Quinoline Alkaloids. Part 28.[1] The Biosynthesis of Furoquinolines and Other Hemiterpenoids in *Ptelea trifoliata*

Charles F. Neville,*,†,[a] (the late) Michael F. Grundon, Venkataraman N. Ramachandran,[a] Geisla Reisch[b] and Johannes Reisch[b]

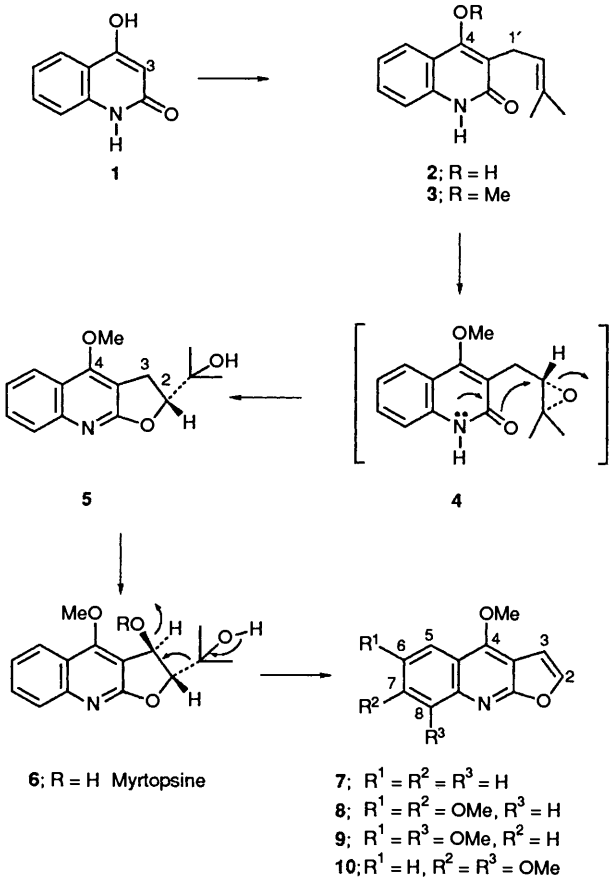
[a] *Department of Applied Physical Sciences, The University of Ulster, Coleraine, Northern Ireland, UK*
[b] *Institut für Pharmazeutische Chemie, West-Wilhems Universität, 58-62 Hittorfstrasse, 4400 Münster, Germany*

Feeding experiments with doubly labelled 4-methoxy-3-prenyl-2-quinolones have shown that the biosynthesis of kokusaginine **8** and maculosidine **9** in *P. trifoliata* also involves a myrtopsine-like derivative. Contrary to our proposed pathways, the biosynthesis of hemiterpenoid olefines such as ptelefoline **15**, in the same plant species, does not appear to involve directly platydesminium salt but possibly also a myrtopsine derivative. On the basis of the results obtained, alternative biosynthetic pathways to these alkaloids are discussed.

The Rutaceae species *Ptelea trifoliata* (hop tree) is known[2] to contain a diverse array of quinoline alkaloids including furoquinolines, 2-(1*H*)-quinolones, and dihydrofuroquinolines. Dictamnine **7** and its dimethoxy derivatives kokusaginine **8**, maculosidine **9** and skimmianine **10** are typical representatives of the furoquinoline complement in this plant. These alkaloids occur widely in rutaceous plants and their synthesis and biosynthesis has been studied on numerous[3] occasions. Extensive studies on *P. trifoliata* by Reisch *et al.*[4–9] resulted in the isolation of several 2-(1*H*)-quinolones and dihydrofuroquinolines which possess an unusual terminal olefinic double bond. Examples of such alkaloids include *O*-methylptelefolonium salt **14**, ptelefoline **15** and ptelefolone **16**. Subsequent studies[10] were to show that some of these alkaloids exhibit antimicrobial activity and marked cytotoxicity against animal and plant tumours. Hence methods for their synthesis have been developed;[11–13] their biosynthesis however has not yet been investigated. Recently we have conducted some preliminary studies in an attempt to elucidate possible biosynthetic pathways to account for the occurrence of alkaloids of this type in *P. trifoliata*. In parallel we have also had the opportunity to check, for the first occasion, the biosynthesis of the trimethoxyfuroquinolines kokusaginine **8** and maculosidine **9** in this particular Rutaceae species.

As stated earlier the biosynthesis of trimethoxyfuroquinolines have been studied previously in other rutaceous plants. Studies by the Grundon group and others[2,14] have shown that these alkaloids arise *via* the biosynthetic sequences outlined in Scheme 1. These pathways were usually elucidated by feeding radioactive-labelled potential precursors to shoots of plants and studying their incorporation in isolated alkaloids.

3-Prenyl-2-quinolones‡ of the type **2** and **3**, that are apparently derived by C-3 prenylation of 4-hydroxy-2-quinolone **1**, were shown[15] to be suitable precursors of the simplest furoquinoline dictamnine **7** in *Skimmia japonica*. Platydesmine **5**, which has been synthesised[16] by peracid oxidative cyclisation of **3**, persumably *via* the epoxide **4**, was thought to be the sole intermediate involved in the formation of compound **7**. Although compound **5** has not been isolated from many rutaceous plants, it was shown[15] to be derived from the 3-prenyl-2-quinolone **3** and concomitantly incorporated into dictamnine **7**. Incorporation of doubly-labelled precursors of compound **3**, [1′-$^3H_2$] and [1′-^{14}C], however occurred[17] with loss of approximately half the tritium label. This suggested that the transformation of compound **3** into compound **6** involved a further intermediate in addition to platydesmine. It was proposed[18] that a benzylic alcohol of the type **6** which could undergo retro-aldol cleavage to give dictamnine was also implicated in the biosynthesis of furoquinolines. Aromatic oxygenation of compound **7** was subsequently proposed to be



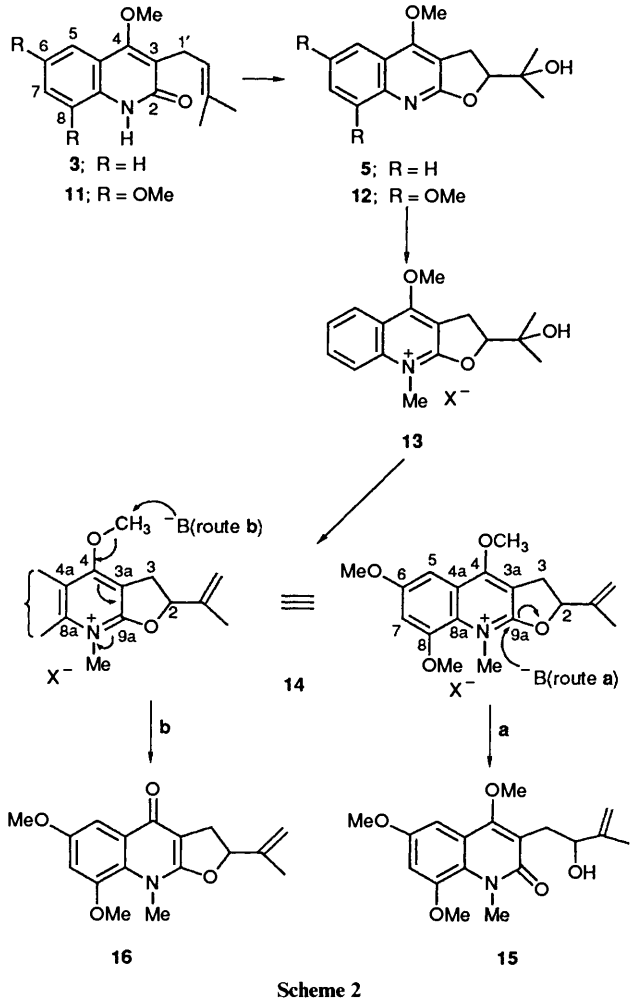
Scheme 1

† *Present address:* Daiichi Pure Chemicals Company Limited, Tokai Research Laboratories, No. 2117 Muramatsu, Tokai-mura, Ibaraki-Ken 319-11, Japan.

‡ The term 'prenyl' refers to 3-methylbut-2-enyl and is used throughout for convenience.

the ultimate step in the formation of trimethoxyfuroquinolines as exemplified by skimmianine[19] **10**. In summary, the biosynthesis of trimethoxyfuroquinolines in rutaceous plant species is thus thought to involve the biosynthetic sequences **1**→**2**→**3**→**4**→**5**→**6**→**7**→**8**, **9**, **10**, *etc.*, in Scheme 1.

We initially contemplated that a biosynthetic pathway analogous to the aforementioned, may also be in operation in *P. trifoliata* to account for the co-occurrence of trimethoxyfuroquinolines and the terminal olefinic alkaloids **14**–**16** in this plant species. Our proposed pathway to the latter alkaloids which is essentially an extension of that in Scheme 1, is outlined in Scheme 2. *N*-Methylplatydesminium salt **13** is also known[15] to



Scheme 2

be derived from 4-hydroxy-2-quinolone **1** and the 3-prenyl-2-quinolones **2** and **3** in *S. japonica*. In the same studies platydesmine **5** itself was shown to be a good precursor of **13**. We had postulated that it may also play a significant role in the biosynthesis of **14**–**16**. Oxygenation and dehydration (or *vice versa*) of **13** may lead to the formation of the ptelefolonium salt **14**. Alternatively **14** could be derived by quaternisation and dehydration of dimethoxyplatydesmine **12** which arises due to the oxidative cyclisation of the 3-prenyl-6,8-dimethoxy-2-quinolone **11**; in analogy with the methods which have been applied[11] in its laboratory synthesis. The point at which *in vivo* oxygenation occurs obviously determines which of these biosynthetic routes is applicable.

Rearrangements of the ptelefolnium salt **14** were recognised as possible pathways for the biosynthesis of ptelefoline **15** and/or ptelefolone **16**; see also Scheme 2. Thus enzyme-base induced cleavage in **14** at the tertiary carbon centre C-9a could result in the opening of its furan ring to give ptelefoline **15** (route **a**). Evidence in support of this theory was obtained in our laboratories when it was observed[20] that treatment of a crude isolated sample of compound **14** with basic anion exchange resin afforded ptelefoline **15**. In contrast cleavage of the methoxy group at C-4, in a reaction analogous to that involved[14] in the rearrangement of furoquinolines to isofuroquinolines, would result in formation of ptelefolone **16** (route **b**). The actual mechanisms assigned to routes **a** and **b** in Scheme 2 are merely postulated as recently it was shown[21] that reactions of this type may involve pseudo-base intermediates.

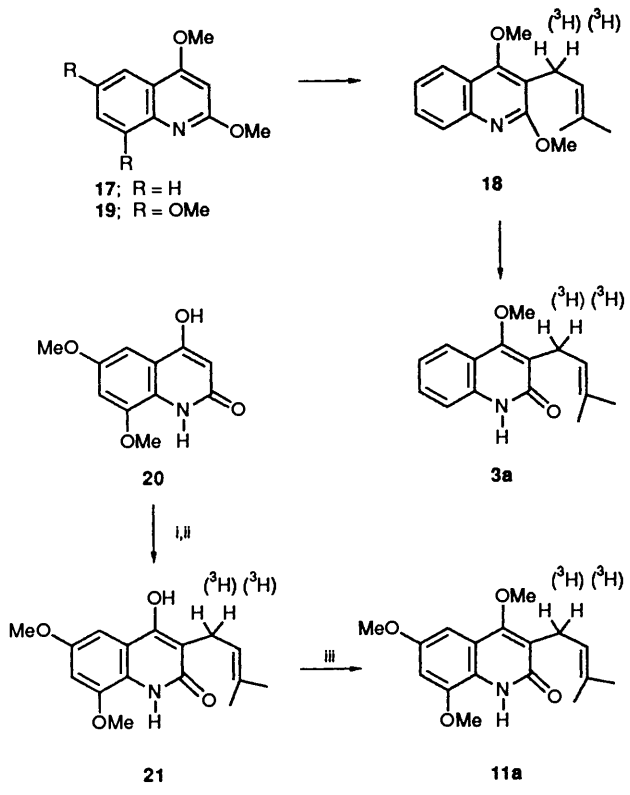
Discussion

In an attempt to determine if the biosynthetic sequences outlined in Schemes 1 and 2 are operating in parallel in *P. trifoliata* we have synthesised, administered and studied the incorporation of radioactive-labelled precursors of 4-methoxy- and 4,6,8-trimethoxy-3-prenyl-2-quinolones (structures **3** and **11** respectively) into the alkaloidal constituents of a plant of this species. As in earlier studies[17] of this type, we have again conducted our experiments using mixtures of the 4-methoxyquinolones labelled at C-1′ with tritium; **3a**, **11a**, and with ^{14}C in the C-4 methoxy group; **3b** and **11b**. A study of the ratio of these labels, in alkaloids incorporating any of these precursors would allow us to clarify the mechanisms involved in their biosynthesis.

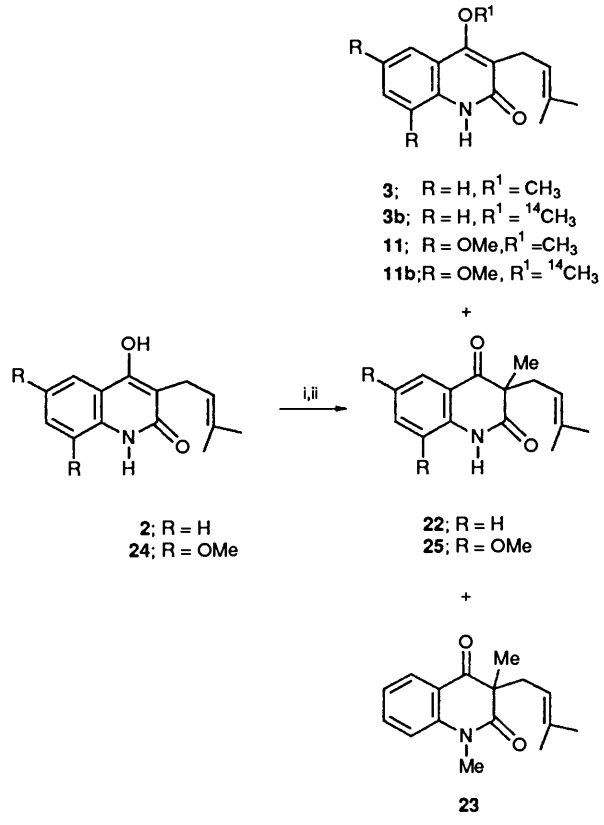
Our first aim was to develop suitable methods for the synthesis of the required precursors. Traditional methods[11,16] for the synthesis of compounds **3** and **11** involve condensation of the appropriate aromatic amine with prenyl malonate ester to afford the relevant 4-hydroxy-3-prenyl-2-quinolones; *cf.* structure **2**. Selective methylation of these 4-hydroxyquinolones with diazomethane then gives the corresponding 4-methoxy ethers. These syntheses however are plagued by low yields of the 4-hydroxy-2-quinolones in the preliminary condensation reactions due to difficulties with their purification from what is often a complex mixture of by-products.[22] Similarily, these latter reactions are sensitive to the substitution pattern prevailing in the aromatic amine which no doubt becomes significant in the case of the synthesis of the 6,8-dimethoxy compound **11**. We thus decided to pursue more direct approaches to synthesise compounds of this type.

In the case of the 4-methoxy precursor **3a**, application of such methods were straightforward since these compounds could be prepared[23] by prenylation of 2,4-dimethoxyquinoline **17** with tritium-labelled prenyl bromide (Scheme 3). *O*-Demethylation of the resulting derivative **18** afforded **3a** in good yield. We persisted with these methods for our synthesis. Preparation of the corresponding 6,8-dimethoxy precursor **11a** was more problematic since attempted selective prenylation of the tetramethoxyquinoline **19** was unsuccessful. Prenylation of 4-hydroxy-6,8-trimethoxy-2-quinolone **20**, using sodium methoxide as base, was more satisfactory. A base-soluble product of this reaction was the 4-hydroxy-2-quinolone **21**. It was obtained in a disappointingly low yield (10%) but unlike earlier attempts to prepare this product, it was essentially pure and it could be used directly for subsequent diazomethane methylation to give the desired tritiated trimethoxy precursor **11a**. The tritiated prenyl bromide (1-bromo-3-methyl[1′-$^3H_2$]but-2-ene) used in the previous reactions was synthesised using slight modifications of previously reported procedures.[17]

4-Methoxy-3-prenyl-2-quinolones such as **3** and **11** can be readily obtained by diazomethane methylation of the corresponding 4-hydroxy compounds. Hence in a typical example the [^{14}C]4-methoxyquinolone **3b** could be prepared by treatment of compound **2** with labelled diazomethane generated from a reagent such as [^{14}C]Diazald®. As diazomethane is such a volatile reactant its use for this purpose was not a very



Scheme 3 *Reagents and conditions:* i, reflux in NaOMe, 1 h; ii, $Me_2C{=}CHCH_2(^3H_2)$-Br, reflux for further 3 h; iii, excess (4 mol equiv.) ethereal CH_2N_2, stir at room temp. 4 h



Scheme 4 *Reagents and conditions:* K_2CO_3, reflux in acetone, 30 min; ii, CH_3I–$^{14}CH_3I$, reflux in acetone for further 5 h

pleasant proposition. It was realised that [^{14}C]methyl iodide may be a more attractive methylating agent. To the best of our knowledge however this reagent has not been used previously for the methylation of 4-hydroxy-3-prenyl-2-quinolones. Thus it was deemed necessary to conduct some pilot experiments with cold material to characterise the reaction and its resulting products (Scheme 4). In such a typical study, reaction of the 4-hydroxyquinolone **2** with methyl iodide and powdered potassium carbonate in refluxing acetone yielded three methylation products. The 4-methoxyquinolone **3**, obtained in 21% yield, was identical in all respects with that reported[16] and with other synthetic samples of this compound. A second product (27% yield) was identified as the 3-methylated derivative **22**. Its NMR spectrum lacked any reasonance characteristic of a methoxy group which occurred at δ 3.96 in the case of compound **3**. Instead a further three-proton singlet was apparent at δ 1.5 which was overlapping with one of the $=CMe_2$ signals of the prenyl substituent. IR absorptions at ν 1660 and 1690 cm^{-1} were further indication of 2-quinolone and 4-quinolone carbonyl groups. This product obviously arises due to alkylation of the ambient ion formed at C-3 in compound **2**. Similarly, the third product was distinguished as the *N*-methylquinolone **23**; its NMR spectrum also showed a 3 H singlet at δ 3.45 typical of an NMe. Similar treatment of the 6,8-dimethoxy-2-quinolone **24** gave the trimethoxy-2-quinolone **11** and the 3-methylated by-product **25** in yields of 48% and 31% respectively. Although the 4-methoxyquinolones were only obtained in moderate yields, purification of the products were readily achieved by chromatography. Our desired 4-[^{14}C]-methoxy-2-quinolones **3b** and **11b** were thus prepared using methyl iodide admixed, with [^{14}C]methyl iodide. The precursors thus obtained had very satisfactory specific radioactivity and were of ideal quality for our feeding experiments.

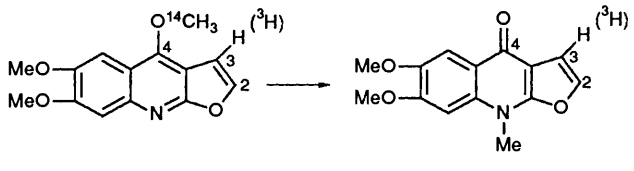
Mixtures of the precursors **3a**, **3b** and **11a**, **11b**, with a precisely known ^{3}H:^{14}C ratio, were then administered to shoots of *P. trifoliata*. After the feeding period had elapsed, the shoots used in each set of experiments were combined and extracted. The extracts were then separated further into acid-soluble, base-soluble, neutral and aqueous fractions (see Experimental section).

Preparative thin-layer chromatography (PLC) of the acid-soluble fraction resulted in the separation of two bands which when examined by GC–MS were found to be isomeric trimethoxyfuroquinolines with a molecular mass of 259 as their base peaks (M^+, 100%). Comparison of the mass spectral fragmentation patterns with those reported[24] for trimethoxyfuroquinolines, resulted in the immediate identification of one of the isolated furoquinolines kokusaginine **8**. Although the second component isolated had similar GC properties to **8**, its mass fragmentations were different. Most notably were the occurrence of substantial fragments at m/z = 258 (M^+ − H) and at m/z = 239 (M^+ − CHO). These were noted[24] to be characteristic of furoquinolines containing an aromatic methoxy substituent at C-8. The isomeric furoquinolines maculosidine **9** and skimmianine **10** have an aromatic substitution which fulfils these requirements. A mass spectrum of an authentic synthetic sample of compound **10**, ruled out the possibility that we had isolated this alkaloid since its base peak occurs at m/z = 244 (M^+ − CH_3, 100%). We were thus able to conclude that the second acid-soluble component isolated was maculosidine **9**. When available, GC–MS spectra of synthetic samples of these furoquinolines were also recorded and found to be in good accord with those observed from the isolated alkaloids. The isolation of the furoquinoline maculosidine **9** was particularly rewarding since (a) this would be the first reported study on the biosynthesis of this particular furoquinoline, and (b) it conveniently contains the same substitution pattern (*i.e.* 6,8-dimethoxy) as that present in the precursors **11a** and **11b**, and in the terminal olefins **14**–**16**; hence a study of the incorporation of our precursors into these alkaloids should allow us to deduce if their biosynthesis follow parallel routes in *P. trifoliata*.

Table 1 Incorporation rates and labelling patterns from a typical set of feeding experiments. Results were comparable with those recorded from a duplicate set of experiments. ND—not determined; the incorporation level in the isolated alkaloids was too low to allow accurate determination of their radioactivity and/or the inherent labelling patterns.

Precursors fed (^3H:^{14}C ratio)	^{14}C Activity fed/ MBq	Incorporation of ^{14}C label in isolated alkaloids (%) (^3H:^{14}C ratio)		
		8	9	15
[1′-$^3H_2$]:**3a** [4-$\overset{+}{O}$$^{14}CH_3$]:**3b** (4.2:1)	0.03	0.2 (2:1)	0.2 (2.6:1)	0.2 (2:1)
[1′-$^3H_2$]:**11a** [4-$\overset{+}{O}$$^{14}CH_3$]:**11b** (2.5:1)	0.055	ND	0.018 (2:1)	0.002 ND

The alkaloids **8** and **9** were then purified further by recrystallisation and/or HPLC. Percentage incorporation of the precursors into the alkaloids and the inherent labelling pattern observed, were then determined by standard scintillation procedures. The results thus obtained are summarised in Table 1. In general, incorporation rates were low when compared to those reported[14] with other plant species. They were, however, comparable in both alkaloids and were similar to those which we observed in preliminary experiments. These findings may be attributed to the presence of a much larger pool of alkaloidal constituents in this plant species resulting in pronounced dilution and distribution of the administered precursors. The data, however, clearly showed that incorporation of the 4-methoxy precursors **3a** and **3b** occurred to a much higher extent than that observed with the corresponding trimethoxy derivatives **11a** and **11b**. This indicated that aromatic oxygenation during the biogenesis of **8** and **9** occurs quite late in their biosynthesis (Scheme 1), probably after the formation of the furan ring in platydesmine **5**. In contrast, incorporation of the precursors **3a** and **3b** occurred with retention of the 4-methoxy group which suggests that this particular methoxy group is introduced earlier during the biosynthesis of these alkaloids as depicted in the sequences outlined in Scheme 1. Rearrangement of a sample of isolated kokusaginine **8** to isokokusaginine **26** with methyl iodide, as shown below, resulted in cleavage of the 4-methoxy group. The isoalkaloid **26** thus obtained was essentially lacking any of the ^{14}C whilst in contrast it had retained approximately 95% of its tritium label at C-3. Attempts to trap efficiently the cleaved ^{14}C were unsuccessful since the methyl iodide (reactant–solvent) collected after the reaction only contained about 30% of this label. However this result provided further evidence that no randomisation of the labels had occurred during incorporation of compounds **3a** and **3b** into this alkaloid.



8 **26**

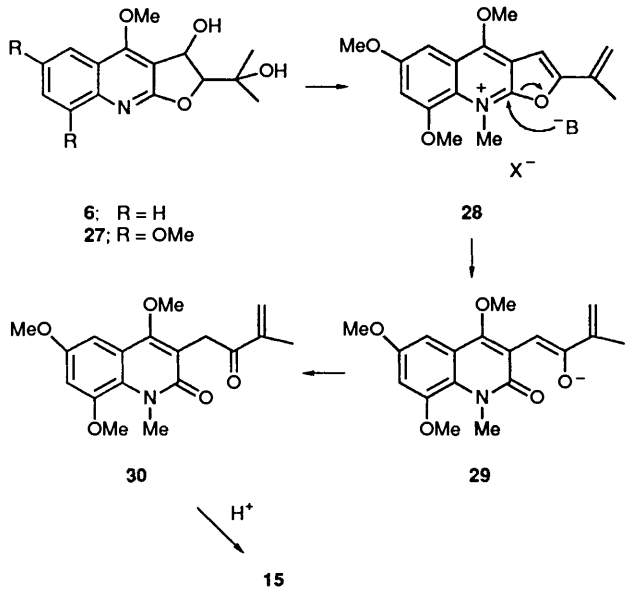
Similarly, the results also showed that during incorporation of these precursors into compounds **8** and **9** approximately half of their tritium label was lost. As stated previously, this suggests that an additional intermediate was involved in the interconversion of compound **5** into compounds **7**, **8**, **9**, *etc.*, whose formation involves loss of a benzylic proton at C-3, or tritium as in the case of precursor-derived molecules.

A benzylic alcohol derivative with the structure **6** (R=H) (Scheme 1) has been isolated[25] from a rutaceous species. Commonly known as myrtopsine, it is, as summarised in Scheme 1, thought[18] to be the immediate biogenetic precursor of furoquinolines such as dictamnine. It could be similarly implicated, in the biosynthesis of furoquinolines **8** and **9** in *P. trifoliata*.

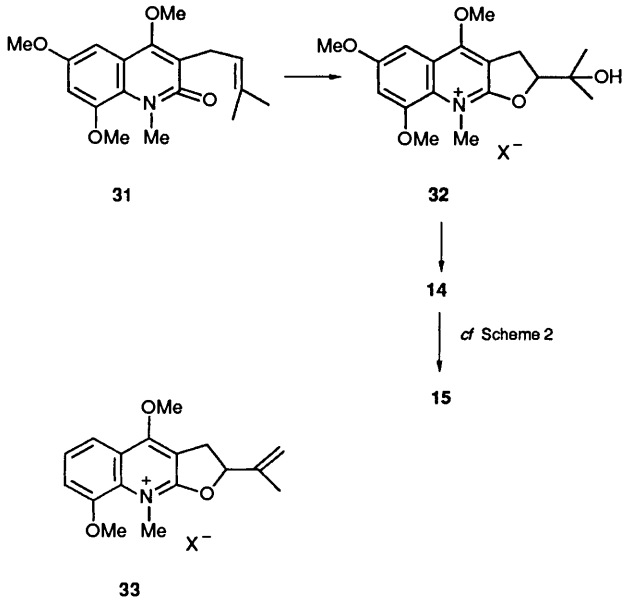
Preparative TLC of the crude neutral fraction resulted in the isolation of its one major component which was again identified by GC–MS to be the terminal olefin ptelefoline **15**. Its mass fragmentation pattern showed a molecular mass of 333 (M^+, 19%) and a base peak at m/z = 248 ($M^+ - C_4H_6O$, CH_3) which was comparable with that reported[7] and recorded from a synthetic sample of the alkaloid. The rate of incorporation of the administered precursors into **15** are also listed in Table 1. As observed with the furoquinolines **8** and **9**, the monomethoxy precursors **3a** and **3b** were incorporated to a greater extent than the 4,6,8-trimethoxy derivatives **11a** and **11b**. Incorporation levels were again low but comparable to that observed with the isolated furoquinolines. These results also suggest that aromatic oxygenation during the biosynthesis of alkaloids of this type occurs late in their biogenesis. A most interesting aspect of the results was that incorporation of compounds **3a** and **3b** again apparently proceeded with loss of approximately half of their tritium label. Our proposed biosynthetic pathway to such alkaloids (Scheme 2) does not account for such a result as it requires that the benzylic protons (tritiums) at C-1′ in compound **3** are retained. Hence it appears that although compounds **3a** and **3b** are potential precursors of ptelefoline **15**, they must participate in different pathways to that which we have postulated.

Possible alternative pathways which would explain the aforementioned results is to consider that myrtopsine **6** or its dimethoxy derivative **27** are implicated in the biosynthesis of **15**. Possible routes which involve the participation of such compounds are depicted below. Dehydration of dimethoxymyrtopsine **27**, followed by quaternisation would give the salt **28**. Base-induced ring cleavage of the latter [*cf.* route **b** (Scheme 2)] would then give the intermediate **29** that would be expected to spontaneously rearrange to the ketone **30**. Reduction of this ketone would ultimately afford ptelefoline **15**. This sequence of reactions, which result in loss of a benzylic proton at C-1′ in compound **3**, may equally apply with myrtopsine **6** as starting point; aromatic oxygenation occurring later in the series of reactions. It is noteworthy that ketones of the type **30** are also known[6,26,27] constituents of *P. trifoliata*. Recent studies[21] on the synthesis of such compounds were deemed to have involved intermediates with a structure analogous to **29**.

It is also conceivable that other alternative pathways to those which we have discussed may be operating in *P. trifoliata* to account for the occurrence of terminal olefins as exemplified by **15**. The *N*-methylquinolone **31** has also been isolated from this plant.[28] In analogy with previous synthetic studies,[29] oxidative cyclisation of this prenylquinolone should result in the



formation of the alcohol **32**. Dehydration of this compound would afford *O*-methylptelefolonium salt **14**, which could then be transformed to ptelefoline **15** by the mechanisms already described in Scheme 2. Similarly a French group have isolated[30] a new alkaloid ptelecultinium **33** from cell cultures of *P. trifoliata*. In the same study they also obtained the ptelefolonium salt **14** and noted that the relative amounts of this alkaloid present were smaller than ptelecultinium **33**, whereas in intact stems the reverse situation was the case, **33** being essentially absent. They subsequently concluded that the absence of **33** *in vivo* may suggest that in such a system it only acts as an intermediate in the biosynthesis of more complex alkaloids such as ptelefolonium salt **14**.



Ptelefolone **16** could not be isolated from any of our feeding experiment extracts. Examination of the aqueous fraction resulted in the isolation of *O*-methylptelefolonium salt **14**. However quantities obtained after purification were too small to allow accurate measurement of incorporation of the administered precursors.

Summarising, our results from these experiments with *P. trifoliata* indicate that the biosynthesis of the trimethoxyfuroquinolines **8** and **9** proceed *via* oxidative cyclisation of 4-methoxy-3-prenyl-2-quinolones and again involve participation of an intermediate such as myrtopsine **6**. The biosynthesis of the terminal olefin **15** also appears to involve a myrtopsine derivative, and not platydesminium salt **13** as proposed by us in Scheme 2. In the future we plan to carry out some feeding experiments with radioactive-labelled precursors of myrtopsine. Results from such studies would help us to confirm their participation in the biosynthesis of furoquinolines and if they play a role in the formation of terminal olefins such as ptelefoline. Feeding experiments with *in vitro* cell cultures may also be worthwhile since it was shown[30] that relatively fewer alkaloids are present and dilution of administered precursors would not be problematic. Resulting incorporation levels would thus be expected to be higher and their distribution should be more easily monitored.

Experimental

General Directions.—M.p.s were recorded on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded (KBr discs unless otherwise indicated) on a Perkin-Elmer 457 spectrometer. ^{1}H NMR spectra were obtained from a Perkin-Elmer R32 (90 MHz). Deuteriochloroform was employed as solvent; chemical shifts are assigned on the δ scale with tetramethylsilane as internal standard, J values are given in Hz. High resolution mass spectra were recorded on an AEI MS9 instrument fitted with an Elliot 905 computer system. GC–MS was conducted with a Hewlett Packard system consisting of a 5890 series gas chromatographer and a 5970 mass spectrometer integrated with the workstation, model 5970A. A Hewlett Packard fused silica capillary column (25 cm × 0.22 mm i.d.), cross-linked with a methyl silicone phase was employed with argon as carrier gas. Initial temperature of the injection port was 40 °C; it was increased to 150 °C (at 40 °C min^{-1}) and then finally to 240 °C (at 6 °C min^{-1}).

Preparative TLC was performed on precoated silica gel plates, Type $60F_{254}$, 20 cm × 20 cm, layer thickness 1 mm; Merck, Art No. 13895. Semipreparative HPLC was carried out on a Varian model 5000, with UV detection at λ 254 nm. It was connected to a LKB fractionator (type 2070), Ultrorac II®. The stationary phase was of reversed-phase octadecylsilane type with dimensions 25 × 0.1 cm i.d. Methanol used as mobile phase, with doubly distilled water, was of analytical grade and also supplied by Merck; solvents were degassed prior to use in the normal fashion.

Radioactivity determinations were measured with a Tricarb® 300G scintillation counter, using a dual programme for ^{3}H and ^{14}C, that was standardised internally for quenching. Quickszint® 212 (supplied by Zinser Anlalytik, Frankfurt) was utilised as scintillation cocktail. [^{14}C]Methyl iodide, purchased from Amersham International plc, had specific activity of 1.85–2.2 GBq mmol^{-1}. Ether refers to diethyl ether and light petroleum to the boiling fraction 40–60 °C. All other reagents and solvents were obtained from commercial sources and purified when necessary by standard laboratory procedures.[31]

4-Methoxy-3-(3-methyl[1-$^3H_2$]but-2-enyl)-2-quinolone **3a**.—This tritiated precursor was prepared as previously described.[23] Hence reaction of 2,4-dimethoxyquinoline **17** (1.0 g, 3.8 mmol) with tritiated prenyl bromide (2.4 g, 16 mmol), in the presence of butyllithium gave 2,4-dimethoxy-3-(3-methyl[1-$^3H_2$]but-2-enyl)quinoline **18** as a straw coloured oil (1.1 g, 81%). *O*-Demethylation of this quinoline (560 mg) in dry refluxing diisopropyl ether, saturated with dry hydrogen chloride gas, afforded compound **3a** as colourless plates (310

mg, 59%), m.p. 132–134 °C (from ether–light petroleum) (lit.,[16] 127–130 °C); specific activity 1.7 MBq mmol^{-1}.

4-*Hydroxy*-6,8-*dimethoxy*-3-(3-*methyl*[1-$^3H_2$]*but*-2-*enyl*)-2-*quinolone* **21**.—The 2-quinolone **20**, prepared by the methods of Storer and Young[32] (0.8 g, 3.6 mmol) was stirred and heated under reflux in a solution of sodium methoxide (from 1.0 g Na in 50 cm^3 MeOH) for 1 h. Tritiated prenyl bromide (1.6 g, 10.7 mmol) was added in one batch and reflux continued for 3 h. The solution was then concentrated under reduced pressure and poured into a suspension of HCl (2 mol dm^{-3}; 150 cm^3) and dichloromethane (100 cm^3). Filtration gave unchanged 2-quinolone **20** (645 mg, 80%).

The remaining organic layer was separated from the filtrate and the aqueous layer extracted further with dichloromethane (3 × 30 cm^3). After combining the organic layers, they were extracted with Na_2CO_3 (2 mol dm^{-3}; 3 × 30 cm^3). Careful acidification (conc. HCl) of the alkaline extracts afforded compound **21** (80 mg, 10%), m.p. 208–210 °C (lit.,[11] 210–212 °C).

4,6,8-*Trimethoxy*-3-(3-*methyl*[1-$^3H_2$]*but*-2-*enyl*)-2-*quinolone* **11a**.—The quinolone **21** (80 mg, 3 mmol) in AnalaR methanol (30 cm^3) was treated with an excess of ethereal diazomethane, as previously reported.[11] PLC on silica gel and elution (× 2) ether–chloroform (20:80 v/v) gave compound **11a** (R_F 0.4) (40 mg, 48%), crystallising as colourless needles, m.p. 130–132 °C; (from ether–light petroleum) (lit.,[11] 132–133 °C); with specific activity 1.2 MBq mmol^{-1}.

4-[^{14}C]*Methoxy*-3-(3-*methylbut*-2-*enyl*)-2-*quinolone* **3b**.—In preliminary cold experiments the hydroxyquinolone **2** (prepared by the method of Boulanger *et al.*[33]) (110 mg, 0.5 mmol) and dry powdered potassium carbonate (345 mg, 2.5 mmol) were stirred and heated under reflux in dry acetone (30 cm^3) for 30 min. Methyl iodide (142 mg, 1 mmol) in acetone (1 cm^3) was added in one batch and reflux continued for a further 5 h. After removal of the solvent under reduced pressure, the residue was suspended in chloroform (30 cm^3) and K_2CO_3 (1 mol dm^{-3}). The organic layer was separated and washed further with aliquots of K_2CO_3 (2 × 10 cm^3) and briefly with water (2 × 20 cm^3). It was then dried ($MgSO_4$), evaporated and the resulting oily residue applied to PLC. Elution (× 2) in ether–chloroform (20:80 v/v) gave three components. The lowest band (R_F 0.4) proved to be the desired 4-methoxyquinolone **3** (25 mg, 21%), separating as colourless plates, m.p. 132–134 °C (from ether–light petroleum) (lit.,[16] 127–130 °C); identical in all other respects with that reported for the quinolone.

The second component (R_F 0.5), showing strong fluorescence, was identified as 3-*methyl*-3-(3-*methylbut*-2-*enyl*)-1H-*quinoline*-2,4-*dione* **22** (32 mg, 27%) also obtained as colourless plates m.p. 102–105 °C (from ether–light petroleum) (Found: M$^+$, 243.1255. $C_{16}H_{17}NO_2$ requires *M*, 243.1259); ν_{max}/cm^{-1} 3190 (NH) and 1695, and 1660 (C=O); δ_H 1.28 (3 H, s, =CMe), 1.53 (6 H, br s, =CMe, COCMe), 2.70 (2 H, d, CH_2CH), 4.98 (1 H, br t, CH$_2$C*H*), 7.10 (2 H, m, ArH), 7.52 (2 H, m, ArH) and 9.87 (1 H, br s, NH; exchangeable with D_2O); *m*/*z* (EI) 243 (M$^+$, 15), 228 (12), 175 (100). A third component (R_F 0.9), obtained as a pale-yellow oil, was shown to be 1,3-*dimethyl*-3-(3-*methylbut*-2-*enyl*)-1H-*quinoline*-2,4-*dione* **23** (16 mg, 13%) (Found: M$^+$, 257.1408. $C_{16}H_{19}NO_2$ requires *M*, 257.1416); ν_{max}/cm^{-1} (NaCl plates) 1690 and 1660 (C=O); δ_H 1.47 (1 H, s, =CMe), 1.52 (6 H, br s, =CMe, COCMe), 2.60 (2 H, d, CH_2CH), 3.45 (3 H, s, NMe), 4.87 (1 H, br t, CH$_2$C*H*), 7.15 (2 H, m, ArH) and 7.98 (2 H, dd, ArH); *m*/*z* (EI) 257 (M$^+$, 21%), 242 (31) and 189 (100).

Repeat of the above procedure using compound **2** (400 mg, 1.7 mmol), K_2CO_3 (1.2 g, 8.5 mmol), and methyl iodide (240 mg, 1.7 mmol) admixed with [^{14}C]methyl iodide (12 mg; *ca.* 37 MBq) followed by chromatography gave the precursor **3b** (95 mg, 22%) which was crystallised until it showed constant radioactivity. It had a final specific activity of 12.7 kBq mmol^{-1} and was identical in all other respects to compound **3**.

4[^{14}C]-6,8-*Trimethoxy*-3-(3-*methylbut*-2-*enyl*)-2-*quinolone* **11b**.—The quinolone **24** (prepared as earlier described for **21**) (85 mg, 0.3 mmol), K_2CO_3 (207 mg, 1.5 mmol) and methyl iodide (85 mg, 0.6 mmol) was treated as above. PLC and elution (× 2), ether–chloroform (20:80 v/v) resulted in the separation of two principal components. The major product (R_F 0.4) was the methoxyquinolone **11** (43 mg, 48%) crystallising as colourless plates, m.p. 129–132 °C (from ether–light petroleum) (lit.,[11] 132–133 °C) and was identical in all other respects with that reported.

A second component (R_F 0.7) was identified as 4,6,8-*trimethoxy*-3-*methyl*-3-(3-*methylbut*-2-*enyl*)-1H-*quinoline*-2,4-*dione* **25** (34 mg, 38%) separating as light fluffy yellow crystals, m.p. 124–127 °C (from ether–light petroleum) (Found: M$^+$, 303.1504 $C_{17}H_{21}NO_4$ requires *M*, 303.1470); ν_{max}/cm^{-1} 3200 (NH), 1680 and 1645 (C=O); δ_H 1.45 (3 H, s, =CMe), 1.55 (6 H, br s, =CMe, COCMe), 3.65 (2 H, d, CH_2CH), 3.88 (3 H, s, OMe), 3.79 (3 H, s, OMe), 4.91 (1 H, br t, CH$_2$C*H*), 6.67 (1 H, d, *J* 2), 6.91 (1 H, d, *J* 2) and 8.90 (1 H, br t, NH; exchangeable with D_2O); *m*/*z* (EI) 303 (M$^+$, 31%), 235 (54) and 234 (15). Similar treatment of compound **24** (300 mg, 1 mmol) with K_2CO_3 (690 mg, 5 mmol), methyl iodide (270 mg, 1.9 mmol), and [^{14}C]methyl iodide (12 mg, *ca.* 37 MBq) afforded **11b** (95 mg, 30%). When purified by repeated crystallisation it had specific activity of 15.6 KBq mmol^{-1} and was identical in all other aspects with **11**.

Feeding Experiments.—Batches (usually 30) of fresh leafy shoots from a mature tree of *P. trifoliata*, growing in the botanical gardens of the Institut für Pharmazeutische Chemie in Münster, Germany were collected during the active growth period; May–August. They were immediately transferred to test-tubes, acting as feeding vials, that contained Hoaglands plant nutrient medium (6 cm^3). Mixtures of the precursors **3a**, **3b** and **11a**, **11b**, containing precisely known ratios of ^{3}H:^{14}C labels were prepared in 50% aqueous dimethyl sulphoxide (60 cm^3). An equal aliquot (2 cm^3) of the appropriate feeding mixture was added to each shoot-containing vial. The tubes, in suitable racks, were placed in a plant laboratory where they were shielded from an excess of strong sunlight. They were observed periodically and supplemented when necessary with further aliquots of Hoaglands solution. After 3 d the leaves of the shoots were notably discoloured. They were then removed from the feeding vials, their tips thoroughly washed with distilled water, and dried in an air oven at 40 °C. The washings and residual feeding solutions were combined and the remaining activity determined. Each set of feeding experiments was conducted in duplicate.

Extraction, Identification and Purification of Alkaloids.—The dried plant shoots were powdered in a blender and exhaustively extracted at room temperature with portions (4 × 1 dm^3) of aqueous methanol (H_2O–MeOH; 20:80 v/v). Excess of methanol was removed under reduced pressure (water-bath temp. 40 °C) and the concentrate diluted with water (200 cm^3). The suspension was then repeatedly extracted with portions (5 × 60 cm^3) of ethyl acetate. These organic layers were combined and re-extracted with 5% HCl (4 × 30 cm^3). The acid layers were carefully adjusted to pH 7 by the addition of dilute ammonia (2 mol dm^{-3}) and then back-extracted with chloroform (5 × 30 cm^3). After being briefly washed with water (3 × 30 cm^3), the chloroform layers were dried ($MgSO_4$) and evaporated to give a crude acid-soluble fraction F_A.

The organic layers remaining after acid extraction were then

washed with aqueous Na_2CO_3 (5 × 50 cm^3). These alkaline layers were also adjusted to pH 7 by dropwise addition of dilute HCl (2 mol dm^{-3}) and back-extracted with chloroform as described above. Evaporation of the dried organic extracts gave a crude base-soluble fraction F_B. The organic layers remaining from base extraction were similarly dried and evaporated to afford a crude neutral fraction F_N. Each of the crude extracts were briefly examined by TLC using various reported[34] solvent systems. The base-soluble fraction F_B did not appear to contain any fluorescent material characteristic of alkaloids and was not further investigated. In contrast F_A was observed to contain two fluorescing components whilst one was apparant in F_N.

PLC of F_A and elution (×2) with ethyl acetate–benzene (30:70 v/v) resulted in the isolation of the two components. A resulting subfraction F_{A1} (8 mg) appeared as a bright fluorescent band at R_f 0.65. A 10 mm^3 sample of this material (1 mg cm^{-3} in MeOH) was examined by GC–MS and found to consist of a component with a retention time of 21.5 min. Its resulting mass fragmentation pattern was characteristic, as previously discussed, of kokusaginine. Hence crystallisation afforded compound **8** as colourless plates, m.p. 172–174 °C (from ethanol) (lit.,[35] 170–171 °C). The second subfraction F_{A2} (11 mg) had a R_F 0.50. Although GC–MS showed it had a similar retention time as compound **8**, its mass spectrum identified it as maculosidine. Crystallisation afforded compound **9** as fine colourless crystals, m.p. 184–186 °C (from chloroform–light petroleum) (lit.,[32] 184 °C). Separation of the neutral fraction F_N by PLC and elution (×2) with ethyl acetate–benzene (20:80 v/v) allowed the isolation of its single component, R_F 0.45, subfraction F_{N1} (6.5 mg). GC–MS of this material gave a component with a retention time of 27.8 min and a mass spectrum consistent with ptelefoline. Crystallisation thus gave **15** as colourless plates, m.p. 92–93 °C (from aqueous methanol) (lit.,[4] 91–93 °C).

The mother liquors remaining from the crystallisation of kokusaginine and ptelefoline were subjected to semipreparative HPLC. With a mobile phase of water–methanol (20:80 v/v), ptelefoline and kokusaginine had retention times of 10.4 and 12.0 respectively. The eluates were fractionated; evaporation of the appropriate fractions gave further amounts of the pure alkaloids **8** and **15**.

Radioactivity Determinations.—Samples of the residual feeding solutions and the purified alkaloids (1–3 mg), dissolved in methanol (1 cm^3) were mixed with scintillation cocktail and the radioactivity was determined with a scintillation counter. Absolute incorporation rates, which are defined as the ratio of the total activity in the isolated alkaloids to that uptaken from the fed precursors, were then calculated. Values obtained and labelling patterns observed in a typical set of experiments are summarised in Table 1.

Rearrangement of [3-$^3H_2$,4-O$^{14}CH_3$]-*kokusaginine.*—Labelled isolated kokusaginine **8** (2 mg), admixed with cold kokusaginine (5 mg), was dissolved in methanol (5 cm^3) and transferred to a glass combustion tube. The solvent was then carefully removed under a stream of nitrogen gas. Methyl iodide (1 cm^3) was added and the tube sealed and heated in an oil-bath at 120 °C for 12 h. The tube, cooled in an ice-bath, was opened and the liquid placed in a microdistillation apparatus. Further aliquots of methyl iodide (2 × 0.3 cm^3) were then added to the tube to wash the residue and also transferred to the distillation flask. The methyl iodide was then carefully distilled off with the receiver being cooled in an ice-bath. After the distillate had been collected, the distillation flask was cooled; an additional portion of methyl iodide was added and similarly distilled off.

The residues from the sealed tube and the distillation flask were dissolved in methanol (5 cm^3) and evaporated to give a dark solid. It was taken-up in chloroform (10 cm^3) and washed with 5% aqueous sodium thiosulphate (3 × 30 cm^3) and then with water (2 × 20 cm^3). The resulting organic layer was dried $MgSO_4$ and evaporated to give [3-$^3H_2$]-isokokusaginine **26** (6.5 mg, 93%), as colourless plates, m.p. 244–247 °C (from chloroform–hexane) (lit.,[36] m.p. 247–248 °C). The pure isoalkaloid, dissolved in methanol (2 cm^2), and the methyl iodide distillate were mixed with scintillation cocktail and their radioactivity determined.

Acknowledgements

The authors are grateful to the Ministry for Science and Research of Nordrhein-Westfalen, Germany for providing a Heinrich Hertz-Stiftung (fellowship) in support of these studies.

References

1 Part 27, C. F. Neville, M. F. Grundon, V. N. Ramachandran and J. Reisch, *J. Chem. Soc., Perkin Trans. 1*, 1991, 259.
2 I. Mester, in *Chemistry and Chemical Taxonomy of the Rutales*, Phytochemical Society of Europe Symposia Series No. 22, ch. 3, 31, 1983; London Academic Press.
3 M. F. Grundon, in *The Alkaloids; Quinoline Alkaloids Related to Anthranilic Acid*, ch. 2, 105, 1979; ch. 5, **32**, 341, 1988; London Academic Press.
4 J. Reisch, K. Szendrei, I. Novák, E. Minker, J. Körösi and K. Csedö, *Tetrahedron Lett.*, 1972, 449.
5 J. Reisch, K. Szendrei, I. Novák and V. Papáy, *Tetrahedron Lett.*, 1969, 3803.
6 J. Reisch, J. Körösi, K. Szendrei, I. Novák and E. Minker, *Phytochemistry*, 1975, **14**, 2722.
7 J. Reisch, K. Szendrei, V. Papáy, E. Minker and I. Novák, *Tetrahedron Lett.*, 1970, 1945.
8 J. Reisch, K. Szendrei, V. Papáy, I. Novák and E. Minker, *Tetrahedron Lett.*, 1970, 3365.
9 J. Reisch, Y. W. Mirhom, J. Körsi, K. Szendrei and I. Novák, *Phytochemistry*, 1973, **12**, 2552.
10 M. Rideau, C. Verchere, P. Hibon, J-C. Cheniéux, P. Maupas and C. Veil, *Phytochemistry*, 1979, **18**, 155.
11 J. L. Gaston, M. F. Grundon and K. J. James, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1136.
12 J. L. Gaston and M. F. Grundon, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2294.
13 M. F. Grundon, D. M. Harrison and S. A. Surgenor, *Tetrahedron Lett.*, 1979, 1713.
14 M. F. Grundon, in *Alkaloids: Chemical and Biological Perspectives; Quinoline, Acridone, and Quinalozine Alkaloids: Chemistry, Biosynthesis, and Biological Properties*, ch. 5, 6, 339, 1985; J. Wiley & Sons Inc.
15 J. F. Collins, W. J. Donnelly, M. F. Grundon and K. J. James, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2177.
16 R. M. Bowman and M. F. Grundon, *J. Chem. Soc. C*, 1966, 1504.
17 M. F. Grundon, D. M. Harrison and C. G. Spyropoulous, *J. Chem. Soc., Perkin Trans. 1*, 1975, 302.
18 M. F. Grundon, D. M. Harrison and C. G. Spyropoulous, *J. Chem. Soc., Chem. Comm.*, 1974, 51.
19 M. F. Grundon, D. M. Harrison and C. G. Spyropoulous, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2181.
20 C. F. Neville and M. F. Grundon, unpublished results, 1989.
21 J. L. Gaston and M. F. Grundon, *J. Chem. Soc., Perkin Trans. 1*, 1989, 905.
22 R. Oels, R. Storer and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2546.
23 J. F. Collins, G. A. Gray, M. F. Grundon, D. M. Harrison and C. G. Spyropoulous, *J. Chem. Soc., Perkin Trans. 1*, 1973, 94.
24 D. M. Clugston and D. B. MacLean, *Can. J. Chem.*, 1965, **43**, 2516.
25 M. S. Hifnawy, J. Vaquette, T. Sevenet, J. L. Pousett and A. Cavé, *Planta Med.*, 1976, **29**, 346.
26 K. Szendrei, M. Petz, I. Novák, J. Reisch, H. E. Bailey and V. L. Bailey, *Hebra Hung.*, 1974, **13**, 49.
27 L. A. Mitscher, M. S. Bathala, G. W. Clark and J. L. Beal, *Lloydia*, 1975, **38**, 117.
28 J. Reisch, J. Körösi, K. Szendrei, I. Novák and E. Minker, *Phytochemistry*, 1975, **14**, 1678.

29 E. A. Clarke and M. F. Grundon, *J. Chem. Soc.*, 1964, 4196.
30 G. Petit-Paly, M. Montagu, C. Viel, M. Rideau and J. C. Cheniéux, *Plant Cell Rep.*, 1987, **6**, 309.
31 D. D. Perrin and W. L. Armarego, in *Purification of Laboratory Chemicals*, Pergamon, Oxford, 3rd edn., 1988.
32 R. Storer and D. W. Young, *Tetrahedron*, 1973, **29**, 1215.
33 D. Boulanger, B. K. Bailey and W. Steck, *Phytochemistry*, 1973, **12**, 2399.
34 Zs Rózsa, K. Szendrei, I. Novák, E. Minker, M. Koltai and J. Reisch, *J. Chromatogr.*, 1974, **100**, 218.
35 D. L. Dreyer, *Phytochemistry*, 1969, **8**, 1013.
36 J. A. Lamberton and J. R. Price, *Aust. J. Chem.*, 1953, **6**, 66.

Paper 1/00558H
Received 5*th February* 1991
Accepted 18*th April* 1991