Biosynthesis of Aromatic Isoprenoids. Part III.[1] Mechanism of Formation of the Furan Ring and Origin of the 4-Methoxy-group in the Biosynthesis of Furoquinoline Alkaloids [2]

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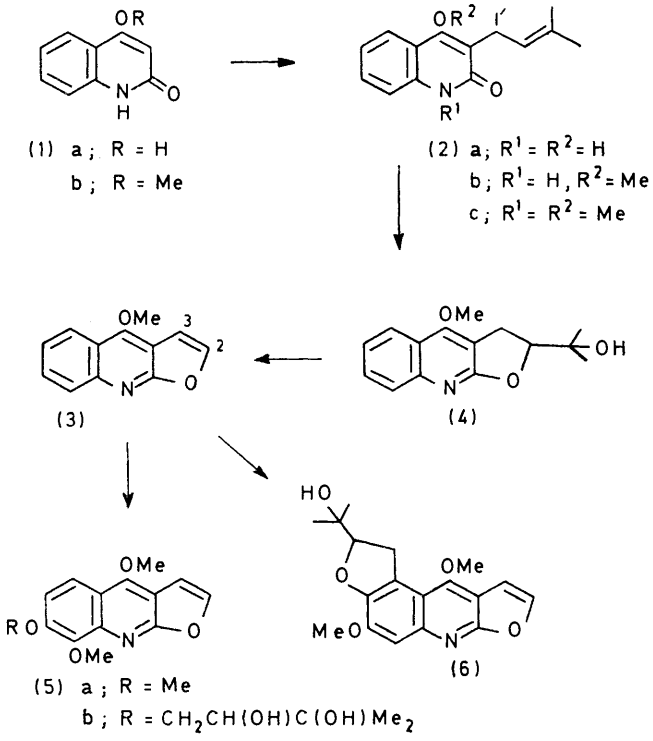
The incorporation of a 3-(3-methylbut-2-enyl)-2-quinolone doubly labelled at the benzylic methylene group into the furoquinoline alkaloids, skimmianine (5a), evoxine (5b), and choisyine (6) in *Choisya ternata* resulted in retention of half the tritium label, showing that a carbonyl derivative is not an intermediate in the formation of a furan ring from platydesmine (4). Feeding experiments also establish that the 4-methoxy-group of a 3-(3-methylbut-2-enyl)quinolone precursor is retained in skimmianine.

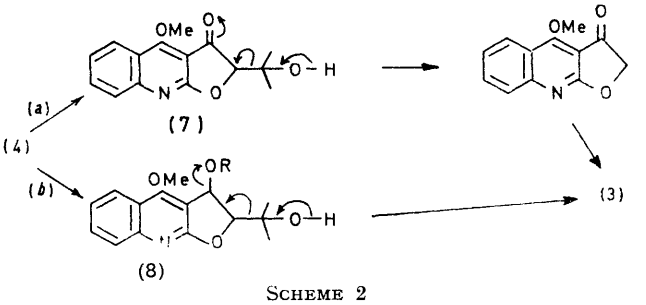
THE biosynthesis of the furan ring, particularly that in furoquinoline alkaloids and in furocoumarins, has been discussed frequently [3-5] but it was only shown recently by tracer feeding experiments that the carbon atoms of



SCHEME 1

the furan ring are isoprenoid in origin.[6] In the furoquinoline alkaloids, 4-hydroxy-2-quinolone (1a), 3-(3-methylbut-2-enyl)-2-quinolones (2), and platydesmine (4) are specific precursors of dictamnine (3),[7] and the latter was incorporated into skimmianine and other furoquinoline alkaloids oxygenated in the homocyclic ring.[1] Thus, the pathway given in Scheme 1 was established. We now report experiments designed to clarify the mechanism of formation of the furan ring by loss of the isopropyl group, (4) ⟶ (3).

Birch and Smith [4] suggested that natural benzofurans might originate from hydroxyisopropyl derivatives by benzylic oxidation to a ketone [*cf.* (7)], followed by a reaction of the retro-aldol type [Scheme 2(*a*), for dictamnine]. Alternatively, the furan ring could be generated by fragmentation either of a benzylic alcohol derivative (8) [Scheme 2(*b*)] or of an electron-deficient species formed during oxidation.[5] The essential structural difference between these pathways concerns the benzylic methylene group of platydesmine (4), both benzylic hydrogen atoms being lost in oxidation to the ketone (7) and only one in the formation of the alcohol derivative (8). The observation that no exchange occurs when [2,3-$^3H_2$]furoquinolines are treated with dilute aqueous acid [1] encouraged us to employ 3-(3-methylbut-2-enyl)-2-quinolone precursors labelled with tritium at the benzylic carbon atom to distinguish between the alternative routes (Scheme 2).



SCHEME 2

4-Methoxy-3-(3-methyl[1,1-$^3H_2$]but-2-enyl)-2-quinolone (2b) was synthesised from 2,4-dimethoxyquinoline

[1] Part II, M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J.C.S. Perkin I*, 1974, 2181.

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and 3-methyl[1,1-$^3H_2$]but-2-enyl bromide by the method described previously for preparation of the [1'-^{14}C]-labelled compound.[7] 3-Methyl[1,1-$^3H_2$]but-2-en-1-ol, required for formation of the bromide, has been made before from [^{3}H]-$LiAlH_4$,[8] but we find that the less expensive procedure employing lithium borohydride with tritiated water [9] can be applied to the reduction of 3-methylbut-2-enal.

The 4-methoxyquinolone (2b) in which both benzylic hydrogen atoms were labelled with tritium was mixed with the [1'-^{14}C]quinolone and administered to excised shoots of *Choisya ternata*. Doubly labelled skimmianine (5a) was isolated (6·2% average incorporation of ^{14}C), the ^{3}H : ^{14}C isotope ratio (Table 1) indicating that approximately half the tritium label was retained. Degradation showed that skimmianine was specifically labelled with ^{14}C at position 3 (Table 2). Thus, oxidation of the alkaloid to skimmianic acid (9a) occurred with retention of ^{14}C and loss of ^{3}H; hydrolytic decarboxylation of the oxidation product afforded radioactive carbon dioxide and essentially inactive 4-hydroxy-7,8-dimethoxy-2-quinolone (9b). The results confirm the biosynthetic sequence (Scheme 1) and eliminate the possibility that the furan rings of dictamnine (3) and skimmianine (5a) arise from platydesmine (4) *via* a ketone (7) [Scheme 2(*a*)]. The most likely pathway involves stereospecific oxidation of platydesmine to an alcohol derivative with the retention of one benzylic hydrogen, followed by loss of the isopropyl group [Scheme 2(*b*)]. On the other hand, there is no evidence to show that a carbon–oxygen bond is formed in the transformation of platydesmine into dictamnine, and our results are also consistent with direct hydride ion abstraction from the methylene group and subsequent

TABLE 1

Tracer feeding experiments with *Choisya ternata*

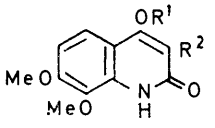
Expt. no.	Compound fed (date)	^{3}H : ^{14}C Atomic ratio	^{14}C Activity fed (disint. s^{-1})	Skimmianine (5a) ^{14}C Incorporation (%)	Skimmianine (5a) ^{3}H : ^{14}C Atomic ratio	Evoxine (5b) ^{14}C Incorporation (%)	Evoxine (5b) ^{3}H : ^{14}C Atomic ratio	Choisyine (6) ^{14}C Incorporation (%)	Choisyine (6) ^{3}H : ^{14}C Atomic ratio
1	[1'-$^3H_2$,1'-^{14}C]-(2b) (July)	2 : 1	4·3 × 10^4	6·0	1·07 : 1	0·20	1·16 : 1	0·23	0·97 : 1
2	[1'-$^3H_2$,1'-^{14}C]-(2b) (August)	2 : 1	3·6 × 10^4	6·4	1·23 : 1	0·34	1·10 : 1	0·27	0·90 : 1
3	[4-*OC*$^3H_3$,1'-^{14}C]-(2b) (April)	3 : 1	2·7 × 10^5	1·1	3·26 : 1	0·01	3·31 : 1	0·0	
4	[4-*OC*$^3H_3$,1'-^{14}C]-(2b) (May)	3 : 1	4·5 × 10^5	1·1	3·24 : 1	0·005	2·97 : 1	0·0	

TABLE 2

Degradation of radioactive skimmianine

Compound	Specific activity (^{14}C) ×10^{-3} (disint. s^{-1} mmol^{-1}) Expt. 1	Specific activity (^{14}C) ×10^{-3} (disint. s^{-1} mmol^{-1}) Expt. 3	Relative specific activity (^{14}C) (%) Expt. 1	Relative specific activity (^{14}C) (%) Expt. 3	^{3}H : ^{14}C Atomic ratios Expt. 1	^{3}H : ^{14}C Atomic ratios Expt. 3
Skimmianine (5a)	4·4	6·9	100	100	1 : 1	3 : 1
Skimmianic acid (9a)	4·1	6·3	94	91·5	<0·1 : 1	2·96 : 1
$BaCO_3$	3·75 *	5·6 *	85 *	81 *		
(9b)	0·08	0·0	<2		<0·05 : 1	

* Calculated from total activity of $BaCO_3$, assuming that all CO_2 was trapped.



(9) a; R^1 = Me, R^2 = CO_2H
b; R^1 = R^2 = H
c; R^1 = H, R^2 = $CH_2CH{:}CMe_2$
d; R^1 = Me, R^2 = $CH_2CH{:}CMe_2$

Origin of the 4-Methoxy-group.—4-Hydroxy-2-quinolone (1a), the 4-hydroxy-3-prenylquinolone (2a), and the 4-methoxy-3-prenylquinolone (2b) are effective precursors (1·3—3·8% incorporation) of dictamnine (3) in *Skimmia japonica*,[7] and the 4-methoxy-derivative (2b) is incorporated (6·2%) into skimmianine (5a) in *Choisya ternata* (see above). These results suggest the biosynthetic sequence (1a) ⟶ (2a) ⟶ (2b) ⟶ (4) ⟶ (3) ⟶ (5a) with methylation of the 4-hydroxy-group occurring at the 3-prenyl stage, but this simple interpretation is not sufficient, since the 4-methoxyquinolone (1b) and the *N*-methyl-2-quinolone (2c) are equally good precursors of dictamnine.[7] The latter observation indicates that the enzyme system can effect *N*-demethylation, and the results pose the question whether *O*-demethylation can occur at one or more fragmentation of the resultant species. Further definition of the biomechanism must await the determination of the stereochemistry of the benzylic proton lost during the conversion of the prenylquinolone (2b) into skimmianine (5a) and studies of the biosynthetic role of alcohol derivatives (8).

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[9] R. H. Cornforth, *Tetrahedron*, 1970, **26**, 4635.

points in the pathway with subsequent remethylation. We decided to study this problem by preparing and feeding the 4-methoxy-3-prenylquinolone (2b) with tritium labelling in the 4-methoxy-group.

Aryl methyl ethers with isotopic labelling in the methyl group are usually prepared by reaction of phenols with labelled diazomethane,[10] but the report that hydrogen–deuterium exchange is subject to acid-catalysis by phenols [11] indicated that our precursor could be prepared simply by reaction of the tritiated phenol (2a) with unlabelled diazomethane. In model experiments with the 4-hydroxyquinolones (2a) and (9c) and with *m*-nitrophenol, the phenols in glyme containing deuterium oxide were treated with diazomethane to give the corresponding labelled methyl ethers. Mass spectrometry showed that the products in each case were mixtures of species $ROCH_3$, $ROCH_2D$, $ROCHD_2$, and $ROCD_3$ (see Experimental section). It is apparent that in the reaction $ArOD + CH_2N_2 \rightleftharpoons ArO^- + CH_2DN_2^+ \longrightarrow ArOCH_2D + N_2$, the initial equilibrium results in exchange of hydrogen atoms in diazomethane leading ultimately to a mixture of mono-, di-, and tri-deuteriated ethers. Since the introduction of a specific number of isotopic hydrogen atoms was not required in our experiments, the 4-[$^3H_3$]methoxy-2-quinolone (2b) was prepared similarly.

The 4-methoxy-3-(3-methyl[1-^{14}C]but-2-enyl)quinolone (2b) [7] mixed with a sample in which the 4-methoxy-group was labelled with tritium was fed to *Choisya ternata*. Extraction afforded doubly labelled skimmianine (5a), the $^3H : {}^{14}C$ ratio in the precursor being maintained in the product (Table 1). Oxidation of skimmianine gave skimmianic acid (9a) without affecting the $^3H : {}^{14}C$ ratio, and degradation of the acid to radioactive carbon dioxide and the inactive 4-hydroxy-2-quinolone (9b) showed that randomisation of ^{14}C had not taken place (Table 2). The results show that in the biosynthesis of skimmianine from the 4-methoxy-3-prenylquinolone (2b), the 4-methoxy-group remains intact in intermediates platydesmine (4) and dictamnine (3). The incorporation of 4-methoxy-2-quinolone into dictamnine suggests, however, that more than one pathway operates in *Skimmia japonica*, and methyl exchange at the 4-hydroxy-2-quinolone stage is not excluded by our results. Since platydesmine (4) is an excellent precursor of dictamnine (18·5% incorporation of the racemate),[7] it appears likely that biosynthetic routes to dictamnine converge at or before the formation of this intermediate.

Biosynthesis of Evoxine and Choisyine.—We showed earlier that dictamnine acts as a specific precursor of the dioxygenated dictamnine derivatives evoxine (5b) and choisyine (6) in *Choisya ternata*, although the incorporation (0·06—0·08%) of [2,3-$^3H_2$,2-^{14}C]dictamnine into the two alkaloids was much lower than into skimmianine (5a) (2·1%).[1] In the present series of feeding experiments we again examined evoxine and choisyine (Table 1). Thus, administration of the 4-methoxy-3-(3-methyl-[1,1-$^3H_2$,1-^{14}C]but-2-enyl)quinolone (2b) afforded radioactive evoxine and choisyine with isotope ratios indicating that approximately half the tritium was retained. The doubly labelled precursor in which the 4-methoxy-group was labelled with tritium was incorporated without change in the $^3H : {}^{14}C$ ratio into evoxine, but choisyine was inactive. The results are similar to those for skimmianine in the same experiments, and confirm that the biosyntheses of all three oxygenated furoquinoline alkaloids follow the same route up to the formation of the common intermediate, dictamnine. It appears (Table 1, Table in Part II [1]) that choisyine is produced in *Choisya ternata* mainly during the later period of the season of active growth (July–August).

EXPERIMENTAL

General directions and the method of feeding labelled precursors to shoots of *Choisya ternata* were as in Part II.[1]

1-Bromo-3-methyl[1,1-$^3H_2$]*but-2-ene.*—Lithium borohydride (236 mg) in tetrahydrofuran (20 ml) containing tritiated water (0·2 ml; 200 mCi ml^{-1}) was refluxed for 2·5 h, 3-methylbut-2-enal [12] (1·25 g) was added, and refluxing continued for a further 0·5 h. Addition of water, then 2N-hydrochloric acid, and extraction with ether afforded 3-methyl[1,1-$^3H_2$]but-2-en-1-ol (1·24 g), which without purification was converted with phosphorus tribromide into 1-bromo-3-methyl[1,1-$^3H_2$]but-2-ene, b.p. 26—33° at 12 mm.

4-[$^3H_3$]*Methoxy-3-(3-methylbut-2-enyl)-2-quinolone and 4-*[$^3H_3$]*Methoxy-compounds.*—The 4-hydroxyquinolone (2a) [13] (147 mg) in dry glyme (5 ml) containing tritiated water (0·8 ml; 200 mCi ml^{-1}) was methylated with diazomethane as described previously [13] to give the methoxyquinolone (2b) (139 mg, 90%), m.p. and mixed m.p. 130—134° (lit.,[13] 132—134°). The [$^2H_3$]methoxy-compounds were prepared similarly from quinolones (2a) and (9c) and from *m*-nitrophenol.

The deuteriated methyl ethers were analysed by integration of n.m.r. methoxy-resonances to give the total proton composition in methoxy-groups, and by mass spectrometry to detect and analyse the species present; the following results were obtained.

		Composition (%)			
	OMe protons	OCH_3	OCH_2D	$OCHD_2$	OCD_3
(2b)	1·2	17	29	29	25
m-nitroanisole (9d)	0·8 7·5	10	17	31	42

Degradation of Skimmianine.[14]—Skimmianine (5a) (120 mg) was degraded as described for dictamnine [7] to give skimmianic acid (9a) (20 mg), m.p. 247—248°, which was converted into carbon dioxide trapped as barium carbonate, and the 4-hydroxy-2-quinolone (9b) (8 mg), m.p. 248—249° (from aqueous methanol).

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