4, 116.3, 119.7, 123.4, 123.5, 131.5, 137.7, 163.7, 166.2; m/z (EI): 235 (M⁺, 4%), 204 (20), 122 (83), 105 (100), 77 (80), 51 (54), 43 (71).

**[c]** Biotransformation of dictamine 12

A small scale biotransformation of dictamine 12 (60 mg), using *S. yanoikuyae* B8/36, followed by extraction with EtOAc yielded a mixture of two dihydrodiol metabolites, [5R,6S]-4-methoxy-5,6-dihydrofuro[2,3-b]quinoline-5,6-diol 16 and [7S,8R]-4-methoxy-7,8-dihydrofuro[2,3-b]quinoline-7,8-diol 17. White solid (41 mg, 29%); m.p. 185–187 ºC (MeOH/CHCl₃); Rf 0.3 (7% MeOH/CHCl₃); [α]D +94 (c 0.78, MeOH); (Found: M⁺ 233.0689. C₁₂H₁₁NO₄ requires 233.0688); δH (500 MHz, CDCl₃) 4.28 (3 H, s, OMe), 4.45 (1 H, dd, J₅,₆ 5.0, J₆,₇ 5.0, 7-H), 4.78 (1 H, d, J₆,₇ 5.0, 8-H), 6.17 (1 H, dd, J₆,₇ 9.8, J₅,₆ 5.0, 6-H), 6.98 (1 H, d, J₉,₈ 2.5, 3-H), 7.02 (1 H, d, J₈,₉ 9.8, 5-H), 7.57 (1 H, d, J₉,₉ 2.5, 2-H); δC (125 MHz, CDCl₃) 58.8, 64.5, 71.1, 105.5, 106.0, 111.7, 123.8, 124.2, 142.2, 151.7, 156.7, 163.1; m/z (EI): 233 (M⁺, 61%), 215 (9), 204 (100); ECD: 27 nm 275.60 (Δ = −0.425), 246.10 (Δ = −2.722), 205.90 (Δ = −0.272).

**[3S,2S]-Dihydroxyethyl]-1,2-dihydro-2-quinolone 11**

A small-scale biotransformation on 4-chlorofuro[2,3-b]quinoline 11 (0.8 g, 3.93 mmol), using *S. yanoikuyae* B8/36, followed by concentration of the aqueous portion under reduced pressure and extraction of the concentrate by ethyl acetate yielded a mixture of products. PLC purification of the mixture (6% MeOH/CHCl₃) gave ([7S,8R]-4-chloro-7,8-dihydrofuro[2,3-b]quinoline-7,8-diol 15 (0.35 g, 38%); Rf 0.6 (6% MeOH/CHCl₃), [5R,6S]-4-chloro-5,6-dihydrofuro[2,3-b]quinoline-5,6-diol 14 (93 mg, 10%); Rf 0.5 (6% MeOH/CHCl₃) and 4-chloro-3-[1,2-dihydroxyethyl]-1,2-dihydro-2-quinolone 20 (23 mg, 25%); Rf 0.4 (6% MeOH/CHCl₃).
20. Brown solid (23 mg, 2%; m.p. 184–186 °C (decomp.); \( \delta_{1}^{1} \) 0.25 (25% EtOAc/hexane); \[ \delta_{1}^{1} \) 136–138 °C (CHCl3/hexane) (lit. < 136–138 °C); \[ \delta_{2}^{1} \) 20.0 (0.49, CHCl3) (lit. < 22, CHCl3); \[ \delta_{2}^{1} \) 195.0684); \[ \delta_{3}^{1} \) 500 MHz, CDCl3) 4.08 (1 H, dd, J2, 3.8, 1.8, 2, 3, 2.57, 3-H), 7.20 (1 H, dd, J3, 2.6, 3-H), 7.75 (1 H, d, J2, 3.2, 2.6, 3-H); \[ \delta_{4}^{1} \) (125 MHz, CDCl3) 18.3, 25.0, 58.6, 66.74, 70.8, 104.9, 106.5, 115.2, 142.2, 151.0, 158.5, 162.8; \[ m/z (El): 235 (M+, 30%), 206 (100), 188 (92), 163 (94), 133 (35).

(7,25,85)-8-Bromo-4-methoxy-5,6,7,8-tetrahydrofuro[2,3-b]quinoline-7-yl acetate 33. \( \delta_{5}^{1} \)urea 2 oxide. Purification of the crude product by PLC (25% EtOAc/hexane) afforded a pure sample of bromoacetate 33 as a yellow oil (0.13 g, 90%); \[ \delta_{6}^{1} \) acetone, 2.14–2.18 (1 H, m, 6′-H), 2.51–2.59 (1 H, m, 6-H), 2.72–2.80 (1 H, m, 5′-H), 2.93–2.98 (1 H, ddd, J5, 17.5, J5, 6.7, J5, 5.6, 2.1, 5-H), 4.27 (3 H, s, OMe), 5.30 (1 H, dd, J7a, 6.8, 3.0, J7b, 5.8, 1.8, 2, 3, 2.57, 3-H), 5.54 (1 H, m, 7-H), 6.95 (1 H, d, J2, 3.2, 2.6, 3-H), 7.58 (1 H, d, J2, 3.2, 2.6, 3-H); \[ \delta_{7}^{1} \) (125 MHz, CDCl3) 17.7, 21.1, 23.1, 48.1, 58.6, 72.4, 104.8, 106.1, 115.7, 143.2, 147.7, 158.4, 162.8, 170.1; \[ m/z (El): 341 (M+, 81), Br, 30%), 339 (M+, 75), Br, 29), 281 (20), 279 (20), 218 (50), 200 (33), 101 (61), 59 (65), 43 (100).

(e) Chemoenzymatic synthesis of dictamine 7,8-oxide 27 and 4-methoxyfuro[2,3-b]quinoline-7,8-diol 29

Dictamine 7,8-oxide 27. To a solution of bromoacetate 33 (0.16 g, 0.47 mmol) in CDCl3 (4 cm3) was added NBS (93 mg, 0.52 mmol) and AIBN (ca. 2 mg). The stirred reaction mixture was heated to 65 °C under nitrogen using a heat lamp, until all the NBS had reacted. The cooled reaction mixture was filtered and the filtrate concentrated under reduced pressure. The crude dibromoacetate 34 obtained was used immediately, without purification, for the next step.

The synthesis of dictamine 7,8-oxide 27 from the crude dibromoacetate 34 (0.15 g, 0.43 mmol) was carried out following the second step of the method described for the synthesis of arene oxide 2, using dry THF (3 cm3) instead of EtOAc as a solvent. The crude sample of 7,8-oxide 27 crystallized out of ether/hexane solution to give a pure sample as a light brown solid (43 mg, 56%); m.p. 125–127 °C (from CHCl3/hexane); \[ \delta_{9}^{1} \) 195.0586. \[ C_{12}H_{12}NO_{2} \) requires 195.0582; \[ \delta_{10}^{1} \) +93.1 (c 0.36, CHCl3); \[ \delta_{11}^{1} \) 500 MHz, CDCl3) 4.18 (1 H, ddd, J7, 3.71, J6, 3.66, J5, 1.84, 4-H), 4.30 (3 H, s, OMe), 4.65 (1 H, d, J6, 3.66, 8-H), 6.73 (1 H, dd, J3a, 6.8, J3b, 5.8, 1.8, 2, 3, 2.57, 3-H), 7.20 (1 H, dd, J3, 2.6, 3-H), 7.62 (1 H, d, J3, 2.57, 3-H); \[ \delta_{12}^{1} \) (125 MHz, CDCl3) 54.4, 58.4, 58.9, 104.6, 105.3, 106.6, 113.5, 122.6, 124.4, 143.0, 157.4; \[ m/z (El): 215 (M+, 100%), 200 (57), 172 (30), 83 (27); \[ ECD: i/\nu/nm 322 (\Delta e) = 0.748), 288 (\Delta e) = 0.867), 241 (\Delta e) = 0.787), 203 (\Delta e) = 6.38).

4-Methoxyfuro[2,3-b]quinoline-7,8-diol 29. A cooled solution (−15 °C) of skimmianine 13 (0.5 g, 1.93 mmol) in dry CH2Cl2 (15 cm3) was treated, drop-wise, with a solution of boron...
tribromide in CH₂Cl₂ (1 M, 4.8 cm³). After leaving the reaction mixture stirred, at room temperature overnight, it was cooled to –60 °C and water (5 cm³) was added and the mixture allowed to warm up to room temperature. The CH₂Cl₂ layer was separated and the remaining aqueous solution was extracted with EtOAc (2 × 10 cm³). The combined organic extract was dried (Na₂SO₄) and concentrated, under reduced pressure, to yield the crude catechol 29. Crystallisation from MeOH furnished catechol 29 as an off-white coloured solid (0.38 g, 85%); m.p. 211 °C (from MeOH); Rf 0.1 (50% EtOAc/hexane); (Found: M⁺, 231.0523. C₁₂H₉NO₄ requires M⁺, 231.0532); δH (500 MHz, CD₂COCD₃) 4.39 (3 H, s, OMe), 6.99 (1 H, d, J₆,₅ = 9.1, 6-H), 7.23 (1 H, d, J₃,₂ = 2.8, 2-H), 7.52 (1 H, d, J₅,₆ = 9.1, 5-H), 7.66 (1 H, d, J₃,₂ = 2.8, 3-H).

(f) Chemoenzymatic synthesis of furoquinoaline alkaloids 30, 31, 36, 37, 38 and 40

Robustine 30. Dihydrodiol 17 (10 mg, 0.04 mmol), dissolved in CHCl₃ solution, was aromatized by the addition of two drops of TFA. The acidic solution was concentrated after adding excess of ammonium hydroxide to yield robustine 17 as a light brown solid (9 mg, 98%); m.p. 147 °C (lit.13e 148–149 °C); δH (300 MHz, CDCl₃) 4.43 (3 H, s, OMe), 7.17 (1H, d, J₂,₃ = 2.8, 3-H), 7.20 (1 H, d, J₆,₅ = 7.8, 6-H), 7.38 (1 H, dd, J₅,₆ = J₆,₅ = 7.8, 6-H), 7.63 (1 H, d, J₃,₂ = 2.8, 2-H), 7.79 (1 H, d, J₅,₆ = 7.8, 5-H); m/z (EI): 215 (M⁺, 100%), 200 (59%).

y-Fagarine 31. Robustine 30 (5 mg, 0.023 mmol) was dissolved in MeOH (1 cm³) and treated (0 °C) with an excess of ethereal diazomethane solution. After leaving the reaction mixture for five minutes, excess of reagent was removed by the addition of a few drops of acetic acid. The crude product, obtained after removal of ether, was purified by PLC (40% EtOAc/hexane) to afford pure pteleine 31 as a white solid (46 mg, 70%); m.p. 152–153 °C (from EtOAc/hexane); Rf 0.2 (50% EtOAc/hexane); (Found: M⁺, 299.1166. C₁₇H₁₇NO₄ requires M⁺, 299.1158); δH (500 MHz, CDCl₃) 1.66 (3 H, s, Me), 1.74 (3 H, s, Me), 4.00 (3 H, s, OMe), 4.43 (3 H, s, OMe), 4.84 (2 H, d, J₁,₂ = 7.2, 1'-H), 5.73 (1 H, t, J₁,₂ = 7.2, H-2'), 7.04 (1 H, d, J₂,₃ = 3.8, 2-H'), 7.22, d, J₆,₅ = 9.4, 6-H), 7.58 (1 H, d, J₂,₃ = 2.8, 2-H), 8.00 (1 H, d, J₅,₆ = 9.4, 5-H); m/z (EI): 313 (M⁺, 20%).

Dihydrodiol 29 (50 mg, 0.22 mmol) was treated (0 °C) with an excess of ethereal diazo methane solution. After leaving the reaction mixture for five minutes, excess of reagent was removed by the addition of a few drops of acetic acid. The crude product, obtained after removal of ether, was purified by PLC (50% EtOAc/hexane), to give haplopine 36 as a white solid (27 mg, 77%); m.p. 124–125 °C (from CH₂Cl₂) (lit.13c 120–121.5 °C); Rf 0.4 (50% EtOAc/hexane); (Found: M⁺, 313.1312. C₁₈H₁₉NO₄ requires M⁺, 313.1314); δH (500 MHz, CDCl₃) 1.66 (3H, s, Me, 1.74 (3 H, s, Me), 4.00 (3 H, s, OMe), 4.43 (3 H, s, OMe), 4.84 (2 H, d, J₁,₂ = 7.2, 1'-H), 5.73 (1 H, t, J₁,₂ = 7.2, H-2'), 7.04 (1 H, d, J₂,₃ = 3.8, 2-H'), 7.22, d, J₆,₅ = 9.4, 6-H), 7.58 (1 H, d, J₂,₃ = 2.8, 2-H), 8.00 (1 H, d, J₅,₆ = 9.4, 5-H); m/z (EI): 313 (M⁺, 20%).

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References


