Synthesis, structure and stereochemistry of quinoline alkaloids from *Choisya ternata*†

Derek R. Boyd,*[a] Narain D. Sharma,[a] Pui L. Loke,[a] John F. Malone,[a] W. Colin McRoberts[b] and John T. G. Hamilton[b,c]

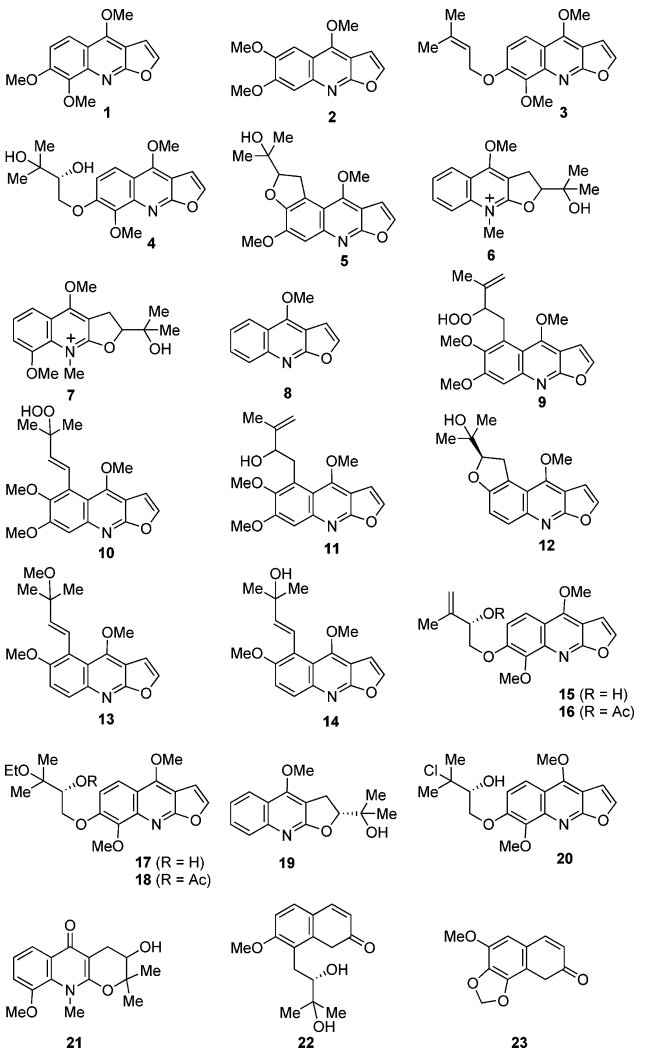
Received 18th May 2007, Accepted 10th July 2007
First published as an Advance Article on the web 7th August 2007
DOI: 10.1039/b707576f

A range of seventeen quinoline alkaloids, involving several types of oxidations during their biosynthetic pathways, have been isolated from leaves of *Choisya ternata*. In addition to the nine known quinoline alkaloids, eight new members of the furoquinoline family, derived mainly from prenylation at C-5 (including two novel hydroperoxides), have been identified. The absolute configurations and enantiopurity values of all chiral quinoline alkaloids have been determined. One of the isolated alkaloids, 7-isopentenyloxy-γ-fagarine, has been used as a precursor for the chemical asymmetric synthesis of the enantiopure alkaloids: evoxine, anhydroevoxine and evodine. The possible roles of oxygenase and other oxygen-atom-transfer enzymes, in the biosynthetic pathways of the *C. ternata* alkaloids, have been discussed.

Introduction

The common ornamental shrub *Choisya ternata* (Mexican orange), a member of the *Rutaceae* family, had earlier been shown to contain seven quinoline alkaloids: skimmianine **1**, kokusaginine **2**, 7-isopentenyloxy-γ-fagarine **3**, evoxine **4**, choisyine **5**, platydesminium methosalt **6** and balfourodinium methosalt **7**.[1–3] During the course of our continuing study of the synthesis and metabolism of quinoline alkaloids, and their isolation from plant sources,[3–9] it became evident that neither the enantiomeric excess (ee) values nor absolute configurations, of many known chiral quinoline alkaloids from *C. ternata* (*e.g.* evoxine **4** and choisyine **5**) and other *Rutaceous* plants, had been determined. Upon completion of our synthesis of chiral quinoline alkaloids in enantiopure form,[8,9] it was found that a significant proportion of earlier assignments were incorrect, *e.g.* platydesminium methosalt **6** and possibly balfourodinium methosalt **7**. After the isolation of eleven chiral quinoline alkaloids from *C. ternata* (including compounds **4**, **5** and **7**) in the current study, the requirement for a generally applicable, sensitive and reliable method of determination of both enantiopurity and absolute configuration of quinoline alkaloids, became essential. In a preliminary communication of this work,[3] a generally applicable method, involving a combination of ozonolysis and chiral stationary phase-GC-MS (CSPGC-MS), was reported and has been utilised herein.

The role of bacterial dioxygenase enzymes in *cis*-dihydroxylation at the 2,3-, 5,6- and 7,8-bonds of the biosynthetic precursor and parent furoquinoline alkaloid, dictamnine **8**, has recently been reported from these laboratories.[9] This study has prompted us to investigate the potential involvement of oxygenase or other oxygen-atom-transfer enzymes in the formation of



[a]*School of Chemistry and Chemical Engineering, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[b]*Agri-food Biosciences Institute for Northern Ireland, Newforge Lane, Belfast, UK BT9 5PX*
[c]*School of Agriculture and Food Science, The Queen's University of Belfast, Newforge Lane, Belfast, UK BT9 5PX*

† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/b707576f

phenol, epoxide, hydroperoxide and other derivatives of quinoline alkaloids during their biosynthesis in *C. ternata*.

The major objectives of this chemical investigation of *C. ternata* leaves are thus: (i) to establish the presence of known alkaloids and the structure of new quinoline alkaloids including the first two hydroperoxide members of the family, (ii) to utilise appropriate methods for the determination of ee values and absolute configurations of the isolated chiral alkaloids, (iii) to suggest feasible biosynthetic pathways for the formation of quinoline alkaloids in *C. ternata*, based upon rigorous structure and absolute configuration determinations, chemotaxonomy considerations, biomimetic syntheses and earlier literature reports of labelling studies.

Results and discussion

(i) Isolation and identification of quinoline alkaloids from *C. ternata*

The methods used for the isolation of quinoline alkaloids were similar to those reported earlier.[2–9] The identification of compounds was facilitated by the availability of authentic samples and spectroscopic data for quinoline alkaloids obtained both from chemical synthesis and from earlier studies of *C. ternata* and *Skimmia japonica*.[2–10] A crude mixture of quinoline alkaloids and coumarins was obtained in the initial step by aqueous ethanol extraction of dried leaves of *C. ternata* (2 kg) followed by concentration. Further purification was achieved by sequential extraction using solvents of increasing polarity including diethyl ether (extract A), ethyl acetate (extract B) and water (extract C). Isolation of alkaloids from extract A (30 g) by conversion into their hydrochloride salts followed base treatment and flash chromatography of the mixture on silica gel yielded less polar components including most of the quinoline alkaloids and a coumarin. Four of the major quinoline alkaloids, skimmianine **1**, 7-isopentenyloxy-γ-fagarine **3**, evoxine **4**, and choisyine **5**, had been reported earlier as metabolites of *C. ternata*.[1,2] Flash chromatography and multiple elution PLC yielded, in addition to the anticipated quinoline alkaloids **1**, **3**, **4** and **5**, thirteen additional alkaloids. The identified components are discussed in order of increasing polarity as they were eluted from the chromatography column.

The least polar compound (R_f 0.35, $CHCl_3$), a very minor component (0.010 g), contained an unprecedented hydroperoxide group as part of quinoline alkaloid structure **9**, which was established with the aid of X-ray crystallography (Fig. 1) and spectral analysis.‡ While the X-ray analysis showed the allylic hydroperoxide group to be attached directly to a stereogenic centre, it also confirmed that the new alkaloid **9** was racemic. A further new minor alkaloid of similar polarity (0.015 g, R_f 0.35, $CHCl_3$) was found to be an achiral allylic hydroperoxide isomer of alkaloid **9**; its structure **10** was determined by NMR spectral analysis and MS data.

The next quinoline alkaloids to be eluted from the chromatography column were the known achiral compounds 7-isopentenyloxy-γ-fagarine **3** (0.3 g, R_f 0.8, 80% EtOAc in hexane), skimmianine **1** (1.8 g, R_f 0.3, 1% MeOH in $CHCl_3$) and kokusaginine **2** (0.05 g, R_f 0.35, 1% MeOH in $CHCl_3$,). The ^{1}H-NMR spectrum of a slightly more polar quinoline alkaloid (0.015 g, R_f 0.2, 2% MeOH in $CHCl_3$) showed a marked similarity to that of hydroperoxide **9** and was found to be the racemic deoxygenation product having structure **11**.

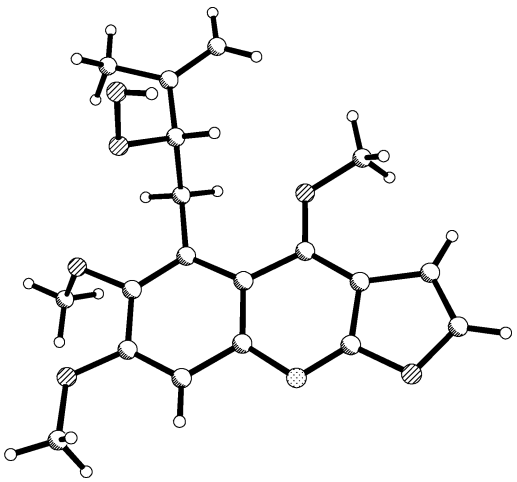


Fig. 1 X-Ray crystal structure of alkaloid **9**.

Quinoline alkaloid **12** (0.25 g, R_f 0.15, 2% MeOH in $CHCl_3$) and choisyine **5** (2.70 g, R_f 0.10, 2% MeOH in $CHCl_3$), although of similar polarity, proved to be separable. Alkaloid **12** and choisyine **5** also showed very similar ^{1}H-NMR spectral characteristics except for the absence of a methoxy group at C-4 in compound **12**. The previously unreported alkaloid **12**, now identified as 4-desmethoxychoisyine, was found to be enantiomerically enriched ($[\alpha]_D$ −43.8, $CHCl_3$). No optical rotation was observed for the major alkaloid choisyine **5** isolated from *C. ternata* in this or earlier studies and on this basis it was assumed to be racemic.

The next two alkaloids (**13** and **14**) to be eluted showed ^{1}H-NMR spectra similar to that of the achiral hydroperoxide **10** and also gave no measurable optical rotations. They were identified as the corresponding achiral alcohol **14** (0.010 g, R_f 0.3, 1% MeOH in $CHCl_3$) and its methyl ether derivative **13** (0.015 g, R_f 0.5, 1% MeOH in $CHCl_3$).

An inseparable mixture of three quinoline alkaloids (**15**, **17** and **19**) was obtained in a single chromatographic fraction despite using multiple-elution PLC. However, on treatment of the mixture with acetic anhydride, separation was achieved when the monoacetate derivatives **16** and **18** (from alkaloids **15** and **17** respectively) were formed. Alkaloid derivative **16** (0.007 g, R_f 0.5, 50% EtOAc in hexane) was optically active ($[\alpha]_D$ −7.9, $CHCl_3$) and was identified as evodine acetate **16** indicative of the presence of evodine **15** as a very minor component of *C. ternata*. The second acetate **18** (0.008 g, R_f 0.2, 1% MeOH in $CHCl_3$) proved to be a derivative of the ethyl ether of evoxine **17** and also gave an optical rotation ($[\alpha]_D$ −8.8, $CHCl_3$). The third component of the mixture of quinoline alkaloids was identified as platydesmine **19** (0.010 g, R_f 0.15, 1% MeOH in $CHCl_3$) with an excess of one enantiomer ($[\alpha]_D$ −42.0, MeOH) whose tertiary alcohol group was not acetylated.

The next alkaloids to be eluted proved to have similar chiral structures and were identified as a chlorohydrin **20** (0.210 g, R_f 0.20, 80% EtOAc in hexane, $[\alpha]_D$ +14.2, MeOH) and evoxine **4**

‡ CCDC reference numbers 644683 and 644684. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b707576f

(0.80 g, R_f 0.2, EtOAc, $[\alpha]_D$ +15.0, EtOH) which had earlier been reported from *C. ternata*. The structure and absolute configuration of the unusual chlorohydrin alkaloid **20** was confirmed by X-ray crystallography (Fig. 2).‡ While the asymmetric unit consisted of four crystallographically independent molecules, all were shown unambiguously to have the (*R*) configuration and differed only in the conformation of the side-chains.

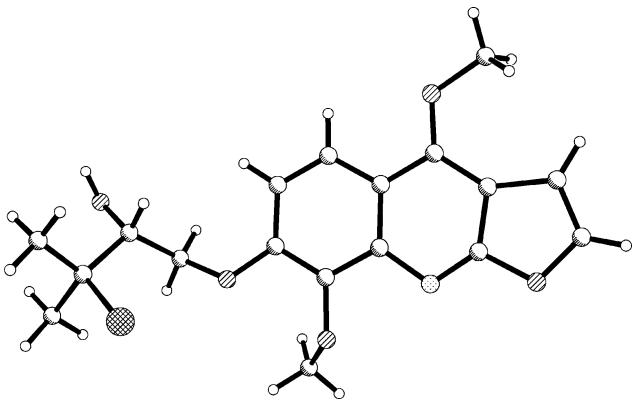


Fig. 2 X-Ray crystal structure of alkaloid **20**.

The most polar alkaloid isolated was identified as isobalfourodine **21** (0.80 g, R_f 0.15, EtOAc). Although isobalfourodine **21** is chiral, and had been isolated earlier with an excess of one enantiomer, in this study it appeared to be racemic.

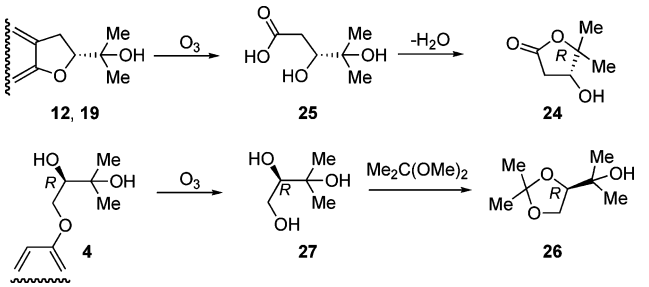
From extract A, a known coumarin, meranzin hydrate **22** (0.005 g, R_f 0.15, 80% EtOAc in hexane, $[\alpha]_D$ −33.0, $CHCl_3$,), was also isolated; a similar observation was made earlier where coumarin **22** was again found among the quinoline alkaloid fractions in *S. japonica*.[10] The EtOAc extract (extract B) yielded a further achiral coumarin **23** (0.010 g, R_f 0.3, 50% EtOAc in hexane) which had also been reported earlier from other plants.

The water-soluble quaternary alkaloid fraction in extract C was precipitated as an insoluble reineckate salt and then converted into the corresponding perchlorate anion using an ion exchange column. Thus, a pure sample of *O*-methylbalfourodinium perchlorate **7** was isolated (2.30 g) but was found to be optically inactive.

(ii) Determination of absolute configurations and enantiopurity values of alkaloids

From the seventeen quinoline alkaloids isolated from *C. ternata*, eleven were found to contain one chiral centre. In this category five gave no measurable optical rotation and were thus assumed to be racemic (**5**, **7**, **9**, **11**, **21**) while six (**4**, **12**, **15**, **17**, **19**, **20**) were found to have optical rotations. Prior to our preliminary report[3] of a new approach, to the determination of the absolute configuration and enantiopurity for chiral hemiterpenoids involving ozonolysis and chiral stationary phase GC-MS (O_3–CSP-GC-MS), no reliable and generally applicable method was available. Through this approach and the use of stereochemical correlation methods, the absolute configurations of the enantiomerically enriched alkaloids **4**, **12**, **15**, **17**, **19**, **20** were determined, and the racemic nature of the other alkaloids (**5**, **7**, **9**, **11**, **21**) confirmed.

Using the O_3–CSP-GC-MS analysis method, alkaloids **12** and **19** were converted to the corresponding (+)-lactone **24** *via* spontaneous dehydration and cyclisation of the dihydroxycarboxylic acid

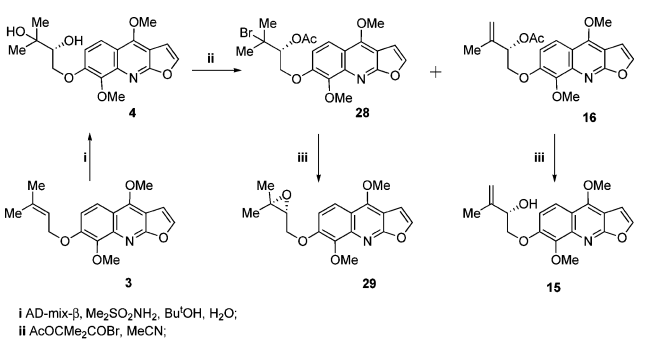


Scheme 1

25 (Scheme 1). The (*R*) absolute configuration of (+)-3-hydroxy-4,4-dimethyl-4-butyrolactone **24** was unequivocally determined, by X-ray crystallographic study of the corresponding camphanate and CSP-GC-MS analysis.[3] The (*R*) lactone **24** eluted earlier than the corresponding (*S*) enantiomer from a Sulpelco γ-DEX 120 capillary column and this observation allowed the absolute configurations of (−)-desmethoxychoisyine **12** (64% ee) and (−)-platydesmine **19** (90% ee) to be unequivocally assigned as (*R*). Ozonolysis of (+)-evoxine **4** gave a triol **27**, which was, in turn, converted to the corresponding (+)-acetonide **26** of known absolute configuration. CSP-GC-MS analysis showed a preponderance of the later eluting (*R*) enantiomer of compound **26** (86% ee) which confirmed the (*R*) configuration and ee value for (+)-evoxine **4** (Scheme 1). A similar ee value (91%) was obtained for (+)-evoxine **4** by ^{1}H-NMR analysis of its acyclic boronate diastereoisomers, from the *vic*-diol moiety, using (+)-(*R*)- and (−)-(*S*)-2-(1-methoxyethylbenzene boronic acid) (MEBBA).[11] On this basis, an error of ±5% should be assumed with these ee determinations The MEBBA method was also used to estimate the ee value (95%) for (−)-meranzin hydrate **22**.

The O_3–CSP-GC-MS method[3] was also employed to establish an (*S*) configuration, and to confirm the enantiopurity value of (−)-meranzin hydrate **22** (92% ee). This method also confirmed that samples of choisyine **5**, *O*-methylbalfourodinium perchlorate **7** and isobalfourodine **21** isolated from the leaves of *C. ternata* were indeed racemic.

The absolute configurations of the optically active alkaloids **15** and **20** from *C. ternata* were obtained by stereochemical correlation (Scheme 2) and X-ray crystallography (Fig. 2) respectively. The limited quantity of (*R*)-evoxine **4** (86–91% ee) isolated from *C. ternata* was supplemented by asymmetric dihydroxylation of the dimethylallyl group of 7-isopentenyloxy-γ-fagarine **3** using the β-form of AD-mix as a chiral oxidant (Scheme 2). The



Scheme 2

chemically-derived sample of (*R*)-evoxine **4** (93% ee) was combined with the plant-derived material and recrystallised to give an enantiopure sample which was subsequently used as a synthetic precursor of (*R*)-anhydroevoxine **29** *via* bromoacetate **28**, and of (*S*)-evodine **15**, *via* the evodine acetate **16**. This approach provided a reliable stereochemical correlation route between alkaloids **4**, **15**, **16** and **29** (Scheme 2).

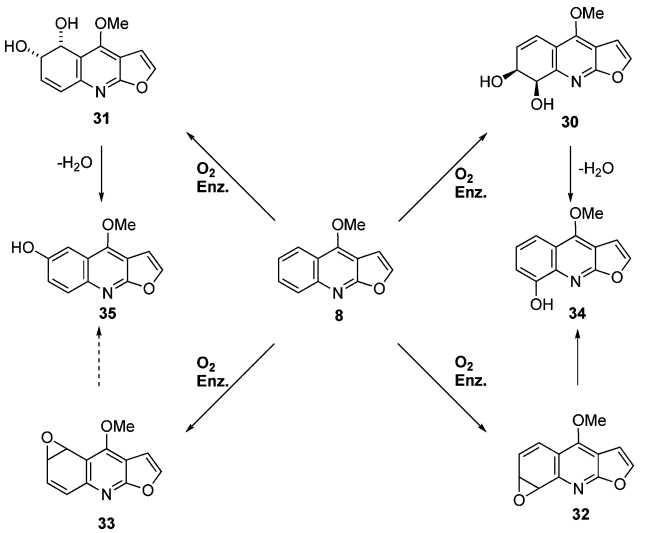
The absolute configuration of the new chlorohydrin alkaloid (+)-**20** was assigned as (*R*) by X-ray crystallography using the Bijvoet method (Fig. 1). It is probable that (*R*)-evoxine **4**, (*S*)-evodine **15**, (*R*)-chlorodesoxyevoxine **20** and ethylevoxine **17** were all obtained by ring opening of the transient intermediate (*R*)-anhydroevoxine **29**. Using this stereochemical correlation method, a similar (*R*) configuration was assumed for ethylevoxine **17** and its acetate derivative **18**.

It is difficult to rationalise the formation of some alkaloids, as racemates *e.g.* choisyine **5**, as single enantiomers *e.g.* platydesmine **19**, and as enantiomeric mixtures *e.g.* desmethoxychoisyine **19**. However, as the occurrence of racemates had been reported earlier for quinoline alkaloids from different members of the *Rutaceae* family, it is tempting to speculate about possible reasons for these observations. Among factors that could be responsible are the (i) variable stereoselectivity of plant monooxygenase enzymes catalysing the key epoxidation step where chirality is introduced, (ii) partial racemisation of the single chiral centre during the acid–base isolation procedure, (iii) formation of epoxide intermediates from racemic or achiral oxidants *e.g.* hydroperoxides **9** and **10**. Based on our earlier experience of examining the stereoselectivity associated with both monooxygenase and dioxygenase enzymes, the dominant factor is likely to be number (i).

(iii) Potential biosynthetic pathways for the formation of alkaloids from *C. ternata*

Biosynthetic studies of quinoline alkaloids found in *C. ternata*, using [3–^{14}C] and [2,3–$^{3}H_2$:3–^{14}C]- labelled dictamnine **8**, have shown isotope incorporation into skimmianine **1**, evoxine **4** and choisyine **5** where substitution has occurred at C-5, C-6, C-7 and C-8 positions.[2] These studies suggest that dictamnine **8** may also be a key precursor of other furoquinoline alkaloids found in *C. ternata.* The dioxygenase-catalysed formation of *cis*-dihydrodiol intermediates, *e.g.* **30** and **31**, or monooxygenase-catalysed formation of transient arene oxides, *e.g.* **32** and **33**, which could yield hydroxydictamnine isomers, *e.g.* **34** and **35** from dictamnine **8** (*via* dehydration of dihydrodiols or isomerisation of arene oxides), had been postulated more than thirty years ago[2] (Scheme 3).

It is probable that the major enzymes responsible for oxidation of dictamnine **8** and its derivatives in *C. ternata*, in common with other eukaryotic systems (fungi, animals), are monooxygenases rather than dioxygenases. These enzymes can catalyse the oxidation of arenes to arene oxides or phenols and alkenes to alkene oxides. By contrast, the ring hydroxylating dioxygenase enzymes are generally associated with prokaryotic systems (bacteria), and catalyse the oxidation of arenes to *cis*-dihydrodiols and phenols (after spontaneous dehydration) and alkenes to *vic*-diols. Recent studies of the enzyme-catalysed oxidation of dictamnine **8**, using a biphenyl dioxygenase, present in whole cells of a mutant strain (B8/36) of the soil bacterium *Sphingomonas yanokuyae*, have



Scheme 3

shown that *cis*-dihydroxylation occurs preferentially at the 7,8-bond to yield *cis*-diol **30**. Only a very minor proportion of oxidation occurred at the 5,6- bond to give *cis*-diol **31** (Scheme 3) or at the 2,3- bond to yield an unstable furan *cis*-diol, a precursor of the isolated acyclic 2-quinolone diol.[9] The major *cis*-dihydrodiol metabolite **30** was then used as a precursor for the four-step synthesis of dictamnine 7,8-oxide **32**. This compound, which had earlier been proposed as a transient biosynthetic intermediate,[2] in practice proved to be a relatively stable arene oxide. Dehydration of the *cis*-dihydrodiols **30** and **31**, under acidic conditions (trifluoroacetic acid), gave mainly the corresponding phenols 8-hydroxydictamnine (robustine) **34** and 6-hydroxydictamnine **35**. Similar treatment of arene oxide **32** resulted in isomerization to yield robustine **34** again as the major product.[9]

Phenols, 7-hydroxydictamnine (confusameline), 8-hydroxydictamnine (robustine) **34**, and the corresponding methyl ether derivatives 7-methoxydictamnine (evolitrine) and 8-methoxydictamnine (γ-fagarine) are known alkaloids in other members of the *Rutaceae* family. However, neither 5-hydroxydictamnine nor 6-hydroxydictamnine have yet to our knowledge been found as natural products. Nevertheless, the isolation of the corresponding methyl ethers, *i.e.* 5-methoxydictamnine from *Ruta chalepensis* and 6-methoxydictamnine (pteleine), from more than twelve different types of *Rutaceous* plants, supports the view that phenol **35** is probably a common transient biosynthetic intermediate in the formation of several furoquinoline alkaloids in *C. ternata*.

It is noteworthy that: (i) phenolic alkaloids **34** and **35** are the major products obtained from dehydration of the corresponding *cis*-dihydrodiols **30** and **31**, (ii) aromatisation of arene oxide **32** also yields mainly phenol **34** and it is expected that arene oxide **33** would rearrange to give phenol **35**, (iii) both *trans*-dihydrodiol (derived from hydrolysis of arene oxides) and *cis*-dihydrodiol metabolites of quinoline, on dehydration, have been shown to give the same phenolic products.[12] On this premise, the biosynthetic pathways shown in Schemes 4–6 utilize compounds **34** and **35** as potential precursors for many of the furoquinoline alkaloids found in *C. ternata* rather than the alternative 5- or 7-hydroxydictamnine isomers.

While the relevant biosynthetic labelling studies remain to be carried out on the new alkaloid products from *C. ternata*, based on the earlier reports and chemotaxonomic support,[2] it is possible to speculate on the potential role of the hydroxydictamnine isomers **34** and **35** as intermediates, and of enzyme-catalysed oxidation steps involved during the biosynthesis of alkaloids **1**, **3**, **4**, **15**, **17**, **20** (from robustine **34**, Scheme 4) and **2**, **5**, **9**, **10**, **11**, **12**, **13**, **14** (from 6-hydroxydictamnine **35**, Schemes 5 and 6).

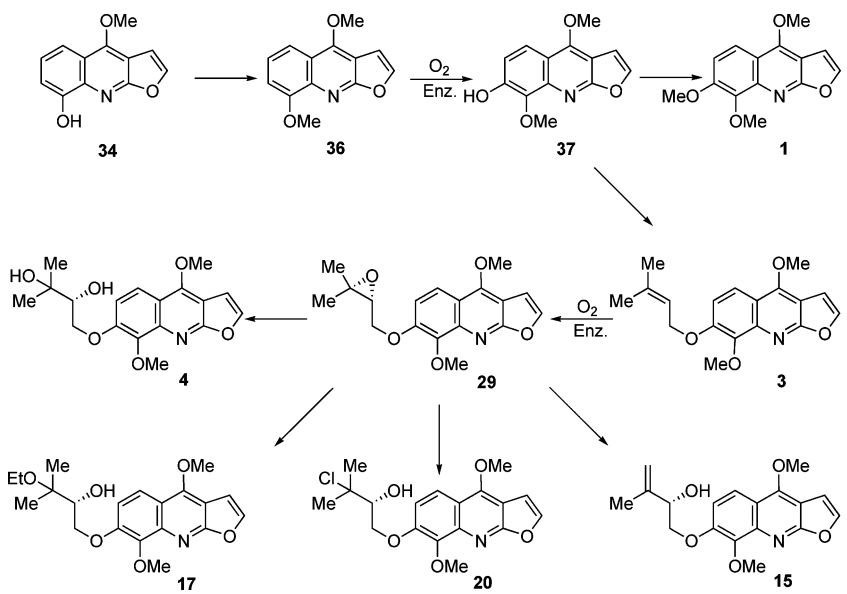
Although the biotransformation of dictamnine **8** to skimmiamine **1** has been established by labelling studies,[2] the exact sequence of enzyme-catalysed *O*-methylation and aromatic hydroxylation steps has not. The biosynthetic sequence for skimmiamine **1** from robustine **34** shown in Scheme 4 assumes the involvement of the known quinoline alkaloids γ-fagarine **36** and haplopine **37**. Prenylation of haplopine **37** to form 7-isopentenyloxy-γ-fagarine **3**, followed by monooxygenase-catalysed asymmetric epoxidation, provides a logical route to (*R*)-anhydroevoxine **29**. While it was not isolated from *C. ternata* during the present study due to the isolation procedure adopted, (*R*)-anhydroevoxine **29** has been obtained directly from furoquinoline **3** by asymmetric epoxidation in other plants, and indirectly by chemical asymmetric dihydroxylation in this study (Scheme 2). The (*R*)-enantiomer of epoxide **29** appears to be the obvious precursor of the enantiopure quinoline alkaloids (*R*)-evoxine **4**, (*S*)-evodine **15**, (*R*)-ethylevoxine **17** and (*R*)-chlorodesoxyevoxine **20**. Thus, the absolute configuration at each of the stereogenic centres in furoquinolines **4**(*R*), **15**(*S*), **17**(*R*) and **20**(*R*) is consistent with the ring-opening reactions of the (*R*) enantiomer of epoxide **29** and retention of configuration.

The non-racemic furoquinolines anhydroevoxine **29**, evoxine **4** and evodine **15** had all been identified earlier as plant alkaloids (of unspecified absolute configuration) while ethylevoxine **17** and chlorodesoxyevoxine **20** appear to be new members of the quinoline alkaloid family. The isolation of the alkaloids methylnkolbisine **39** and chlorodesnkolbisine **40** from *Teclea nobilis*,[13] having structural similarities to the quinoline alkaloids **17** and **20** from *C. ternata* is noteworthy. Both contain a tertiary alkylether group or tertiary chlorine substituent possibly derived from opening of a prenyl epoxide, *i.e.* alkaloids **39** and **40** from tecleoxine **38** (also isolated from *T. nobilis*) and compounds **17** and **20** from anhydroevoxine **29**. While the possibility of chlorodesnkolbisine **40** being formed as an artefact from the epoxide tecleoxine **38** was considered and excluded during the latter study,[13] the formation of furoquinoline products (*S*)-**15**, (*R*)-**17** and (*R*)-**20** as artefacts, derived from (*R*)-evoxine **4** and (*R*)-anhydroevoxine **29** in *C. ternata*, cannot be ruled out.

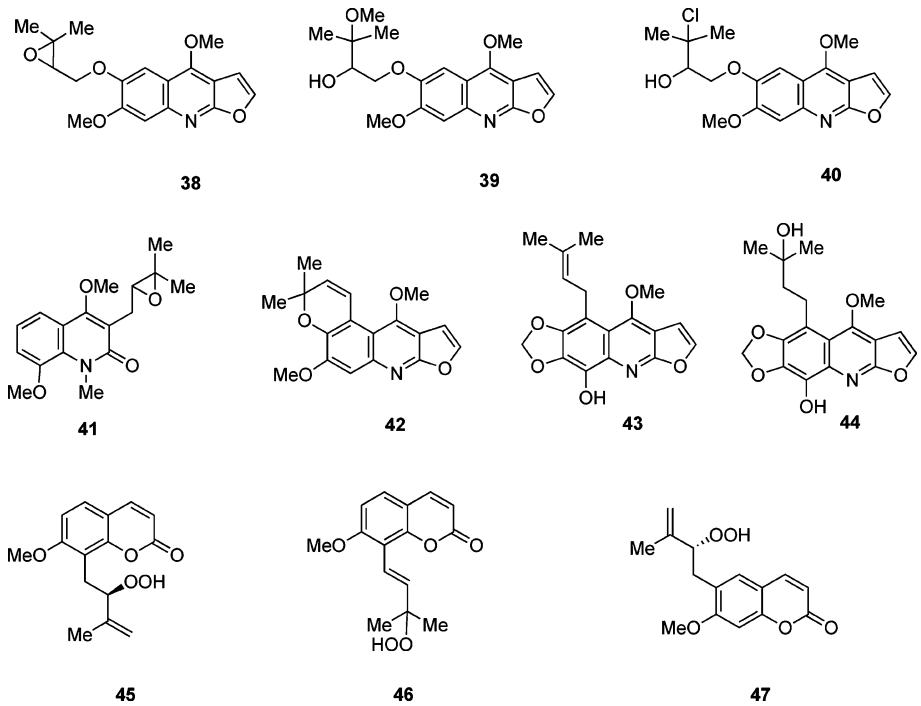
On the basis of labelling studies[2] and absolute stereochemistry correlation, the enantiomerically enriched (>90% ee) furoquinoline alkaloids **4**, **15**, **17** and **20** in *C. ternata* could all result from the initial hydroxylation and *O*-methylation of dictamnine **8** at C-8 (**34** → **36**, Scheme 4). This pathway may not, however, be involved in the biosynthesis of other chiral alkaloids bearing a methoxyl group at C-8, *i.e.* the balfourodinium methosalt **7** and isobalfourodine **21**. Based on their shared racemic nature, it is possible that alkaloids **7** and **19** were derived from a common racemic epoxide precursor, *e.g.* **41**, which may be formed during their biosynthesis *via* aromatic hydroxylation–methylation at C-8 of a 2-quinolone precursor followed by monooxygenase-catalysed epoxidation of a dimethylallyl substituent at C-3.

From a survey of the literature, it is evident that extensive oxygenation can occur at all positions in the carbocyclic aromatic ring of the parent furoquinoline alkaloid dictamnine **8** but C-prenylation is rare and generally occurs only at C-5. Among the few other examples of furoquinoline alkaloids, where C-prenylation has been found at the C-5 position, are: acronidine **42** (from *Acronychia baueri*),[14] tecleaverdoornine **43**, and tecleaverdine **44** (both from *Teclea verdoorniana*).[15] It is thus unusual to find seven furoquinoline alkaloids (**5**, **9–14**), each resulting from C-prenylation at the C-5 position, present in a single plant source (*C. ternata*).

Without direct evidence from labelling studies, it is not possible to confirm the biosynthetic sequences for the C-prenylated and derivatised furoquinoline alkaloids **5**, **9–14** in *C. ternata*. However,

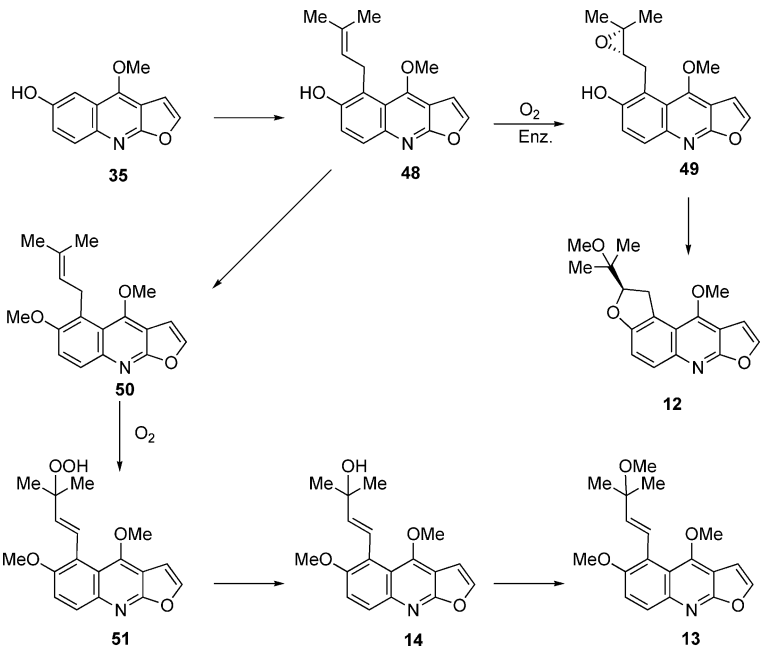


Scheme 4

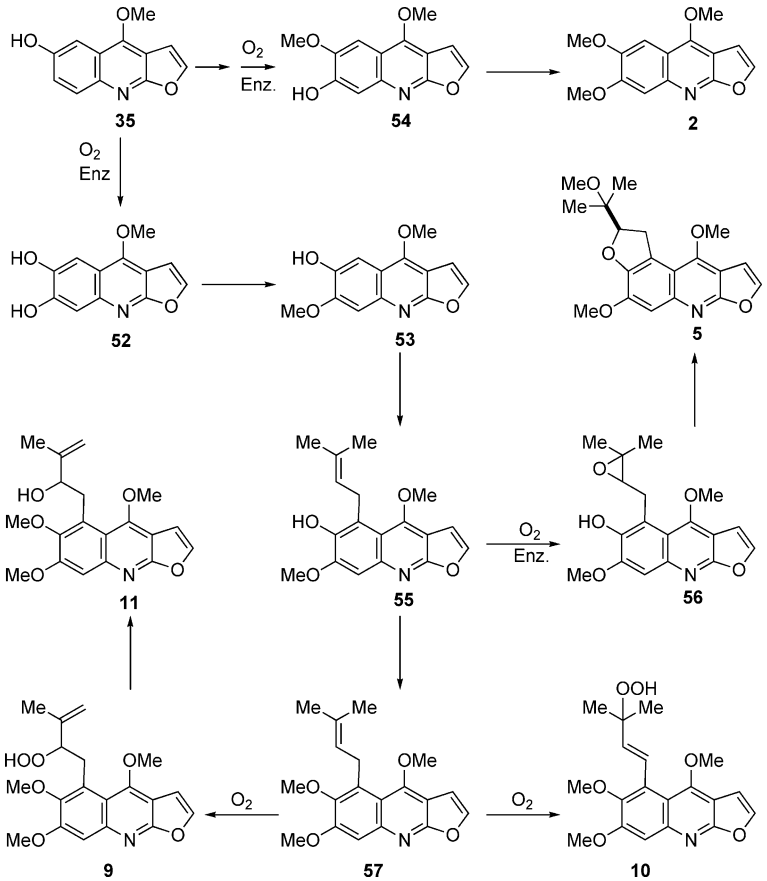


in view of the isolation of previously unknown hydroperoxide derivatives **9** and **10** of furoquinoline alkaloids, it is considered important to address the question of their formation. Potential biogenetic routes involving epoxidation and hydroperoxidation steps, that could account for the presence of both racemic and non-racemic furoquinolines, are shown in Scheme 5 (**12**–**14**) and Scheme 6 (**2**, **5**, **9**–**11**).

The initial step in Scheme 5 (**35** → **48**) assumes the insertion of a dimethylallyl group at C-5 adjacent to the phenolic OH group in 6-hydroxydictamnine **35**. Monooxygenase-catalysed asymmetric epoxidation of the resulting furoquinoline **48** would then yield, mainly, (*S*)-epoxide **49** prior to an intramolecular cyclisation with inversion of configuration (**48** → **49** → **12**) to yield the new alkaloid (*R*)-desmethoxychoisyine **12** (64% ee). The formation of the allylic alcohol **14**, and its methyl ether **13** could, in principle, be accounted for by methylation of furoquinoline **48** to give alkene **50** followed by the formation of a transient achiral hydroperoxide **51**. Deoxygenation of this hydroperoxide (**51** → **14**) could also account for the presence of the tertiary alcohol **14** among the range of plant alkaloids from *C. ternata*. The intermediacy of hydroperoxide **51** has been postulated in view of the isolation of the furoquinoline hydroperoxide **10** having a very similar structure



Scheme 5



Scheme 6

from the same plant source (Scheme 6). While the lipoxygenase-catalysed formation of hydroperoxides during the oxidation of allylic carbon atoms in non-conjugated dienes has been widely observed *in vivo*, some hydroperoxides isolated from natural sources have been considered to be artefacts.[16] A small number of earlier examples of hemiterpene hydroperoxide derivatives have been reported, *e.g.* coumarins **45**–**47**.

Enzymes appear to have been involved in the formation of enantiomerically enriched hydroperoxides from oxidation of a dimethylallyl group in coumarins, including peroxyauraptenol **45** (from *Melicope stipitata*)[17] and peroxytamarin **47** (from *Citrus sulcata*).[18] The allylic hydroperoxide peroxymurraol **46**, an achiral isomer of hydroperoxide **45**, was isolated from *Murraya exotica*.[19] The postulated hydroperoxide intermediate **51**, the isolated hydroperoxides **9** and **10** (Scheme 5), and racemic coumarins **46** and **47** could thus all be genuine new alkaloids resulting from enzyme-catalysed oxidation or artefacts formed through a non-enzymatic reaction of oxygen with a dimethylallyl group due to aerial autoxidation reactions.

Further evidence of the possible involvement of different types of oxidation reactions, occurring in *C. ternata*, is shown in Scheme 6. Aromatic hydroxylation (**35** → **52** and **35** → → **54**), epoxidation (**55** → **56**) and hydroperoxidation (**57** → **9** and **57** → **10**) steps are all postulated to account for the formation of the furoquinoline alkaloids **2**, **5**, **9**, **10** and **11** from 6-hydroxydictamnine **35**. Although the postulated biosynthetic intermediates 6-methoxydictamnine, delbine **53**, heliparvifoline **54**, and kokusaginine **2**, are known alkaloids, furoquinolines **35**, **52**, **55** and **57**, to our knowledge, have not yet been reported as plant alkaloids.

On account of the novel hydroperoxide group present in alkaloid **9**, unequivocal identification by spectroscopic methods proved difficult until X-ray crystallographic analysis firmly established both its structure and racemic nature. As shown earlier, while hydroperoxide derivatives from other hemiterpenoids have been isolated as plant products, furoquinolines **9** and **10** are, to our knowledge, the first examples of hydroperoxide derivatives to be reported from the quinoline alkaloid family. It should thus be reemphasised that the formation of the hemiterpenoid hydroperoxides **9** and **10** could occur through either enzyme-catalysed oxidation or non-enzymatic aerial autoxidation and at present it is not possible to distinguish between these alternative oxidation mechanisms. The possibility that other hydroperoxide intermediates may be involved during the biosynthesis of furoquinoline alkaloids *e.g.* compound **51** (Scheme 5), or in further oxygen-atom-transfer processes, cannot be excluded at present.

Conclusion

A series of new quinoline alkaloids (**9**–**14**, **17** and **20**), isolated from the leaves of *C. ternata*, have been characterized. Among the novel features associated with these alkaloids are: C-prenylation, hydroperoxidation and chlorohydrin formation. The absolute configurations and ee values for the chiral alkaloids **4**, **5**, **6**,

7, **9**, **11**, **12**, **15**, **17**, **19**, **20** and **21** have been established by stereochemical correlation, X-ray crystallography, ^{1}H-NMR analysis and chiral stationary phase GC-MS methods. Biosynthetic pathways involving multiple oxidation steps that could account for the formation of the quinoline alkaloids present in *C. ternata*, have been presented.

Experimental

^{1}H-NMR spectra were recorded at 300 MHz (Bruker Avance DPX-500) and at 500 MHz (Bruker Avance DRX-500) in $CDCl_3$ solvent, unless stated otherwise. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. Accurate mass measurements were recorded at 70 eV on a VG Autospec, using a heated inlet system. Accurate molecular weights were determined by the peak matching method with perfluorokerosene used for standard reference. The GC-MS instrument used was an Agilent 6890 gas chromatograph directly attached to a 5971 Mass Selective Detector. CSP-GC separation of enantiomers **23** and **25** was carried out using a Supelco γ-DEX 120 capillary column (30 m × 0.25 mm × 0.25 μm film thickness) using helium as carrier gas at a flow rate of 2.3 mL min^{-1}. The GC injector port and transfer line were maintained at 250 °C and 230 °C respectively. The oven temperature was initially held at 80 °C for 1 min and then ramped at 2 °C per min to 200 °C. The mass detector was operated in the EI full scan mode measuring ion currents between m/z 30 and 550 amu. The ee values were calculated using the integrated total ion current for each isomer. Optical rotation ($[\alpha]_D$) measurements were performed on a Perkin-Elmer polarimeter at ambient temperature (*ca.* 20 °C) and are expressed in units of 10^{-1} deg cm^2 g^{-1} in the specified solvent.

Extraction of leaves

The dried and powdered leaves of *Choisya ternata* (2.0 kg) were extracted, three times at room temperature, with 95% EtOH in water (3 × 5 L), over a period of two weeks. The combined ethanolic extract was concentrated to give a viscous dirty green coloured gum (*ca.* 250 g). The gummy mass was extracted with Et_2O (5 × 200 mL) to give extract A. The residual gum was extracted with EtOAc (2 × 125 mL) to give extract B. The gummy mass left after EtOAc extraction, was extracted with warm water (200 mL), filtered and the aq. solution was labelled as extract C.

Isolation of compounds

From extract A. Extract A was evaporated to half its volume and the concentrated solution extracted with aq. HCl solution (2 M, 2 × 250 mL). The combined aq. extract was cooled in an ice bath and the solution made alkaline with the drop-wise addition of 2 M aq. NaOH. The mixture was extracted with EtOAc (3 × 200 mL), the combined extract washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give a mixture of crude products (*ca.* 30 g). Partial separation of the mixture was carried out by flash chromatography (20% EtOAc in hexane → 10% MeOH in EtOAc) followed by multi-elution preparative layer chromatography (PLC) of the separated fractions containing mixtures of compounds. Alkaloids **9**, **10**, **3**, **1**, **2**, **11**, **12**, **5**, **13** and **14** from early fractions (30% EtOAc in hexane), alkaloid **20** from middle fractions (50% EtOAc in hexane), alkaloid **4** and coumarin **22** from late fractions (80% EtOAc in hexane), and alkaloid **21** from the last fractions (10% MeOH in EtOAc) were isolated. A mixture of three alkaloids, present in early column fractions, could only be separated by PLC (2% MeOH in $CHCl_3$) after acetylation (Ac_2O–pyridine). Thus, alkaloids **16** and **18** (acetyl derivatives) along with alkaloid **19** were also isolated.

From extract B. Purification of extract B (0.250 g) by PLC (50% EtOAc in hexane) gave coumarin **23**.

From extract C. The aq. extract C was treated, drop-wise, with a saturated solution of ammonium reineckate (tetra-isothiocyanatodiammine chromate) until no further precipitation occurred. After allowing the precipitates to stand overnight, the mixture was centrifuged, and the precipitates were collected as a pellet. The pellet was dissolved in a minimum amount of MeOH (*ca.* 20 mL) and the solution loaded onto an ion exchange column [Amberlite IRA-420(Cl^-) resin washed thoroughly with 2 M perchloric acid followed by MeOH]; the column was eluted with MeOH. The methanolic fractions, on concentration, yielded the perchlorate salt of **7**.

(±)-2-Methyl-1-[(4,6,7-trimethoxyfuro[2,3-*b*]quinolin-5-yl)methyl]allyl hydroperoxide 9

White crystalline solid (0.011 g); mp 169–170 °C (from CH_2Cl_2); R_f 0.35 ($CHCl_3$); $[\alpha]_D$ 0.0 (*c* 0.5, $CHCl_3$); (Found: M$^+$ + 1, 360.1446. $C_{19}H_{22}NO_6$ requires 360.1447); δ_H (500 MHz, $CDCl_3$) 1.97 (3 H, s, Me), 3.66 (1 H, dd, $J_{1a,1b}$ 13.8, $J_{1a,1'}$ 9.6, H-1′a), 3.79 (1 H, dd, $J_{1b,1a}$ 13.8, $J_{1b,1'}$ 3.1, H-1b), 3.93 (3 H, s, OMe), 4.00 (3 H, s, OMe), 4.29 (3 H, s, OMe), 4.55 (1 H, dd, $J_{1',1a}$ 9.7, $J_{1',1b}$ 3.1, H-1′), 5.11 (2 H, s, CH_2), 7.02 (1 H, d, $J_{3,2}$ 2.8, H-3), 7.30 (1 H, s, H-8), 7.55 (1 H, d, $J_{2,3}$ 2.8, H-2), 9.20 (1 H, br s, OOH); δ_C (125 MHz, $CDCl_3$) 19.4, 29.0, 55.7, 59.3, 61.2, 78.4, 88.6, 103.6, 105.2, 107.1, 112.7, 113.1, 127.1, 142.5, 145.3, 146.1, 146.5, 154.4, 158.7; m/z (FAB) 360.2 (M$^+$ + 1, 32%), 272.1 (37), 147.1 (100).

(*E*)-1,1-Dimethyl-3′-(4,6,7-trimethoxyfuro[2,3-*b*]quinolin-5-yl)-2-propenyl hydroperoxide 10

Colourless crystalline solid (0.215 g); mp 138–139 °C (from EtOAc–hexane); R_f 0.30 ($CHCl_3$); $[\alpha]_D$ 0.0 (*c* 0.7, $CHCl_3$); (Found: M$^+$, 359.1353. $C_{19}H_{21}NO_6$ requires 359.1369); δ_H (500 MHz, $CDCl_3$) 1.54 (3 H, s, Me), 2.17 (3 H, s, Me), 3.77 (3 H, s, OMe), 4.01 (3 H, s, OMe), 4.26 (3 H, s, OMe), 5.83 (1 H, d, *J* 16.4, *trans* ArCH=C*H*), 6.99 (1 H, d, $J_{3,2}$ 2.7, H-3), 7.10 (1 H, d, *J* 16.4, *trans* ArC*H*=CH), 7.33 (1 H, s, H-8), 7.57 (1 H, d, $J_{2,3}$ 2.7, H-2) 8.02 (1 H, s, OOH); δ_C (125 MHz, $CDCl_3$) 24.4, 30.9, 55.8, 59.5, 60.8, 82.5, 104.4, 104.8, 107.3, 112.9, 127.1, 135.1, 142.8, 145.1, 145.4, 155.1, 158.4, 163.5; m/z 359 (M$^+$, 50%), 343 (20), 272 (50), 43 (100).

(±)-3′-Methyl-1-(4,6,7-trimethoxyfuro[2,3-*b*]quinolin-5-yl)-3′-buten-2′-ol 11

White crystalline solid (0.015 g); mp 95 °C (from $CHCl_3$); R_f 0.20 (2% MeOH in $CHCl_3$); $[\alpha]_D$ 0.0 (*c* 0.5, $CHCl_3$); (Found: M$^+$, 343.1430. $C_{19}H_{21}NO_5$ requires 343.1420); δ_H (500 MHz, $CDCl_3$) 1.93 (3 H, s, Me), 3.42 (1 H, dd, $J_{1'a,1'b}$ 13.4, $J_{1'a,2'}$ 9.9, H-1′a), 3.73 (1 H, dd, $J_{1'b,1'a}$ 13.4, $J_{1'b,2'}$ 3.1, H-1′b), 3.92 (3 H, s, OMe), 3.99–4.12 (3 H, s, OMe and 1 H, m, H-2′), 4.37 (3 H, s, OMe), 4.90

and 5.04 (1 H each, s, C=CH_2), 7.04 (1 H, d, $J_{2,3}$ 2.8, H-2), 7.34 (1 H, s, H-8) and 7.56 (1 H, d, $J_{3,2}$ 2.8, H-3); *m/z* 343 (M^+, 15%), 272 (100).

(−)-(*R*)-Desmethoxychoisyine 12

Colourless crystals (0.250 g); mp 184–186 °C (from acetone); R_f 0.15 (2% MeOH in $CHCl_3$); $[\alpha]_D$ −43.8 (*c* 0.6, $CHCl_3$); (Found: M^+, 299.1156. $C_{17}H_{17}NO_4$ requires 299.1158); δ_H (500 MHz, $CDCl_3$) 1.29 (3 H, s, Me), 1.39 (3 H, s, Me), 1.95 (1 H, broad s, OH), 3.68 (1 H, dd, $J_{1a,1b}$ 16.8, $J_{1a,2}$ 9.0, H-1a), 3.77 (1 H, dd, $J_{1b,1a}$ 16.8, $J_{1b,2}$ 9.8, H-1b), 4.37 (3 H, s, OMe), 4.74 (1 H, dd, $J_{2,1b}$ 9.8, $J_{2,1a}$ 9.0, H-2), 7.02 (1 H, d, $J_{9,8}$ 2.8, H-9), 7.26 (1 H, d, $J_{4,5}$ 8.9, H-4), 7.59 (1 H, d, $J_{8,9}$ 2.8, H-8), 7.80 (1 H, d, $J_{5,4}$ 8.9, H-5); δ_C (125 MHz, $CDCl_3$) 24.1, 26.0, 33.5, 58.9, 72.1, 89.9, 104.2, 104.6, 115.0, 117.2, 117.8, 128.4, 142.3, 143.7, 155.7, 156.5, 162.4; *m/z* 299 (M^+, 80%), 240 (100).

4,6-Dimethoxy-5-[(*E*)-3′-methoxy-3′-methyl-1′-butenyl]furo[2,3-*b*]quinoline 13

White crystalline solid (0.015 g); mp 94 °C (from EtOAc–hexane); R_f 0.5 (1% MeOH in $CHCl_3$); $[\alpha]_D$ 0.0 (*c* 0.2, $CHCl_3$); (Found: M^+, 327.1470. $C_{19}H_{21}NO_4$ requires 327.1471); δ_H (500 MHz, $CDCl_3$) 1.46 (6 H, s, 2 × Me), 3.35 (3 H, s, OMe), 3.88 (3 H, s, OMe), 4.26 (3 H, s, OMe), 5.78 (1 H, d, $J_{2',1'}$ 16.5, H-2′), 6.94 (1 H, d, $J_{1',2'}$ 16.5, H-1′), 7.02 (1 H, d, $J_{2,3}$ 2.8, H-2), 7.49 (1 H, d, $J_{7,8}$ 9.3, H-7), 7.60 (1 H, d, $J_{3,2}$ 2.8, H-3), 7.93 (1 H, d, $J_{8,7}$ 9.3, H-8); δ_C (125 MHz, $CDCl_3$) 26.0, 26.2, 50.6, 57.3, 59.4, 75.5, 104.7, 106.2, 118.4, 118.9, 121.6, 125.5, 128.5, 137.6, 142.6, 143.8, 153.3, 158.4, 162.5; *m/z* 327 (M^+, 5%), 312 ($M^+ - CH_3$, 6%), 43 (100).

(*E*)-4′-(4,6-Dimethoxyfuro[2,3-*b*]quinolin-5-yl)-2′-methyl-3′-buten-2′-ol 14

White crystalline solid (0.010 g); mp 144–145 °C (from EtOAc–hexane); R_f 0.3 (1% MeOH in $CHCl_3$); $[\alpha]_D$ 0.0 (*c* 0.2, $CHCl_3$); (Found: $M^+ + 1 - H_2O$, 296.1287. $C_{18}H_{18}NO_3$ requires 296.1287); δ_H (500 MHz, $CDCl_3$) 1.50 (6 H, s, 2 Me), 3.90 (3 H, s, OMe), 4.23 (3 H, s, OMe), 5.97 (1 H, d, $J_{3',4'}$ 16.3, H-3′), 6.99 (1 H, d, $J_{2,3}$ 2.7, H-2), 7.04 (1 H, d, $J_{4',3'}$ 16.3, H-4′), 7.47 (1 H, d, $J_{7,8}$ 9.3, H-7), 7.59 (1 H, d, $J_{3,2}$ 2.7, H-3) and 7.92 (1 H, d, $J_{8,7}$ 9.3, H-8).

(−)-1-[(4,8-Dimethoxyfuro[2,3-*b*]quinolin-7-yl)oxy]methyl-2′-ethoxy-2′-methylpropyl acetate 18

Viscous oil (0.008 g); R_f 0.2 (1% MeOH in $CHCl_3$); $[\alpha]_D$ −8.8 (*c* 0.2, $CHCl_3$); (Found: M^+, 417.1796. $C_{22}H_{27}NO_7$ requires 417.1788); δ_H (500 MHz, $CDCl_3$) 1.17 (3 H, t, *J* 4.65, CH_2CH_3), 1.25 (3 H, s, Me), 1.27 (3 H, s, Me), 2.10 (3 H, s, OCOMe), 3.51 (2 H, m, CH_2CH_3), 4.06 (3 H, s, OMe), 4.29 (1 H, dd, $J_{1a,1b}$ 10.8, $J_{1a,1'}$ 8.6, H-1a), 4.43 (3 H, s, OMe), 4.59 (1 H, dd, $J_{1b,1a}$ 10.8, $J_{1b,1'}$ 2.6, H-1b), 5.46 (1 H, dd, $J_{1',1a}$ 8.6, $J_{1',1b}$ 2.6, H-1′), 7.04 (1 H, d, $J_{2,3}$ 2.8, H-2), 7.21 (1 H, d, $J_{6,5}$ 9.3, H-6), 7.59 (1 H, d, $J_{3,2}$ 2.8, H-3), 7.97 (1 H, d, $J_{5,6}$ 9.3, H-5); δ_C (125 MHz, $CDCl_3$) 15.9, 21.0, 21.6, 23.3, 57.2, 58.9, 61.4, 69.2, 75.1, 75.9, 102.2, 104.5, 114.6, 115.3, 117.8, 141.7, 143.0, 151.3, 157.0, 170.4; *m/z* 417 (M^+, 30%), 113 (100).

(+)-(2′*R*)-3-Chloro-1′-[(4,8-dimethoxyfuro[2,3-*b*]quinolin-7-yl)oxy]-3′-methylbutan-2′ol (chlorodesoxyevoxine) 20

Colourless crystals (0.210 g); mp 137–138 °C [from $(CH_3)_2CO$–hexane]; R_f 0.2 (80% EtOAc–hexane); $[\alpha]_D$ +14.2 (*c* 0.33, EtOH); (Found: C, 59.3; H, 5.4; N, 3.6. $C_{18}H_{20}ClNO_5$ requires C, 59.1; H, 5.5; N, 3.8%); δ_H (500 MHz, $CDCl_3$) 1.67 (3 H, s, Me), 1.68 (3 H, s, Me), 3.46 (1 H, br s, OH), 4.03 (1 H, dd, $J_{2',1'a}$ 7.9, $J_{2',1'b}$ 3.1, H-2′), 4.13 (3 H, s, OMe), 4.25 (1 H, dd, $J_{1'a,1'b}$ 10.1, $J_{1'a,2'}$ 7.9, H-1′a), 4.42 (3 H, s, OMe), 4.55 (1 H, dd, $J_{1'b,1'a}$ 10.1, $J_{1'b,2'}$ 3.1, H-1′b), 7.04 (1 H, d, $J_{2,3}$ 2.7, H-2), 7.22 (1 H, d, $J_{6,5}$ 9.1, H-6), 7.59 (1 H, d, $J_{3,2}$ 2.7, H-3), 7.99 (1 H, d, $J_{5,6}$ 9.1, H-5); δ_C (125 MHz, $CDCl_3$) 28.2, 29.7, 59.0, 61.9, 71.0, 72.3, 76.5, 102.5, 104.7, 115.2, 115.8, 118.6, 141.5, 143.2, 143.5, 151.1, 157.2, 164.3.

Acknowledgements

We thank the Queen's University of Belfast, Oxford Glycosciences and the Overseas Research Student programme for financial support (PLL) and Christopher Monaghan for assistance with the alkaloid isolation.

References

1 S. R. Johns, J. A. Lamberton and A. A. Sioumis, *Aust. J. Chem.*, 1967, **20**, 1975.
2 M. F. Grundon, D. M. Harrison and C. G. Spyropoulos, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2181.
3 D. R. Boyd, N. D. Sharma, P. L. Loke, J. F. Malone, W. C. McRoberts and J. T. G. Hamilton, *Chem. Commun.*, 2002, 3070.
4 D. R. Boyd and M. F. Grundon, *J. Chem. Soc. C*, 1970, 556.
5 S. A. Barr and D. R. Boyd, *J. Chem. Soc., Chem. Commun.*, 1994, 153.
6 S. A. Barr, D. R. Boyd, N. D. Sharma, T. A. Evans, J. F. Malone and V. D. Mehta, *Tetrahedron*, 1994, **50**, 11219.
7 S. A. Barr, C. F. Neville, M. F. Grundon, D. R. Boyd, J. F. Malone and T. A. Evans, *J. Chem. Soc., Perkin Trans. 1*, 1995, 445.
8 D. R. Boyd, N. D. Sharma, S. A. Barr, J. G. Carroll and J. F. Malone, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3397.
9 D. R. Boyd, N. D. Sharma, C. O'Dowd, J. G. Carroll, P. L. Loke and C. C. R. Allen, *Chem. Commun.*, 2005, 3989.
10 E. Atkinson, D. R. Boyd and M. F. Grundon, *Phytochemistry*, 1974, **13**, 835.
11 K. Burgess and A. M. Porte, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1182.
12 S. A. Barr, N. Bowers, D. R. Boyd, N. D. Sharma, L. Hamilton, R. A. S. McMordie and H. Dalton, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3443.
13 A. J. Al-Rehaily, M. S. Ahmad, I. Muhammad, A. A. Al-Thukair and H. P. Peranowski, *Phytochemistry*, 2003, **64**, 1405.
14 J. A. Lamberton and J. R. Price, *Aust. J. Chem.*, 1953, **6**, 66.
15 J. F. Ayafor and J. I. Okogun, *J. Chem. Soc., Perkin Trans. 1*, 1982, 909.
16 D. A. Casteel, *Nat. Prod. Rep.*, 1999, **16**, 55.
17 I. C. Parson, A. I. Gray, T. G. Hartley and P. G. Waterman, *Phytochemistry*, 1994, **37**, 565.
18 C. Ito, K. Fujiwara, M. Kajita, M. Ju-ichi, Y. Takemura, Y. Suzuki, K. Tanaka, M. Omura and H. Furukawa, *Chem. Pharm. Bull.*, 1991, **39**, 2509.
19 C. Ito and H. Furukawa, *Chem. Pharm. Bull.*, 1989, **37**, 819.