Biosynthesis of Aromatic Isoprenoids. Part II.[1] Aromatic Hydroxylation in the Biosynthesis of the Furoquinoline Alkaloids, Skimmianine, Evoxine, and Choisyine [2]

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Dictamnine (5) is shown to be an efficient biosynthetic precursor of skimmianine (6) by feeding [3-^{14}C]- and [2.3-$^{3}H_2$:3-^{14}C]-dictamnine to *Choisya ternata* and to a *Skimmia* species; dictamnine is also incorporated specifically into evoxine (7a) and into choisyine (8) in *C. ternata*. A g.l.c. method for the analysis of quinoline alkaloids of rutaceous plants is described.

PARENT members of the furoquinoline alkaloids and the furocoumarins, *e.g.* dictamnine (5) and psoralen (1a), respectively, usually co-occur in plants of the Rutaceae and Umbelliferae families with derivatives containing oxygen substituents in the homocyclic rings, for example, skimmianine (6) and bergapten (1b). The incorporation of 4-hydroxy-2-quinolone (2) into skimmianine (6) in *Skimmia japonica* [1] and in *Ruta graveolens* [3] and the effectiveness of 7-hydroxycoumarin as a precursor of oxygenated furocoumarins [4] show that biological aromatic hydroxylation can occur after the formation of the heterocyclic nucleus. The point in the biosynthetic routes to oxygenated furoquinoline alkaloids and furocoumarins at which aromatic hydroxylation takes place has not been established, however, although the balance of available evidence suggests that the oxidation occurs late in the pathway. Thus, platydesmine (3), which is a highly efficient precursor of dictamnine (5), was incorporated to a small extent into skimmianine (6) in *Skimmia japonica*.[1] In the coumarin series, 7,8-dihydroxycoumarin is a less effective precursor of xanthotoxin (1c) than 7-hydroxycoumarin, suggesting that prenylation at position 6 precedes hydroxylation at position 8.[4] By using precursors generally labelled with tritium it was shown that psoralen (1a) was incorporated into bergapten (1b) and into xanthotoxin (1c) in *Ruta graveolens*, but biological demethoxylation was also detected.[5] In order to clarify the role of biological aromatic hydroxylation

[1] Part I, J. F. Collins, W. J. Donnelly, M. F. Grundon, and K. J. James, preceding paper.

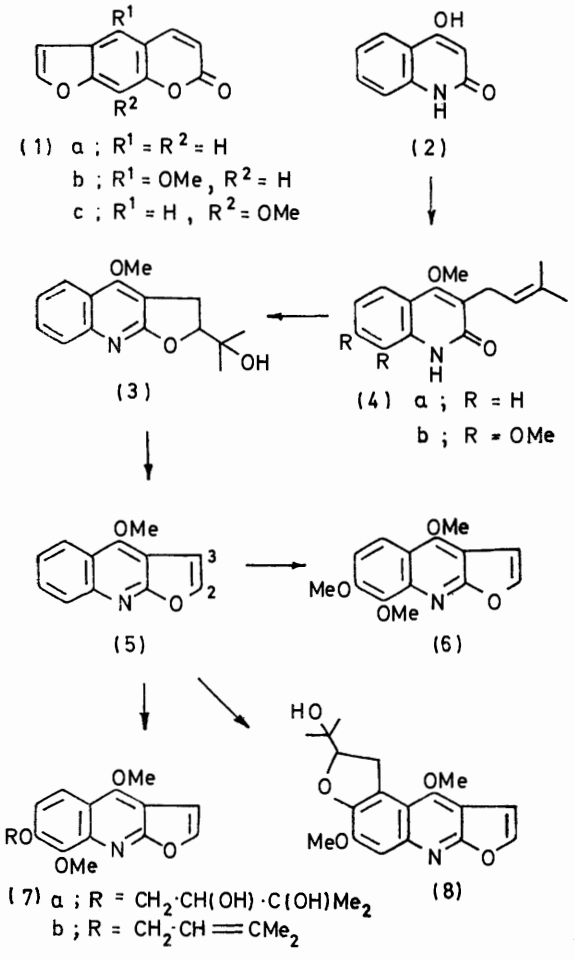
[2] Preliminary communications, J. F. Collins, W. J. Donnelly, M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J.C.S. Chem. Comm.*, 1972, 1029; M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J.C.S. Chem. Comm.*, 1974, 51.

[3] M. Cobet and M. Luckner, *European J. Biochem.*, 1968, **4**, 74; *Phytochemistry*, 1971, **10**, 1031.

[4] S. A. Brown, M. El-Dekhakhny, and W. Steck, *Canad. J. Biochem.*, 1970, **48**, 863; W. Steck and S. A. Brown, *ibid.*, 1971, **49**, 1213.

[5] G. Caporale, F. Dall'Acqua, S. Marcioni, and C. Capozzi, *Z. Naturforsch.*, 1970, **25b**, 700.

in the formation of aryl isoprenoids we decided to study the biosynthesis of skimmianine (6) and related quinoline alkaloids by feeding specifically labelled dictamnine (5) to appropriate rutaceous plants.



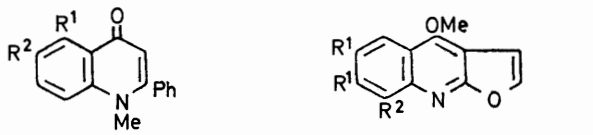
The constituents of the leaves and shoots of three rutaceous shrubs were examined, namely *Choisya ternata*, *Skimmia japonica* (which was used in our previous biosynthetic studies [1]), and another *Skimmia* variety. The latter apparently is a hybrid bred for horticultural purposes and, unlike *S. japonica*, contains male and female flowers on the same bud.

The alkaloids were extracted as described earlier.[6] We developed a g.l.c. method (see Experimental section) for the detection and quantitative analysis of the alkaloids.

As previously reported, skimmianine (6) is the major alkaloid of *Choisya ternata*.[7] In the mature bush that we examined, the three alkaloids of particular interest, skimmianine (6), choisyine (8), and evoxine (7a), were present in relative yields of 100 : 46 : 37. Dictamnine (5) was not detected. A minor alkaloid not previously obtained from *C. ternata* was isolated by preparative t.l.c. and identified as the isopentenyloxyfuroquinoline (7b) found in *Ptelea aptera*;[8] the alkaloid is probably a biosynthetic precursor of evoxine (7a).

G.l.c. analysis of the mixture of tertiary alkaloids from *Skimmia japonica* confirmed that dictamnine (5) was the principal alkaloid and that skimmianine (6) and eduline (9a) were present as minor components (relative yields 100 : 4 : 4). γ-Fagarine (10a) was detected, and its presence confirmed by isolation. A more polar constituent was also obtained by preparative t.l.c. and shown by spectroscopy to be 5-hydroxy-1-methyl-2-phenyl-4-quinolone (9b), isolated previously from *Lunasia quersifolia*.[9] Analysis of the tertiary alkaloid fraction of the *Skimmia* variety showed that, in contrast to *S. japonica*,[6] there was more skimmianine present than dictamnine (relative yields 10 : 1). This isolation work indicated that *Choisya ternata* and the *Skimmia* variety in which skimmianine was the principal alkaloid were suitable for our biosynthetic studies.

Preparation of Labelled Precursors.—[3-^{14}C]Dictamnine [*cf.* (5)] was prepared from 2,4-dimethoxyquinoline and 1-bromo-3-methyl[1-^{14}C]but-2-ene by the method already reported.[10] The final stage in the synthesis of dictamnine involves cyclisation of the aldehyde (11) by heating with polyphosphoric acid and when tritiated or deuteriated polyphosphoric acid was employed, [2,3-$^{3}H_2$]- and [2,3-$^{2}H_2$]-dictamnine (15) were obtained, respectively. The n.m.r. spectrum of deuteriated dictamnine showed the absence of the furan hydrogen doublets and analysis of the molecular ion region of the mass spectra of dictamnine and deuteriated dictamnine confirmed that the latter was largely dideuteriated dictamnine with some tri- and tetra-labelled species. Prolonged treatment of deuteriated dictamnine with 2N-hydrochloric acid did not result in exchange of deuterium atoms. A possible mechanism for the formation of [2,3-$^{2}H_2$]dictamnine is shown (Scheme 1), the exchange of ^{2}H between positions 2 and 3 occurring

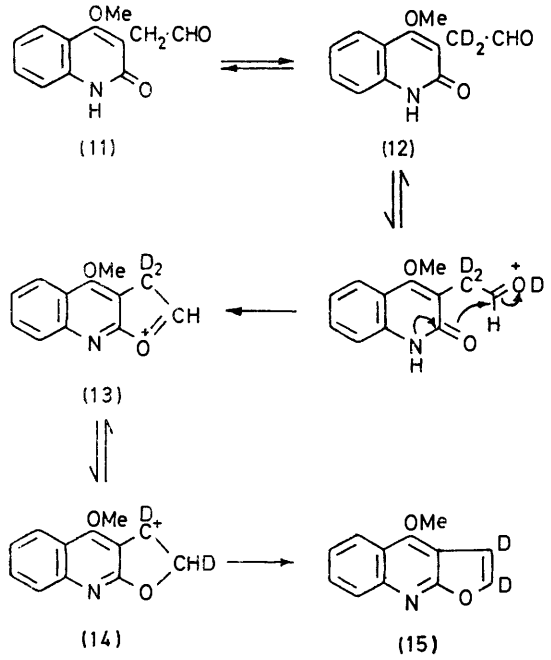


in the equilibrium (13) ⇌ (14) through a 1,2-shift, but our results do not exclude the possibility of exchange of preformed dictamnine with deuteriated polyphosphoric acid.*

* An attractive alternative is exchange of the aldehyde proton of (12) *via* addition of D⁺ to the enol form of the aldehyde (12), assisted by the methoxy-group. We are grateful to a referee for pointing out this possibility.

[6] D. R. Boyd and M. F. Grundon, *J. Chem. Soc.* (*C*), 1970, 556.
[7] S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Austral. J. Chem.*, 1967, **20**, 1975; W. J. Donnelly and M. F. Grundon, unpublished work.
[8] D.L. Dreyer, *Phytochemistry*, 1969, **8**, 1013.
[9] N. K. Hart, S. R. Johns, J. A. Lamberton, and J. R. Price, *Austral. J. Chem.*, 1968, **21**, 1389.
[10] J. F. Collins, G. A. Gray, M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J.C.S. Perkin I*, 1973, 94.

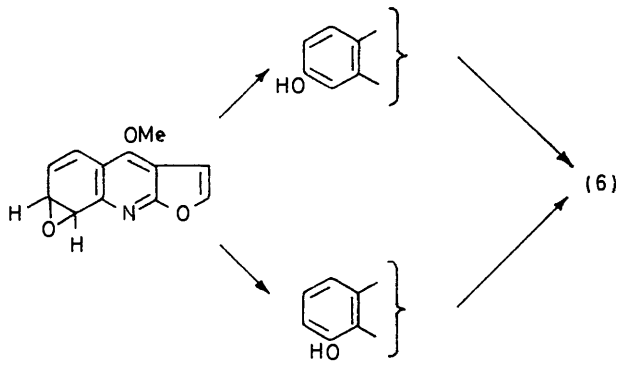
Feeding Experiments and Discussion of Results.—[3-^{14}C]Dictamnine in dimethyl sulphoxide was administered to shoots of the *Skimmia* species and of *Choisya*



SCHEME 1

ternata by the procedure developed for *Skimmia japonica*,[1] and radioactive skimmianine was isolated. The results are given in the Table. The substantial

There are several possible mechanisms for the biosynthetic conversion of dictamnine into skimmianine. Thus, dictamnine may be hydroxylated directly by a dioxygenase. Alternatively, an arene oxide formed from dictamnine in a reaction with a mono-oxygenase may be transformed into a 7,8-dihydroxy-derivative



SCHEME 2

by an epoxide hydrase. Stepwise reaction of the arene oxide through a 7- or 8-hydroxy-dictamnine could also lead to skimmianine (Scheme 2) and is likely to be accompanied by an NIH shift.[11] Hall and Prager [12] studied the incorporation of [4-^{3}H]anthranilic acid into skimmianine in *Acronychia baueri*; the results, although inconclusive, partly because of the low level of incorporation and the difficulty of interpreting the results without evidence from double labelling experiments, suggested that an NIH shift occurred. On this basis,

Feeding experiments

Compound fed	Plant	Month	^{14}C activity fed (disint. $s^{-1} \times 10^{-4}$)	^{3}H : ^{14}C atomic ratio of precursor	Skimmianine Incorporation (%)	Skimmianine ^{3}H : ^{14}C atomic ratio	Evoxine Incorporation (%)	Evoxine ^{3}H : ^{14}C atomic ratio	Choisyine Incorporation (%)	Choisyine ^{3}H : ^{14}C atomic ratio
[3-^{14}C]Dictamnine	*Skimmia* species	April	5·6		1·8					
	Skimmia species	May	5·2		2·4					
	C. ternata	April	6·4		1·3		<0·1		<0·1	
	C. ternata	May	4·1		3·6		0·15		0	
	C. ternata	May	37		3·3		<0·015		<0·01	
[2,3-$^{3}H_2$;3-^{14}C]Dictamnine	*C. ternata*	July	12	2·00 : 1	2·1	2·28 : 1	0·06	2·19 : 1	0·08	2·3 : 1

incorporations (1·3—3·6%) indicate that dictamnine is a biosynthetic precursor of skimmianine in the two plants and in conjunction with earlier results [1] allow the formulation of the biosynthetic pathway (2) ⟶ (4a) ⟶ (3) ⟶ (5) ⟶ (6). In a second series of experiments, the doubly labelled [2,3-$^{3}H_2$;3-^{14}C]-dictamnine [*cf.* (5)] was fed to *Choisya ternata*, and an incorporation of 2·1% of ^{14}C into skimmianine (6) was observed. The results (Table) show that both tritium atoms are retained in the hydroxylation process and confirm that dictamnine is a specific precursor of skimmianine.

a stepwise mechanism seems probable but a more precise formulation must await studies with 7- and 8-hydroxydictamnine.

Although our results establish that an important and probably principal route to skimmianine involves hydroxylation of dictamnine, other minor pathways are not excluded. For instance, the isolation of the 3-(3,3-dimethylallyl)-4,7,8-trimethoxyquinolone (preskimmianine) (4b) from *Dictamnus albus* led to the proposal that hydroxylation occurred earlier, at the 4-hydroxy-2-quinolone or at the 3-prenylquinolone stage; [13] it now appears that preskimmianine either

[11] *Cf.* B. Witkop, *Experientia*, 1971, **27**, 1121.

[12] C. R. Hall and R. H. Prager, *Austral. J. Chem.*, 1969, **22**, 2437.

[13] R. Storer and D. W. Young, *Tetrahedron*, 1973, **29**, 1217; R. B. Herbert, in ' The Alkaloids,' ed. J. E. Saxton, Chem. Soc. Specialist Periodical Reports, 1973, vol. 3, pp. 34, 35.

lies on a subsidiary pathway to skimmianine through a dioxygenated platydesmine or that it is a terminal product of aromatic hydroxylation.

The presence of evoxine (7a) and choisyine (8) in *Choisya ternata* provided the opportunity to study the biosynthesis of these dioxygenated dictamnine derivatives. [2,3-$^3H_2$;3-^{14}C]Dictamnine was incorporated into both alkaloids (Table). The ^3H : ^{14}C ratio was essentially unchanged in the product indicating that dictamnine can serve as a specific precursor of evoxine and of choisyine, but the incorporation of ^{14}C (0·1%) was so low in comparison with that into skimmianine (2·1%) in the same experiment that our results do not establish that this is the major route.

Since the initial publication of our work,[2] Austin and Brown [14] have reported that in the furocoumarin series [3-^{14}C]psoralen (1a) is an efficient precursor of xanthotoxin (1c) and bergapten (1b) in *Ruta graveolens* cell cultures, and in this medium dictamnine has been shown recently to be a precursor of methoxylated furoquinolines.[15] It seems, therefore, that the occurrence of aromatic hydroxylation after the formation of the furo-ring is likely to be a general phenomenon associated with the biosynthesis of furoquinoline alkaloids and furocoumarins.

EXPERIMENTAL

I.r. spectra were measured with Perkin-Elmer 157 and 457 spectrometers, n.m.r. spectra with a Perkin-Elmer 60 MHz R12 spectrometer (solutions in $CDCl_3$ with tetramethylsilane as internal standard), and mass spectra with a Perkin-Elmer RMU-6L spectrometer (unless stated otherwise).

G.l.c. Analysis of Quinoline Alkaloids.—G.l.c. was performed on a Perkin-Elmer F11 instrument using a 6 ft silanised glass column ($\frac{1}{8}$ in diam.) packed with 2% OV-1 on Diatomite CQ (90—100 mesh), with nitrogen pressure of 24 lb in^{-2}. The following retention times (min) were observed: at 200°, dictamnine (5), 3·2; γ-fagarine (10a), 7·4; skimmianine (6), 12·1; kokusaginine (10b), 13·9; platydesmine (3), 9·8; 5-hydroxy-1-methyl-2-phenyl-4-quinolone (9b), 30·8; and at 220°, eduline (9a), 25·1; choisyine (8), 26·3; and evoxine (7a), 33·9.

Isolation of Alkaloids.—The tertiary alkaloids were obtained from the leaves and shoots by the procedure described earlier,[6] and separated by preparative t.l.c. on silica [eluant chloroform–ethyl acetate (4 : 1)]. *Skimmia japonica* yielded dictamnine (5) (R_F 0·74), skimmianine (6) (R_F 0·55), and eduline (9a) (R_F 0·20), as recorded previously.[6] γ-Fagarine (10a) (R_F 0·70) was obtained from ether as prisms, m.p. and mixed m.p. 139—140° (lit.,[16] 142°). Another constituent, R_F 0·78, separating from ethyl acetate as prisms, m.p. 170—173°, was shown to be 5-hydroxy-1-methyl-2-phenyl-4-quinolone (lit.,[9] m.p. 174—176°) by comparison of i.r., n.m.r., and mass spectra with those reported.[9] The relative yields of the five alkaloids were shown by g.l.c. to be dictamnine 100, skimmianine 4·0, γ-fagarine 3·2, eduline 4·3, and 5-hydroxy-1-methyl-2-phenyl-4-quinolone 4·8.

The *Skimmia* variety was reported to contain skimmianine as the principal alkaloid; [17] we identified skimmianine and dictamnine in relative yields 100 : 9·7 (g.l.c. analysis).

Choisya ternata, in addition to skimmianine, choisyine (R_F 0·2), and evoxine (R_F 0·08) reported already,[7] contained 7-isopentenyloxy-8-methoxydictamnine (7b) (R_F 0·60), separating from hexane–ethyl acetate as prisms, m.p. and mixed m.p. 100—103° (lit.,[18] 100—103°). The n.m.r. and i.r. spectra of this last alkaloid corresponded with the published data,[8] and the mass spectrum [*m/e* 313 (M^+), 259, 245 ($M - C_5H_8$), 244, 227, and 217] provides corroborative evidence for the presence of an isopentenyloxy-group.[18] The relative yields of the alkaloids were shown (g.l.c.) to be skimmianine 100, choisyine 45·5, evoxine 35·1, and 7-isopentenyloxy-8-methoxydictamnine 10·2.

[2,3-$^3H_2$]- *and* [2,3-$^2H_2$]-*Dictamnine.*—Crude 3-formyl-methyl-4-methoxy-2-quinolone (11) (80 mg) in freshly prepared tritiated polyphosphoric acid [from phosphorus pentoxide (4 g) and tritiated water (0·6 ml; 200 mCi ml^{-1})] was kept at 150° for 30 min. Work-up in the usual way [10] gave [2,3-$^3H_2$]dictamnine (40 mg, 53%). [2,3-$^2H_2$]Dictamnine was prepared similarly; the mass spectrum (MS — 30 instrument; g.l.c.–mass spectral determination) indicated the composition $^2H_0$ 2%, $^2H_1$ 17%, $^2H_2$ 61%, $^2H_3$ 17%, $^2H_4$ 3%).

Feeding Experiments.—The labelled precursors in dimethyl sulphoxide were administered to young excised shoots of *Choisya ternata* and the *Skimmia* species during April–August 1972 and 1973, as described previously.[1] After 3 days, skimmianine, evoxine, and choisyine were obtained by preparative t.l.c., and crystallised to constant radioactivity. The results are given in the Table.

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[14] D. J. Austin and S. A. Brown, *Phytochemistry*, 1973, **12**, 1657.
[15] D. Boulanger, B. K. Bailey, and W. Steck, *Phytochemistry*, 1973, **12**, 2399.
[16] V. Deulofeu, R. Labriola, and J. De Lauche, *J. Amer. Chem. Soc.*, 1942, **64**, 2326.
[17] D. R. Boyd, personal communication.
[18] J. A. Diment, E. Ritchie, and W. C. Taylor, *Austral. J. Chem.*, 1967, **20**, 1719; E. Ritchie, W. C. Taylor, and J. S. Shannon, *Tetrahedron Letters*, 1964, 1427.