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

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Biotransformation of acridine, dictamnine and 4-chlorofuro[2,3-*b*]quinolone, 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 dictamnine 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 dictamnine have been synthesised, from the corresponding *cis*-dihydrodiols. The achiral furoquinoline alkaloids robustine, γ-fagarine, haploine, isohaplopine-3,3′-dimethylallylether and pteleine have been obtained, from either *cis*-dihydrodiol, catechol or arene oxide metabolites of dictamnine.

Introduction

Polycyclic azaarenes are ubiquitous in the environment as atmospheric pollutants, resulting from incomplete combustion of nitrogen-containing molecules present in fossil fuels or tobacco and also as plant alkaloids.[1a,b] Some larger members of the family of aza-polycyclic aromatic hydrocarbons (APAHs) present a significant hazard to human health, resulting from the mutagenicity/carcinogenicity of their mammalian metabolites.[1c–e] The mineralization of APAHs and alkaloids containing azaaromatic rings by soil bacteria, *via* non-mutagenic/non-carcinogenic metabolites can, therefore, play a useful role in reducing this problem. Earlier bacterial studies from these laboratories have focused on the toluene dioxygenase (TDO)-catalysed biodegradation of bicyclic heterocycles including quinolines,[2a,b] benzo[*b*]thiophenes[2c] and benzo[*b*]furans,[2c] using the UV4 mutant strain of *Pseudomonas putida* (Schemes 1a and 1b). Regioselective *cis*-dihydroxylation of the carbocyclic and the heterocyclic rings in the quinolines (5,6 and/or 7,8 and/or 2,3 bonds), benzo[*b*]thiophenes (4,5 and/or 2,3 bonds) and benzo[*b*]furans (6,7 and/or 2,3 bonds), occurred to give the corresponding *cis*-dihydrodiol metabolites. The 3-hydroxyquinoline and anthranilic acid metabolites of quinoline were assumed to be derived from the undetected heterocyclic *cis*-3,4-dihydrodiol intermediate (Scheme 1a).[2a,b]

Further metabolism of the benzo[*b*]furan 2,3-*cis*-diols involved spontaneous ring opening and enzyme-catalysed carbonyl reduction to give exocyclic phenolic diol products (Scheme 1b).[2c]

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.* benzo[*f*]quinoline, benzo[*h*]quinoline, 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.
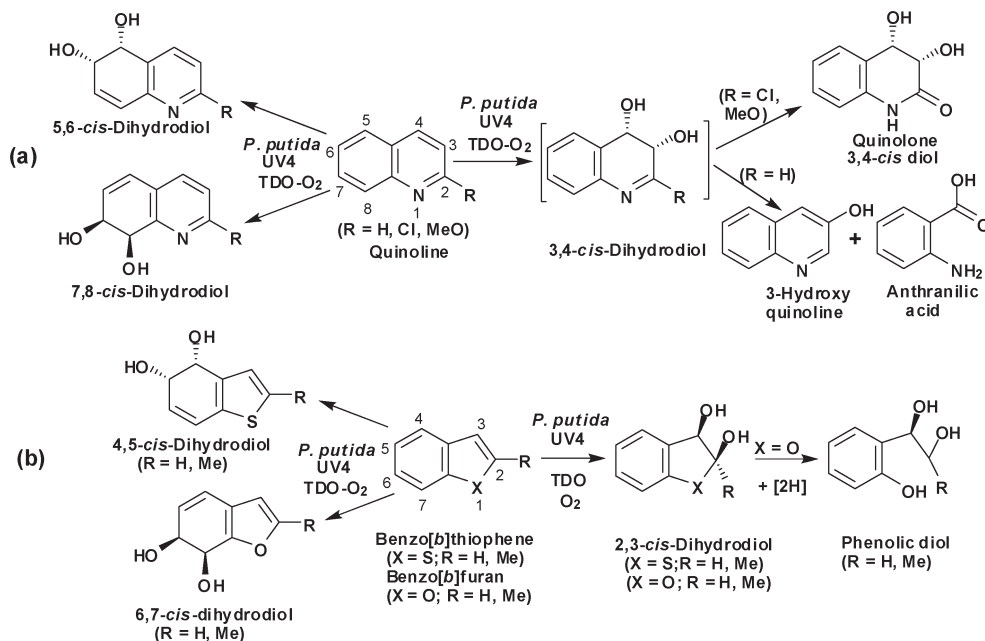
As part of an earlier programme[3b,c] to investigate the ability of BPDO to catalyse the *cis*-tetrahydroxylation of larger polycyclic aromatic rings, it was found that *bis-cis*-dihydrodiols were formed as further metabolites of the initial *cis*-dihydrodiols derived from larger carbocyclic (*e.g.* anthracene, chrysene, benz[*a*]anthracene) and heterocyclic (*e.g.* acridine, phenazine, benzo[*b*]naphtha[2,1-*d*]thiophene) substrates. The

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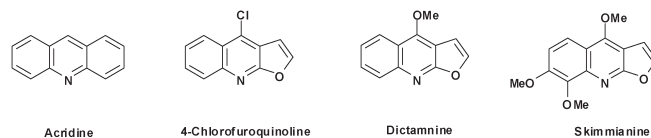[b]*School of Biological Sciences, Queen's University Belfast, Belfast, UK BT9 5AG*
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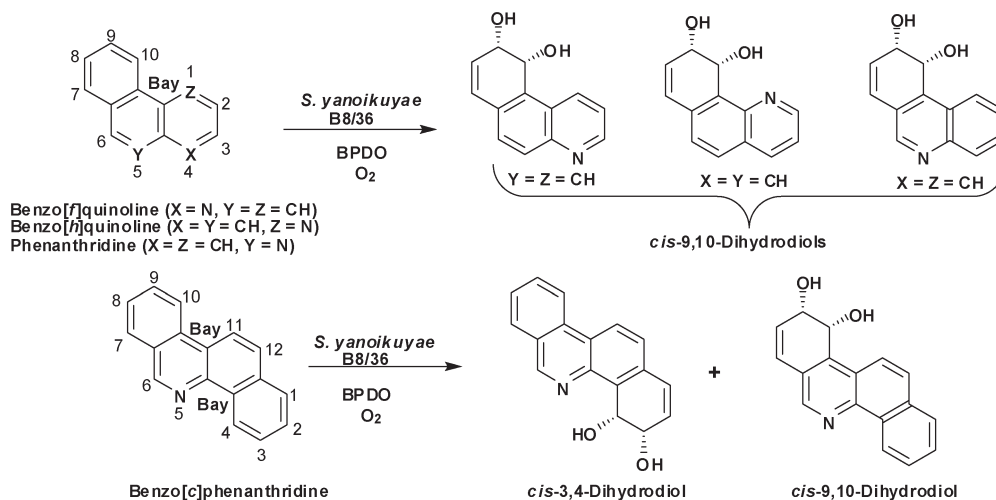
Scheme 1

similarity in size and shape of the linear tricyclic arenes, anthracene and acridine, and their acceptability as substrates for the BPDO enzyme,[3c,d] prompted this comparative biotransformation study of acridine with furo[2,3-b]quinoline substrates. Following our earlier reports on the isolation and synthesis[4a-e] 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.[4d]
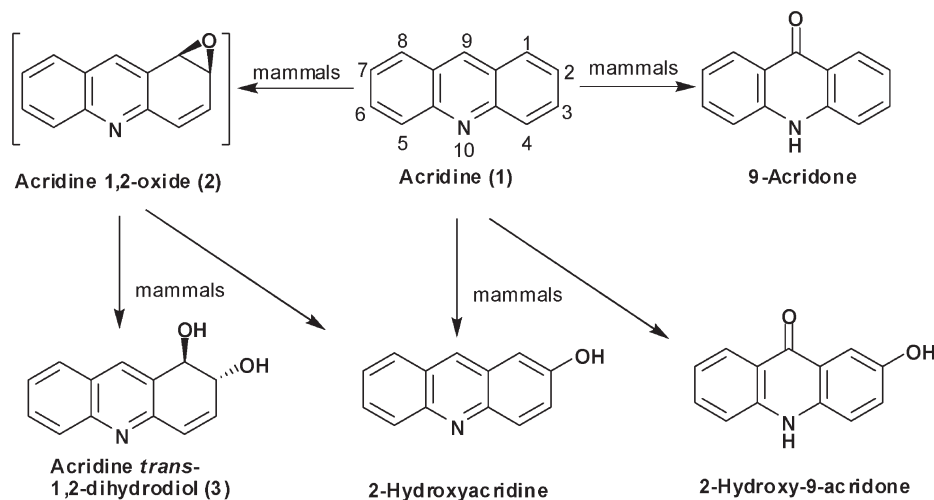

In our preliminary studies of the biotransformations of acridine and dictamnine, using *S. yanoikuyae* B8/36, we had reported[3d,4d] the presence of the corresponding *cis*-dihydrodiol metabolites. This comprehensive study now provides full structural and stereochemical characterization of all new bacterial metabolites and shows how they can be utilized in

Scheme 2

Scheme 3

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.[5a–d] 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 monooxygenase-catalysed oxidation, prompted the preliminary[3d] 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,[3a–d] 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)phenyl-boronic acid (MPBA) and ^{1}H-NMR analysis of the resulting boronates.

The absolute configuration of *cis*-dihydrodiol **4** was initially assigned as (1*R*,2*S*), based on the well established ^{1}H-NMR pattern previously observed for MPBA derivatives from other polycyclic arene *cis*-dihydrodiol metabolites (*e.g.* from naphthalene, anthracene, phenanthrene and their aza-analogues).[2a,b,3a,b] The observation of a larger chemical shift value (δ$_H$ 3.18) for the MeO group protons, using the (*R*)-(+)-MPBA compared with the value obtained using (*S*)-(−)-MPBA (δ$_H$ 3.11), was again assumed to be consistent with a benzylic (*R*)
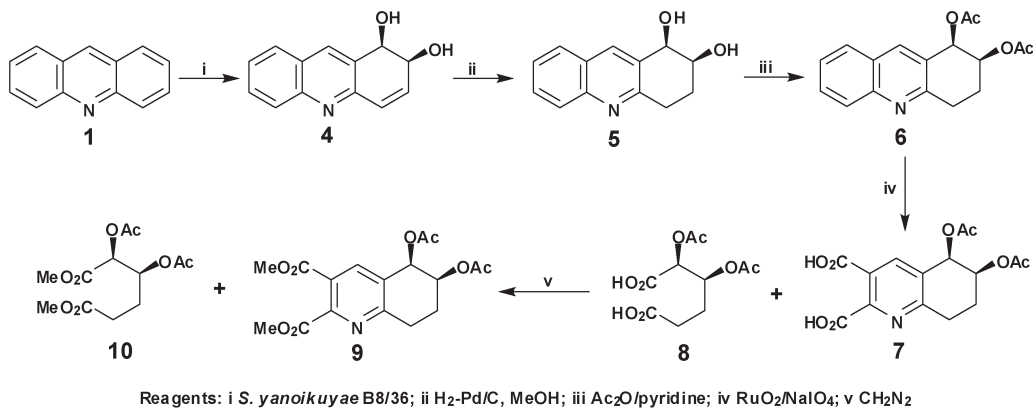
and an allylic (*S*) configuration for *cis*-dihydrodiol **4**. The reliability of the MPBA method for the linear azaarene *cis*-dihydrodiol **4** was confirmed by an unequivocal stereochemical correlation sequence similar to that used for other polycyclic arene *cis*-dihydrodiols (Scheme 4).[3a,6] The sequence involved a catalytic hydrogenation (H$_2$,Pd/C) to yield *cis*-tetrahydrodiol **5** followed by *bis*-acetylation (Ac$_2$O, pyridine) to give *cis*-diacetate **6**. In the final step, an oxidative ring opening reaction (RuO$_2$/NaIO$_4$) 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 (CH$_2$N$_2$) 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 (2*S*,3*S*) configuration[6] and thus the (1*R*,2*S*) 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 monooxygenase-catalysed epoxidation of a carbocyclic ring to yield an arene oxide (*cf.* compound **2**), (ii) an epoxide hydrolase-catalysed hydrolysis of the arene oxide to yield a *trans*-dihydrodiol (*cf.* compound **3**), (iii) a monooxygenase-catalysed epoxidation of the alkene bond in the *trans*-dihydrodiol to yield diastereoisomeric *trans*-diol epoxides and (iv) nucleophilic attack of DNA on the epoxide ring within a bay region to yield a covalent adduct.[1b–f] 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 dictamnine **12** and other furoquinoline alkaloids, *e.g.* γ-fagarine, had been reported earlier.[7a–e] In a more recent study, from these laboratories, the furoquinoline

Reagents: i *S. yanoikuyae* B8/36; ii H_2-Pd/C, MeOH; iii Ac_2O/pyridine; iv RuO_2/$NaIO_4$; v CH_2N_2
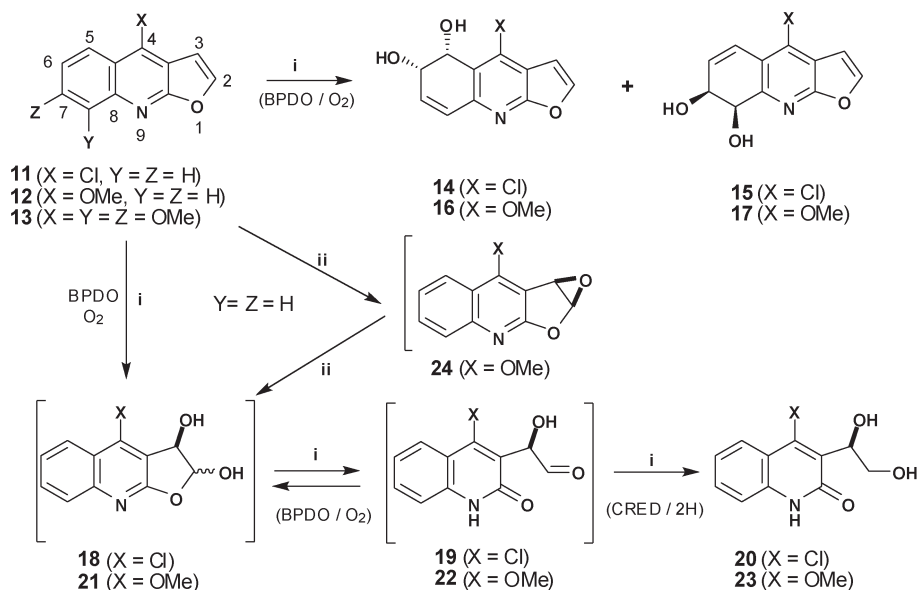
Scheme 4

alkaloid skimmianine **13** was found to be the major compound present in *C. ternata*,[4a] 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[8a–c] which involved 4-chlorofuroquinoline **11** as precursor. Furoquinolines **11** and **12** 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 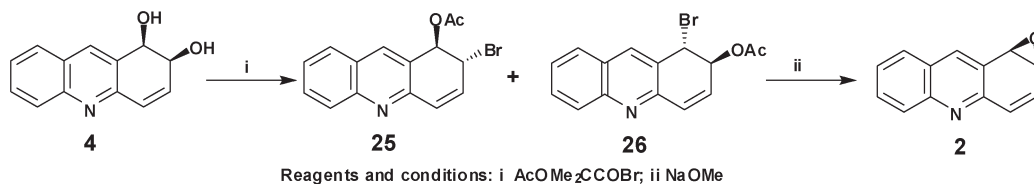a mixture of two *cis*-dihydrodiol products **14–17**, resulting from BPDO-catalysed *cis*-dihydroxylation of the 5,6 and 7,8 bonds of the carbocyclic ring, skimmiamine **13** was not an acceptable substrate. The mixtures of metabolites (**14**/**15** and **16**/**17**) were separated into individual *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 ^{1}H-NMR spectroscopy. As found for the acridine *cis*-dihydrodiol **4**, the larger chemical shift values (δ_H) for the exocyclic MeO group of *cis*-dihydrodiols **14–17**, (δ_H 3.23–3.25) using the (*R*)-(+)-MPBA compared with (*S*)-(−)-MPBA **14–17**, (δ_H 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

Reagents and conditions: i *S. yanoikuyae* B8 /36; ii liver microsomes

Scheme 5

Reagents and conditions: i AcOMe₂CCOBr; ii NaOMe

Scheme 6

(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 dictamnine **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 dictamnine *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 dictamnine **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).[2c]

The origin of mutagenicity associated with dictamnine **13** has not yet been rigorously established.[7a–e] 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,[7f] 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.[7e,f]
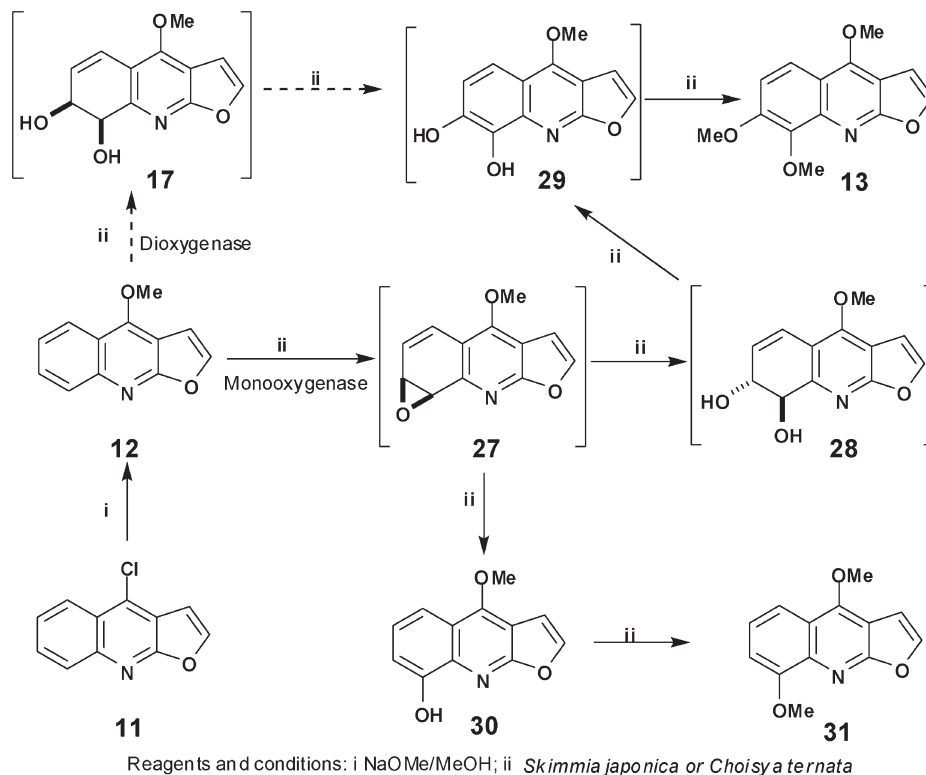
(iii) Application of acridine *cis*-dihydrodiol 4 in the synthesis of arene oxide 2

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-methylethyl acetate, 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 Et₂O 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**.[5a–e] A preliminary study[3d] 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 dictamnine *cis*-dihydrodiols 16 and 17 as precursors in the synthesis of furoquinoline alkaloids

The potential of dictamnine *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 *Rutaceaeous* plants, *e.g. Skimmia japonica* and *Choisya ternata*, have been studied using ¹⁴C-labelled precursors.[10a] These labelling studies showed that enzyme-catalysed hydroxylation could

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

Scheme 7

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 monooxygenase-catalysed epoxidation of dictamnine **12**, to yield the transient arene oxide **27**, followed by epoxide hydrolase-catalysed hydrolysis to yield *trans*-dihydrodiol **28**.[10a] The possibility of an alternative dioxygenase-catalysed *cis*-dihydroxylation of dictamnine **12** to yield *cis*-dihydrodiol **17** was also discussed.[10a] The enzyme-catalysed oxidations of *trans*- and *cis*-dihydrodiols, to yield catechols followed by *O*-methylation, are well established metabolic steps[1a] and, when allied to the earlier labelling studies,[10a] 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*[10a] or found among the furoquinoline alkaloids recently isolated from this[4a] or other plants in the *Rutaceae* family.[10b]
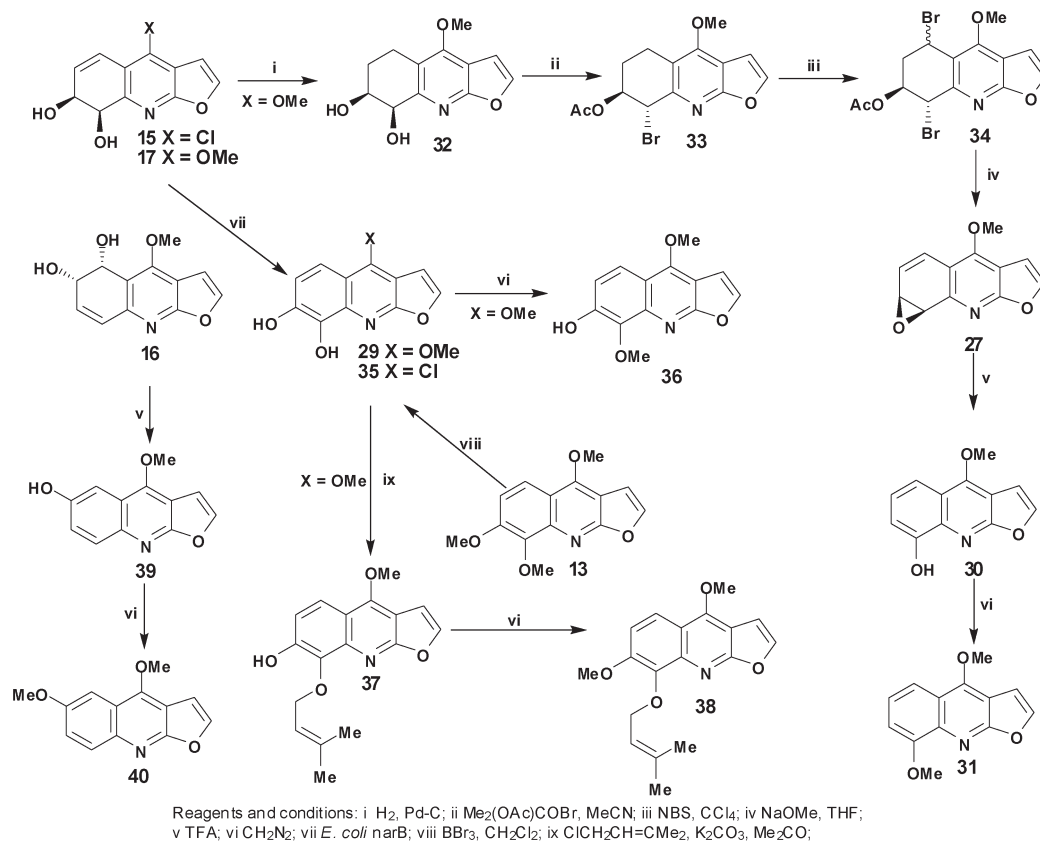
As expected, the B8/36 mutant strain of *S. yanoikuyae* did not yield catechol metabolites *e.g.* compound **29** from dictamnine **12** (Scheme 7). The biphenyl *cis*-diol dehydrogenase (DD) activity required to catalyse the dehydrogenation of *cis*-dihydrodiols to yield catechols, was blocked in the 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.[11] Using *E. coli* narB and *cis*-dihydrodiol **15** as substrate, catechol **35** was detected by ^{1}H-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[4a] from *Choisya ternata* (Scheme 8).

Convincing evidence of monooxygenase-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.[10a,b] Furthermore, monooxygenase-catalysed epoxidation of azaarenes, to yield the corresponding arene oxides, *e.g.* quinoline 5,6-oxide from quinoline, using liver microsomes with inhibition of epoxide hydrolase activity,[12a,b] 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 monooxygenase-catalysed epoxidation sequence, shown in Scheme 7, is currently favoured over the dioxygenase pathway. Epoxidation,

Reagents and conditions: i H₂, Pd–C; ii Me₂(OAc)COBr, MeCN; iii NBS, CCl₄; iv NaOMe, THF;
v TFA; vi CH₂N₂; vii *E. coli* narB; viii BBr₃, CH₂Cl₂; ix ClCH₂CH=CMe₂, K₂CO₃, Me₂CO;

Scheme 8

as an initial step, can readily account for the formation of both monophenols (*e.g.* robustine **30**), catechols (*e.g.* 7,8-dihydroxydictamnine **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 (7*S*,8*R*)-dictamnine 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*-Dihydrodiols **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 dictamnine **12**. *cis*-Dihydrodiol **16** and **17** and arene oxide **27**, as derivatives of dictamnine **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 pteleine **40**.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400, DPX-300 and DRX-500 instruments. Chemical shifts (δ) are reported in ppm relative to SiMe$_4$ 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 the peak matching method, with perfluorokerosene as the standard. CD spectra were recorded in spectroscopic grade acetonitrile using a JASCO J-720 instrument. A PerkinElmer 341 polarimeter was used for optical rotation ($[\alpha]_D$) measurements (*ca.* 20 °C). Flash column chromatography and preparative layer chromatography (PLC) were performed on Merck Kieselgel type 60 (250–400 mesh) and PF$_{254/366}$ plates respectively. Merck Kieselgel type 60F$_{254}$ analytical plates were employed for TLC. Authentic samples of 4-chlorofuro[2,3-*b*]quinoline **11** and skimmianine **13** were available from earlier studies.$^{4a–e}$ As reported,^{2b} *Sphingomonas yanoikuyae* B8/36 was grown on minimal salts medium with 0.5% sodium succinate and 0.5 g L^{-1} of yeast extract. Biphenyl dioxygenase (BPDO) was induced, during the exponential phase of growth, by the addition of *m*-xylene (1 cm^3 L^{-1}) every 0.5 h for 7 h. Substrate concentration was 0.5 mg cm^{-3}.

Synthesis of dictamnine 12

To a solution of 4-chlorofuro[2,3-*b*]quinoline **11** (3 g, 14.7 mmol) in dry methanol (120 cm^3) 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 cm^3). The combined organic extract was dried (Na$_2$SO$_4$), concentrated under reduced pressure, and the crude product purified by flash chromatography (1 : 1, EtOAc : hexane) to afford dictamnine **12** as a yellow solid (1.65 g, 56%); R_f 0.5, (1 : 1 EtOAc/hexane); m.p. 133–134 °C (lit.^{13a} 132–133 °C); δ_H (300 MHz, CDCl$_3$) 4.45 (3 H, s, Me), 7.08 (1 H, d, $J_{3,2}$ 2.8, 3-H), 7.45 (1 H, dd, $J_{6,5}$ 9.1, $J_{6,7}$ 7.0 6-H), 7.62 (1 H, d, $J_{2,3}$ 2.8, 2-H), 7.69 (1 H, dd, $J_{7,8}$ 8.4, $J_{7,6}$ 7.0 J 7.0, 7-H), 8.0 (1 H, d, $J_{8,7}$ 8.4, 8-H), 8.26 (1 H, d, $J_{5,6}$ 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-diol **4** (7.5 g, 42%). The majority of the diol metabolite **4** crystallized out of solution on concentration. The mother liquor was purified by column chromatography (CHCl$_3$ →10% MeOH/CHCl$_3$). Colourless crystalline solid, m.p. 176 °C (EtOH); R_f 0.45 (15% MeOH/CHCl$_3$); $[\alpha]_D$ +71 (*c* 0.53, MeOH); (Found: C, 73.4; H, 5.1; N, 6.4. C$_{13}$H$_{11}$NO$_2$ requires C, 73.2; H, 5.2; N, 6.6%); (Found: M$^+$ 213.07876. C$_{13}$H$_{11}$NO$_2$ requires 213.07898); δ_H (500 MHz, CD$_3$COCD$_3$) 4.13 (1 H, m, OH), 4.35 (1 H, m, OH), 4.42 (1 H, dd, $J_{2,1}$ 4.7, $J_{2,3}$ 4.8 , 2-H), 4.86 (1 H, d, $J_{1,2}$ 4.7, 1-H), 6.54 (1 H, dd, $J_{3,2}$ 4.8, $J_{3,4}$ 10.1, 3-H), 6.77 (1 H, d, $J_{4,3}$ 10.1, 4-H), 7.53 (1 H, dd, $J_{7,8}$ 8.1, $J_{7,6}$ 6.9, 7-H), 7.68 (1 H, dd, $J_{6,7}$ 6.9, $J_{6,5}$ 8.5, 6-H), 7.90 (1 H, d, $J_{8,7}$ 8.1, 8-H), 7.95 (1 H, d, $J_{5,6}$ 8.5, 5-H), 8.28 (1 H, s, 9-H); δ_C (125 MHz, CD$_3$COCD$_3$) 66.6, 70.2, 126.3, 127.7, 127.9, 129.0, 129.1, 130.8, 132.1, 133.2, 136.3, 147.1, 153.0; *m/z* (EI): 213 (M$^+$, 56%), 195 (23), 184 (100); v 3369 cm^{-1} (OH); ECD: λ/nm 311 ($\Delta\varepsilon$ 1.036), 283 ($\Delta\varepsilon$ −1.079), 257 ($\Delta\varepsilon$ 4.644), 243 ($\Delta\varepsilon$ 6.421), 215 ($\Delta\varepsilon$ 4.647), 199.40 ($\Delta\varepsilon$ 0.573).

(1R,2S)-1,2,3,4-Tetrahydroacridine-1,2-diol 5. *cis*-Dihydrodiol metabolite **4** (0.5 g, 2.35 mmol) was catalytically hydrogenated (H$_2$, 10% Pd/C, 50 mg) in MeOH solution (20 cm^3) at atmospheric pressure and room temperature (4 h). 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%); $[\alpha]_D$ +61 (*c* 1.00, MeOH); (Found: M$^+$ 215.0937. C$_{13}$H$_{13}$NO$_2$ requires 215.0946); δ_H (500 MHz, CDCl$_3$) 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, $J_{2,1}$ 3.5, 2-H), 4.93 (1 H, d, $J_{1,2}$ 3.5, 1-H), 7.49 (1 H, dd, $J_{7,8}$ 8.0, $J_{7,6}$ 7.1, 7-H), 7.68 (1 H, dd, $J_{6,7}$ 7.1, $J_{6,5}$ 8.4, 6-H), 7.79 (1 H, d, $J_{8,7}$ 8.0, 8-H), 8.00 (1 H, d, $J_{5,6}$ 8.4, 5-H), 8.30 (1 H, s, 9-H); δ_C (125 MHz, CDCl$_3$) 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); v 3435 cm^{-1} (OH).

(1R,2S)-1,2-Diacetoxy-1,2,3,4-tetrahydroacridine 6. *cis*-Tetrahydrodiol **5** (0.18 g, 0.84 mmol) was acetylated with Ac$_2$O (1.5 cm^3) in dry pyridine (0.7 cm^3) solution by stirring the mixture overnight at room temperature. Excess of pyridine was removed under high vacuum, the residue treated with water (10 cm^3), and the aqueous mixture extracted with Et$_2$O (3 × 15 cm^3). The combined ether extract was dried (Na$_2$SO$_4$) 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 (Et$_2$O/hexane); $[\alpha]_D$ −57 (*c* 0.85, MeOH); (Found: M$^+$ 299.1150. C$_{17}$H$_{17}$NO$_4$ requires 299.1158); δ_H (500 MHz, CDCl$_3$) 2.05 (3 H, s, OCOMe), 2.15 (3 H, s, OCOMe), 2.20 (1 H, m, $J_{3a,2}$ 3.1, 3a-H), 2.44 (1 H, m, 3b-H), 3.23 (1 H, m, 4a-H), 3.40 (1 H, m, 4b-H), 5.43 (1 H, m, $J_{2,1}$ 3.3, $J_{2,3a}$ 3.1, 2-H), 6.32 (1 H, d, $J_{1,2}$ 3.3, 1-H), 7.51 (1 H, dd, $J_{7,8}$ 8.1, $J_{7,6}$ 7.0, 7-H), 7.72 (1 H, dd, $J_{6,7}$ 7.0, $J_{6,5}$ 8.5, 6-H), 7.79 (1 H, d, $J_{8,7}$ 8.1, 8-H), 8.02 (1 H, d, $J_{5,6}$ 8.5, 5-H), 8.12 (1 H, s, 9-H); δ_C (125 MHz, CDCl$_3$) 19.6, 19.7, 22.1, 28.8, 67.8, 68.3, 124.9, 125.3, 125.4, 126.4, 127.0, 128.9, 135.9, 146.5, 155.6, 169.0, 169.2; *m/z* (EI): 299 (M$^+$, 69%), 197 (100); v 1741 cm^{-1} (C=O), 1421.

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 NaIO$_4$ (3.4 g, 16 mmol) in a mixture of CCl$_4$ (2 cm^3), MeCN (2 cm^3) and water (3 cm^3). After stirring the reaction mixture for 4 days at ambient tempera-

ture, dilute HCl (20 cm^3, 1.5 M) was added and the reaction mixture extracted with EtOAc (3 × 30 cm^3). The combined extract was washed with water, dried (Na$_2$SO$_4$), and concentrated under reduced pressure to yield a crude mixture of two dicarboxylic acids. The mixture was dissolved in MeOH (5 cm^3) and treated (0 °C, 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% Et$_2$O/hexane) to yield dimethyl (5R,6S)-5,6-di(acetoxy)-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 °C (from CHCl$_3$); [α]$_D$ −53 (c 0.66, MeOH); (Found: C, 55.7; H, 5.1; N, 3.9. C$_{17}$H$_{19}$NO$_8$ requires C, 55.9; H, 5.2; N, 3.8%); (Found: M$^+$ 365.1105. C$_{17}$H$_{19}$NO$_8$ requires 365.1111); δ$_H$ (500 MHz, CDCl$_3$) 2.04 (3 H, s, OCOMe), 2.15 (3 H, s, OCOMe), 2.16 (1 H, m, J$_{7a,6}$ 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, CO$_2$Me), 3.99 (3 H, s, CO$_2$Me), 5.42 (1 H, m, J$_{6,5}$ 3.4, J$_{6,7a}$ 3.1, 6-H), 6.13 (1 H, d, J$_{5,6}$ 3.4, 5-H), 8.11 (1 H, s, 4-H); δ$_C$ (125 MHz, CDCl$_3$) 21.3 (×2), 23.6, 29.5, 53.3, 53.6, 68.3, 69.0, 123.8, 130.4, 138.8, 151.4, 160.6, 165.5, 167.2, 170.6, 170.7; m/z (EI): 365 (M$^+$, 21%), 43 (100); ν 1746 cm^{-1} (C=O).

(2S,3S)-Dimethyl-(2,3-diacetoxy)-adipate 10. Colourless oil; [α]$_D$ −14 (c 0.74, CHCl$_3$) (lit.[6] −14.1, CHCl$_3$); δ$_H$ (500 MHz, CDCl$_3$) 1.97 (1 H, m, CH$_2$), 2.07 (3 H, s, OCOMe), 2.06–2.15 (1 H, m, CH$_2$), 2.18 (3 H, s, OCOMe), 2.37 (2 H, m, CH$_2$), 3.68 (3 H, s, CO$_2$Me), 3.79 (3 H, s, CO$_2$Me), 5.30 (2 H, m, 2-H and 3-H); m/z (EI): 259 (M$^+$-OMe, 5%), 217 (M$^+$-COMe, 16%); ν 1735 cm^{-1} (C=O).

(b) Biotransformation of dictamnine 12

A small scale biotransformation of dictamnine **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**. These diols were separated by PLC (7% MeOH/CHCl$_3$). A repeat larger scale biotransformation of dictamnine **12** (0.6 g, 3.02 mmol), using S. yanoikuyae B8/36, yielded a mixture of three compounds, diol **16** (8 mg, 1.1%), diol **17** (0.143 g, 20%) and 3-(1,2-dihydroxyethyl)-4-methoxy-1,2-dihydro-2-quinolinone **23** (7 mg, 1.0%).

(5R,6S)-4-Methoxy-5,6-dihydrofuro[2,3-b]quinoline-5,6-diol 16. White solid (2 mg, 3%); m.p. 157–159 °C; R$_f$ 0.26 (7% MeOH/CHCl$_3$); [α]$_D$ −111 (c 0.19, MeOH); (Found M$^+$ 233.0694. C$_{12}$H$_{11}$NO$_4$ requires 233.0688); δ$_H$ (500 MHz, CDCl$_3$) 4.32 (3 H, s, OMe), 4.63 (1 H, m, 6-H), 5.14 (1 H, d, J$_{5,6}$ 5.3, 5-H), 6.19 (1 H, ddd, J$_{7,8}$ 10.0, J$_{7,6}$ = J$_{7,5}$ 1.9, 7-H), 6.57 (1 H, dd, J$_{8,7}$ 10.0, J$_{8,6}$ 2.6 8-H), 6.98 (1 H, d, J$_{3,2}$ 2.5, 3-H), 7.60 (1 H, d, J$_{2,3}$ 2.5, 2-H); δ$_C$ (125 MHz, CDCl$_3$) 58.9, 63.8, 69.7, 105.4, 105.8, 114.1, 128.4, 136.4, 143.0, 147.9, 158.9, 164.4; m/z (EI): 233 (M$^+$, 6%), 215 (9), 43 (100); ECD: λ/nm 286 (Δε 4.808), 245 (Δε −5.670), 229 (Δε 0.415), 216 (Δε −3.007), 204 (Δε 1.004).

(7S,8R)-4-Methoxy-7,8-dihydrofuro[2,3-b]quinoline-7,8-diol 17. White solid (41 mg, 29%); m.p. 185–187 °C (MeOH/CHCl$_3$); R$_f$ 0.3 (7% MeOH/CHCl$_3$); [α]$_D$ +94 (c 0.78, MeOH); (Found: M$^+$ 233.0689. C$_{12}$H$_{11}$NO$_4$ requires 233.0688); δ$_H$ (500 MHz, CDCl$_3$) 4.28 (3 H, s, OMe), 4.45 (1 H, dd, J$_{7,8}$ 5.0, J$_{7,6}$ 5.0, 7-H), 4.78 (1 H, d, J$_{8,7}$ 5.0, 8-H), 6.17 (1 H, dd, J$_{6,5}$ 9.8, J$_{6,7}$ 5.0, 6-H), 6.98 (1 H, d, J$_{3,2}$ 2.5, 3-H), 7.02 (1 H, d, J$_{5,6}$ 9.8, 5-H), 7.57 (1 H, d, J$_{2,3}$ 2.5, 2-H); δ$_C$ (125 MHz, CDCl$_3$) 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: λ/nm 275.60 (Δε −0.425), 246.10 (Δε 2.722), 205.90 (Δε −0.272).

3-(1,2-Dihydroxyethyl)-4-methoxy-1,2-dihydro-2-quinolinone 23. Brown solid (1.1 mg, 1.6%); m.p. 137–140 °C (decomp.); R$_f$ 0.20 (7% MeOH/CHCl$_3$); [α]$_D$ +8.8 (c 0.68, MeOH); (Found: M$^+$ 235.0840. C$_{12}$H$_{13}$NO$_4$ requires 235.0845); δ$_H$ (500 MHz, CDCl$_3$) 3.82 (1 H, dd, J$_{2,2'}$ 11.1, J$_{2,1}$ 4.4, 2-H), 3.97 (1 H, dd, J$_{2',2}$ 11.1, J$_{2',1}$ 7.4, 2'-H), 4.04 (3 H, s, OMe), 5.15 (1 H, dd, J$_{1,2}$ 4.4, J$_{1,2'}$ 7.4, 1-H), 7.30 (1 H, dd, J$_{6,7}$ = J$_{6,5}$ 7.9, 6-H), 7.35 (1 H, d, J$_{8,7}$ 8.2, 8-H), 7.57 (1H, dd, J$_{7,8}$ 8.2, J$_{7,6}$ 7.9, 7-H), 7.81 (1 H, d, J$_{5,6}$ 7.9, 5-H), 11.68 (1 H, br s, NH); δ$_C$ (75 MHz, CDCl$_3$) 62.9, 66.0, 69.0, 116.3, 116.8, 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 4-chlorofuro[2,3-b]quinoline 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/CHCl$_3$) gave (7S,8R)-4-chloro-7,8-dihydrofuro[2,3-b]quinoline-7,8-diol **15** (0.35 g, 38%); R$_f$ 0.6 (6% MeOH/CHCl$_3$), (5R,6S)-4-chloro-5,6-dihydrofuro[2,3-b]quinoline-5,6-diol **14** (93 mg, 10%); R$_f$ 0.5 (6% MeOH/CHCl$_3$) and 4-chloro-3-(1,2-dihydroxyethyl)-1,2-dihydro-2-quinolinone **20** (23 mg, 2.5%); R$_f$ 0.4 (6% MeOH/CHCl$_3$).

(7S,8R)-4-Chloro-7,8-dihydrofuro[2,3-b]quinoline-7,8-diol 15. Brown crystalline solid (0.35 g, 38%); m.p. 121–123 °C (decomp., CHCl$_3$/hexane); [α]$_D$ +138.1 (c 0.53, CHCl$_3$); (Found: M$^+$ 237.0201. C$_{11}$H$_8$NO$_3$ requires 237.0193); δ$_H$ (500 MHz, CDCl$_3$) 4.49 (1 H, dd, J$_{7,8}$ 4.8, J$_{7,6}$ 5.2, 7-H), 4.81 (1 H, d, J$_{8,7}$ 4.8, 8-H), 6.34 (1 H, dd, J$_{6,7}$ 5.2, J$_{6,5}$ 9.9, 6-H), 6.85 (1 H, d, J$_{3,2}$ 2.4, 3-H), 7.05 (1 H, d, J$_{5,6}$ 9.9, 5-H), 7.66 (1 H, d, J$_{2,3}$ 2.4, 2-H); δ$_C$ (125 MHz, CDCl$_3$) 63.8, 70.3, 104.2, 117.6, 120.3, 123.5, 127.5, 133.6, 144.0, 150.9, 159.1; m/z (EI): 239 (M$^+$, ^{37}Cl, 14%), 237 (M$^+$, 43), 219 (M$^+$-H$_2$O, 15), 208 (M$^+$-CHO, 75), 199 (100), 190 (80), 184 (55), 156 (49), 149 (29), 128 (28); ECD: λ/nm 316 (Δε −0.31), 316 (Δε −0.27), 253 (Δε 4.12), 235 (Δε 3.34), 207 (Δε −2.07).

(5R,6S)-4-Chloro-5,6-dihydrofuro[2,3-b]quinoline-5,6-diol 14. Brown crystalline solid (93 mg, 10%); m.p. 172–174 °C (decomp.); [α]$_D$ +189 (c 0.41, MeOH); (Found M$^+$ 237.0199. C$_{11}$H$_8$ClNO$_3$ requires 237.0193); δ$_H$ (500 MHz, CDCl$_3$) 4.72 (1 H, br s, 6-H), 5.21 (1 H, dd, J$_{5,6}$ 5.1, J$_{5,7}$ 1.7, 5-H), 6.23 (1 H, ddd, J$_{7,8}$ 10, J$_{7,6}$ = J$_{7,5}$ 1.7, 7-H), 6.64 (1 H, dd, J$_{8,7}$ 10, J$_{8,6}$ 2.7, 8-H), 6.86 (1 H, d, J$_{3,2}$ 2.5, 3-H), 7.72 (1 H, d, J$_{2,3}$ 2.5, 2-H); δ$_C$ (125 MHz, CDCl$_3$) 66.1, 69.7, 105.3, 118.1, 123.5, 128.17, 136.8, 137.68, 145.8, 148.2, 161.7; m/z (EI): 237 (M$^+$, ^{35}Cl, 5%), 221

(72), 219 (100), 191 (18), 156 (80); ECD: λ/nm 316 ($\Delta\varepsilon$ 3.07), 306 ($\Delta\varepsilon$ 3.98), 290 ($\Delta\varepsilon$ 3.70), 246 ($\Delta\varepsilon$ −5.26), 217 ($\Delta\varepsilon$ −6.16), 201($\Delta\varepsilon$ 0.61).

4-Chloro-3-(1,2-dihydroxyethyl)-1,2-dihydro-2-quinolinone 20. Brown solid (23 mg, 2%); m.p. 184–186 °C (decomp.); $[\alpha]_D$ −5.1 (c 0.43, MeOH); (Found: M$^+$-CH$_2$OH 208.0157. C$_{10}$H$_7$ClNO$_2$ requires 208.0165); δ_H (500 MHz, CD$_3$OD) 3.76 (1 H, dd, $J_{2,2'}$ 11.3, $J_{2,1}$ 5.6, 2-H), 3.87 (1 H, dd, $J_{2',2}$ 11.3, $J_{2',1}$ 7.2, 2'-H), 4.51 (2 H, br s, OH), 5.20 (1 H, dd, $J_{1,2}$ 5.6, $J_{1,2'}$ 7.2, 1-H), 7.32 (2 H, m, 6-H, 8-H), 7.57 (1 H, m, 7-H), 8.00 (1 H, dd, $J_{5,6}$ 8.2, $J_{5,7}$ 1.1, 5-H); δ_C (125 MHz, CD$_3$OD) 65.8, 74.2, 117.1, 120.2, 125.0, 127.0, 129.6, 133.2, 138.7, 144.8, 164.0; m/z (EI): 210 (M$^+$, ^{37}Cl-CH$_2$OH, 31%), 208 (100), 162 (15), 89 (13), 32 (16).

(7S,8R)-4-Methoxy-5,6,7,8-tetrahydrofuro[2,3-b]quinoline-7,8-diol 32. A solution of dictamnine 7,8-diol 17 (0.15 g, 0.64 mmol) in MeOH (10 cm^3) containing 3% Pd/C (15 mg) was stirred (1 h) under hydrogen atmosphere at atmospheric pressure. The reaction mixture was filtered and the solvent removed under reduced pressure to yield the crude product as a brown oil. Purification of the crude product by PLC (4% MeOH/CHCl$_3$), afforded the tetrahydro cis-diol 32 as a white solid (0.115 g, 76%); m.p. 149–151 °C (from EtOAc); R_f 0.5 (4% MeOH/CHCl$_3$); $[\alpha]_D$ −25 (c 0.39, CHCl$_3$); (Found: M$^+$ 235.0843. C$_{12}$H$_{13}$NO$_4$ requires 235.0844); δ_H (500 MHz, CDCl$_3$) 1.82–1.89 (1 H, m, 6'-H), 2.24–2.28 (1 H, m 6-H), 2.64 (1 H, br s, OH), 2.76 (1 H, m, 5'-H), 2.86 (1 H, m, 5-H), 4.20 (3 H, s, OMe), 4.35 (1 H, m, 7-H), 4.70 (1 H, d, $J_{8,7}$ 3.3, 8-H), 6.95 (1 H, d, $J_{3,2}$ 2.6, 3-H), 7.53 (1 H, d, $J_{2,3}$ 2.6, 2-H); δ_C (125 MHz, CDCl$_3$) 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 (EI): 235 (M$^+$, 30%), 206 (100), 188 (92), 163 (94), 133 (35).

(7S,8S)-8-Bromo-4-methoxy-5,6,7,8-tetrahydrofuro[2,3-b]quinolin-7-yl acetate 33. cis-Tetrahydrodiol 32 (0.1 g, 0.43 mmol) was converted into bromoacetate 33, using the first step of the method described for the synthesis of arene oxide 2. Purification of the crude product by PLC (25% EtOAc/hexane) afforded a pure sample of bromoacetate 33 as a pale yellow oil (0.13 g, 90%); R_f 0.25 (25% EtOAc/hexane); $[\alpha]_D$ −37.5 (c 1.16, CHCl$_3$); (Found: M$^+$ 339.0121. C$_{14}$H$_{14}$BrNO$_4$ requires 339.0106); δ_H (500 MHz, CDCl$_3$) 1.99 (3 H, s, OCOMe), 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, $J_{5,5'}$ 17.5, $J_{5,6'}$ 6.7, $J_{5,6}$ 2.1, 5-H), 4.27 (3 H, s, OMe), 5.30 (1-H, dd, $J_{8,7}$ 3.0, $J_{8,6}$ 1.5, 8-H), 5.54 (1 H, m, 7-H), 6.95 (1 H, d, $J_{3,2}$ 2.6, 3-H), 7.58 (1 H, d, $J_{2,3}$ 2.6, 2-H); δ_C (125 MHz, CDCl$_3$) 17.7, 21.1, 21.3, 48.1, 58.6, 72.4, 104.8, 106.1, 115.7, 143.2, 147.7, 158.4, 162.8, 170.1; m/z (EI): 341 (M$^+$, ^{81}Br, 30%), 339 (M$^+$, ^{79}Br, 29), 281 (20), 279 (20), 218 (50), 200 (33), 101 (61), 59 (65), 43 (100).

(d) Chemoenzymatic synthesis of (1R,2S)-1,2-epoxy-1,2-dihydroacridine (acridine 1,2-oxide) 2

To a stirred solution of cis-dihydrodiol 4 (0.1 g, 0.47 mmol) in dry MeCN (4 cm^3) was added, at 0 °C, 1-bromocarbonyl-1-methylethyl acetate (0.109 g, 0.52 mmol). The reaction mixture was stirred (1.5 h) at room temperature, diluted with Et$_2$O (30 cm^3) and the solution washed thoroughly with 5% aqueous NaHCO$_3$ solution (15 cm^3). The organic layer was dried (Na$_2$SO$_4$) and concentrated to yield an inseparable mixture of bromoacetates 25 and 26 in which bromoacetate 25 was the

major component (0.12 g, 81%); (Found: M$^+$ 317.0061. C$_{15}$H$_{12}$NO$_2$^{79}Br requires 317.0051).

(1R,2R)-1-Acetoxy-2-bromo-1,2-dihydroacridine 25. δ_H (500 MHz, CDCl$_3$) 1.99 (3 H, s, OCOMe), 4.93 (1 H, dd, $J_{2,1}$ 2.5, $J_{2,3}$ 5.7, 2-H), 6.35 (1 H, d, $J_{1,2}$ 2.5, 1-H), 6.62 (1 H, dd, $J_{3,2}$ 5.7, $J_{3,4}$ 9.8, 3-H), 7.01 (1 H, d, $J_{4,3}$ 9.8, 4-H), 7.55–8.09 (4 H, m, Aromatic), 8.27 (1 H, s, 9-H). **(1S,2S)-1-Bromo-2-acetoxy-1,2-dihydroacridine 26.** δ_H (500 MHz, CDCl$_3$) 2.00 (3 H, s, OCOMe), 5.51 (1 H, d, $J_{1,2}$ 2.5, 1-H), 5.73(1 H, dd, $J_{2,1}$ 2.5, $J_{2,3}$ 5.4, 2-H), 6.58 (1 H, dd, $J_{3,2}$ 5.4, $J_{3,4}$ 9.7, 3-H), 7.16 (1 H, d, $J_{4,3}$ 9.7, 4-H), 7.54–8.09 (4 H, m, Aromatic), 8.13 (1 H, s, 9-H); m/z (EI): 317 (M$^+$, ^{79}Br, 41%), 238 (80), 196 (100).

A cooled solution (0 °C) of bromoacetates 25 and 26 (0.1 g, 0.31 mmol) in dry Et$_2$O (10 cm^3) was treated with NaOMe (24 mg, 0.44 mmol). The reaction mixture was stirred overnight at room temperature. After addition of water (10 cm^3) to the reaction mixture, it was extracted with Et$_2$O (3 × 15 cm^3), the combined ether extract dried (Na$_2$SO$_4$) and the solvent evaporated under reduced pressure to yield (1R,2S)-acridine-1,2-oxide 2 as a light brown solid (51 mg, 83%); m.p. 136–137 °C (CHCl$_3$/hexane) (lit.[9] 136–138 °C); $[\alpha]_D$ +30 (c 0.49, CHCl$_3$) (lit.[9] +22, CHCl$_3$); (Found: M$^+$ 195.0689. C$_{13}$H$_9$NO requires 195.0684); δ_H (500 MHz, CDCl$_3$) 4.08 (1 H, dd, $J_{2,1}$ 3.8, $J_{2,3}$ 6.1, 2-H), 4.55 (1 H, d, $J_{1,2}$ 3.8, 1-H), 6.78 (1 H, dd, $J_{3,2}$ 6.1, $J_{3,4}$ 10.0, 3-H), 7.03 (1 H, d, $J_{4,3}$ 10.0, 4-H), 7.51 (1 H, dd, $J_{6,7}$ 7.0, $J_{6,5}$ 8.2, 6-H), 7.67 (1 H, dd, $J_{7,6}$ 7.0, $J_{7,8}$ 8.6, 7-H), 7.78 (1 H, $J_{8,7}$ 8.6, 8-H), 8.02 (1 H, $J_{5,6}$ 8.2, 5-H), 8.30 (1 H, s, 9-H); δ_C (125 MHz, CDCl$_3$) 52.4, 56.7, 126.1, 127.4, 127.8, 127.9, 129.8, 130.1, 131.9, 134.5, 137.9, 148.6, 151.8; m/z (EI): 195 (M$^+$, 84%), 167 (100).

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

Dictamnine 7,8-oxide 27. To a solution of bromoacetate 33 (0.16 g, 0.47 mmol) in CCl$_4$ (4 cm^3) 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 dictamnine 7,8-oxide 27 from the crude dibromoacetate 34 (0.15 g, 0.36 mmol) was carried out following the second step of the method described for the synthesis of arene oxide 2, using dry THF (3 cm^3) instead of Et$_2$O 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 CHCl$_3$/hexane); (Found: M$^+$ 215.0586. C$_{12}$H$_9$NO$_3$ requires 215.0582); $[\alpha]_D$ +93.1 (c 0.36, CHCl$_3$); δ_H (500 MHz, CDCl$_3$) 4.18 (1 H, ddd, $J_{7,8}$ 3.71, $J_{7,6}$ 3.66, $J_{7,5}$ 1.84, 7-H), 4.30 (3 H, s, OMe), 4.65 (1 H, d, $J_{8,7}$ 3.84, 8-H), 6.37 (1 H, dd, $J_{6,5}$ 9.86, $J_{6,7}$ 3.66, 6-H), 7.01 (1 H, d, $J_{3,2}$ 2.57, 3-H), 7.20 (1 H, dd, $J_{5,6}$ 9.86, $J_{5,7}$ 1.8, 5-H), 7.62 (1 H, d, $J_{2,3}$ 2.57, 2-H); δ_C (125 MHz, CDCl$_3$) 54.4, 58.4, 58.9, 104.6, 105.3, 106.6, 113.5, 122.6, 124.4, 143.0, 148.8, 157.4; m/z (EI): 215 (M$^+$, 100%), 200 (57), 172 (30), 83 (27); ECD: λ/nm 322 ($\Delta\varepsilon$ 0.747), 288 ($\Delta\varepsilon$ 0.867), 241 ($\Delta\varepsilon$ 7.87), 203 ($\Delta\varepsilon$ −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 CH$_2$Cl$_2$ (15 cm^3) was treated, drop-wise, with a solution of boron

tribromide in CH_2Cl_2 (1 M, 4.8 cm^3). After leaving the reaction mixture stirred, at room temperature overnight, it was cooled to −60 °C and water (5 cm^3) was added and the mixture allowed to warm up to room temperature. The CH_2Cl_2 layer was separated and the remaining aqueous solution was extracted with EtOAc (2 × 10 cm^3). The combined organic extract was dried (Na_2SO_4) 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); R_f 0.1 (50% EtOAc/hexane); (Found: M$^+$, 231.0523. $C_{12}H_9NO_4$ requires M$^+$, 231.0532); δ_H (500 MHz, CD_3COCD_3) 4.39 (3 H, s, OMe), 6.99 (1 H, d, $J_{6,5}$ 9.1, 6-H), 7.23 (1 H, d, $J_{2,3}$ 2.8, 2-H), 7.52 (1 H, d, $J_{5,6}$ 9.1, 5-H), 7.66 (1 H, d, $J_{3,2}$ 2.8, 3-H).

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

Robustine 30. Dihydrodiol **17** (10 mg, 0.04 mmol), dissolved in $CHCl_3$ solution, was aromatised 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.[13b] 148–149 °C); δ_H (300 MHz, $CDCl_3$) 4.43 (3 H, s, OMe), 7.17 (1H, d, $J_{3,2}$ 2.8, 3-H), 7.20 (1 H, d, $J_{7,6}$ 7.8, 7-H), 7.38 (1 H, dd, $J_{6,7} = J_{6,5} =$ 7.8, 6-H), 7.63 (1 H, d, $J_{2,3}$ 2.8, 2-H), 7.79 (1 H, d, $J_{5,6}$ 7.8, 5-H); m/z (EI): 215 (M$^+$, 100%), 200 (59).

γ-Fagarine 31. Robustine **30** (5 mg, 0.023 mmol) was dissolved in MeOH (1 cm^3) and treated (0 °C) with an excess of ethereal solution of diazomethane. The solvents were evaporated under a stream of nitrogen to yield the title compound **31** (4.2 mg, 79%); m.p. 142–143 °C (lit.[13c] 141 °C); δ_H (500 MHz, $CDCl_3$) 4.09 (3 H, s, OMe), 4.46 (3 H, s, OMe), 7.01 (1 H, d, $J_{7,6}$ 7.8, H-7), 7.09 (1 H, d, $J_{3,2}$ 2.7, H-3), 7.35 (1 H, dd, $J_{6,5}$ 8.3 $J_{6,7}$ 7.8, H-6), 7.65 (1 H, d, $J_{2,3}$ 2.7, H-2), 7.85 (1 H, d, $J_{5,6}$ 8.3, H-5); δ_C (125 MHz, $CDCl_3$): 29.6, 56.0, 59.0, 104.5, 107.7, 114.1, 118.9, 123.2, 123.4, 129.7, 143.9, 154.5, 157.2; m/z (EI): 229 (M$^+$, 100%), 214 (18).

Haplopine 36. Catechol **29** (50 mg, 0.20 mmol) was 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 (50% EtOAc/hexane), to give haplopine **36** as a white solid (27 mg, 55%); m.p. 203–205 °C (from EtOAc–hexane) (lit.[13d] 203–204 °C); R_f 0.2 (50% EtOAc/hexane); δ_H (500 MHz, $CDCl_3$) 4.22 (3 H, s, OMe), 4.43 (3 H, s, OMe), 7.05 (1 H, d, $J_{3,2}$ 2.8, 3-H), 7.17 (1 H, d, $J_{6,5}$ 9.2, 6-H), 7.58 (1 H, d, $J_{2,3}$ 2.8, 2-H), 7.97 (1 H, d, $J_{5,6}$ 9.2, 5-H).

4-Methoxy-8-[(3-methyl-2-butenyl)oxy]furo[2,3-b]quinolin-7-ol 37. A mixture of catechol **29** (50 mg, 0.22 mmol), anhydrous K_2CO_3 (50 mg) and 1-chloro-3-methyl-but-2-ene (0.03 cm^3, 0.24 mmol) in dry acetone (5 cm^3) was heated under reflux (2 h). The solvent was removed under reduced pressure, the residue taken up in EtOAc (10 cm^3), the solution washed with water (5 cm^3) and dried (Na_2SO_4). Removal of solvent under reduced pressure yielded the crude product which was purified by multiple elution PLC (30% EtOAc/hexane) to furnish the title compound **37** as a white solid (46 mg, 70%); m.p. 152–153 °C (from EtOAc/hexane); R_f 0.2 (50% EtOAc/hexane); (Found: M$^+$, 299.1166. $C_{17}H_{17}NO_4$ requires M$^+$, 299.1158); δ_H (500 MHz, $CDCl_3$) 1.62 (3 H, s, Me), 1.75 (3 H, s, Me), 4.43 (3 H, s, OMe), 5.01 (2 H, d, $J_{2',3'}$ 7.5, 2'-H), 5.60 (1 H, t, $J_{3',2'}$ 4.5, 3'-H), 7.05 (1 H, d, $J_{3,2}$ 2.8, 3-H), 7.15 (1 H, d, $J_{6,5}$ 9.2, 6-H), 7.57 (1 H, d, $J_{2,3}$ 2.8, 2-H), 7.95 (1 H, d, $J_{5,6}$ 9.2, 5-H); m/z (EI): 299 (M$^+$, 20%), 231 (100).

iso-Haplopine-3,3′-dimethylallylether 38. 4-Methoxy-8-[(3-methyl-2-butenyl)oxy]furo[2,3-b]quinolin-7-ol **37** (20 mg, 0.067 mmol) was treated with an excess of diazomethane as described earlier. The crude methylated product was purified by PLC to give compound **38** as a white solid (19 mg, 90%); m.p. 124–125 °C (from CH_2Cl_2) (lit.[13e] 120–121.5 °C); R_f 0.4 (40% EtOAc/hexane); (Found: M$^+$, 313.1312. $C_{18}H_{19}NO_4$ requires 313.1314); δ_H (500 MHz, $CDCl_3$) 1.66 (3 H, s, Me), 1.74 (3 H, d, Me), 4.00 (3 H, s, OMe), 4.43 (3 H, s, OMe), 4.84 (2 H, d, $J_{1',2'}$ 7.2, 1'-H), 5.73 (1 H, t, $J_{2',1'}$, 7.2, H-2'), 7.04 (1 H, d, $J_{3,2}$ 2.8, 3-H), 7.22, d, $J_{6,5}$ 9.4, 6-H), 7.58 (1 H, d, $J_{2,3}$ 2.80, 2-H), 8.00 (1 H, d, $J_{5,6}$ 9.4, 5-H); m/z (EI): 313 (M$^+$, 20%).

Pteleine 40. cis-Dihydrodiol **16** (7 mg, 0.004 mmol) was dissolved in $CDCl_3$ in an NMR tube and a drop of TFA added to the solution. The tube was kept at 50 °C until the aromatization of diol **16** was complete (¹H-NMR analysis). After removal of solvent the aromatized product was treated with excess of diazomethane and the crude product obtained was purified by PLC (40% EtOAc/hexane) to afford pure pteleine **40** as a white solid (3 mg, 77%); m.p. 132–134 °C (lit.[13f] 134.5 °C); R_f 0.4 (40% EtOAc/hexane); δ_H (500 MHz, $CDCl_3$) 3.95 (3 H, s, Me), 4.60 (3 H, s, Me), 7.07 (1 H, d, $J_{3,2}$ 2.8, 3-H), 7.36 (1 H, dd, $J_{7,8}$ 9.15, $J_{7,5}$ 2.95, 7-H), 7.53 (1 H, d, $J_{8,7}$ 2.95, 8-H), 7.63 (1 H, d, $J_{2,3}$ 2.8, 2-H), 7.92 (1 H, d, $J_{8,7}$ 9.15, 8-H).

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