Biosynthesis of the Furoquinoline Alkaloids, Skimmianine, Evoxine, and Choisyine: Mechanism of Formation of the Furan Ring. The Timing of Aromatic Hydroxylation and of Methylation

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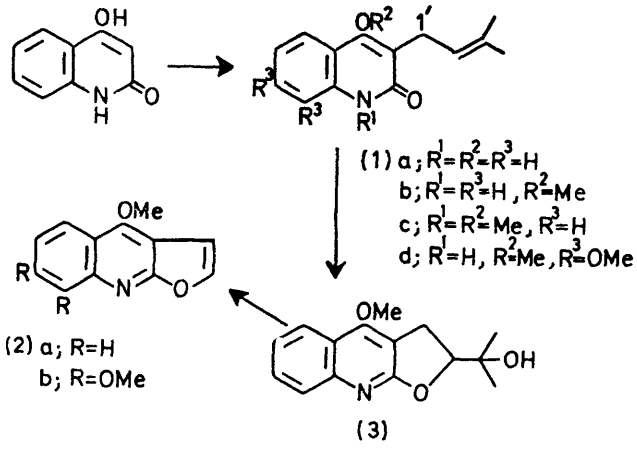
Summary Tracer feeding experiments with *Choisya ternata* using doubly labelled precursors confirm that dictamnine (**2a**) is converted into skimmianine (**2b**), and show that the 4-methoxy-group of a 3-prenylquinolone precursor (**1b**) is retained in skimmianine; in the formation of the furan ring from platydesmine (**3**), a carbonyl derivative is not an intermediate; a similar biosynthetic route applies to the 7,8-dioxygenated quinoline, evoxine (**4**) and to the 6,7-derivative, choisyine (**5**).

STUDIES of the biosynthesis of furoquinoline alkaloids[1] and of furocoumarins[2,3] show that the furan ring in both series arises by oxidative cyclisation of a prenyl derivative to a hydroxyisopropyldihydrofuran, followed by loss of the isopropyl fragment, as summarised in Scheme 1 for dictamnine (**2a**). The isolation of the 7,8-dimethoxyquinolone (**1d**), preskimmianine, from *Dictamnus albus* led to the proposal that the hydroxylated analogues of the 3-prenylquinolone (**1b**) and of platydesmine (**3**) are precursors of skimmianine (**2b**).[4] On the other hand, the incorporation (1·2—3·5%) of specifically labelled ^{14}C-dictamnine (**2a**) into skimmianine in *Choisya ternata* and in a *Skimmia* species[5] indicated that a major pathway to the oxygenated furoquinoline alkaloids involves aromatic hydroxylation after the formation of the furan ring. A similar route has been established recently for furocoumarins.[3] We now present further results related to aromatic hydroxylation in furoquinoline alkaloids and to other aspects of the biosynthesis of this group of alkaloids.

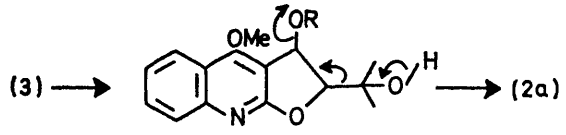


SCHEME 1

Doubly labelled [2,3-$^3H_2$; 3-^{14}C]dictamnine was administered to shoots of *Choisya ternata*, and radioactive skimmianine (**2b**) (2·1% incorporation) was isolated. The ^{3}H:^{14}C ratio was essentially unchanged in the product, and the results corroborate the conclusion[5] that dictamnine is an intermediate in the biosynthesis of skimmianine.

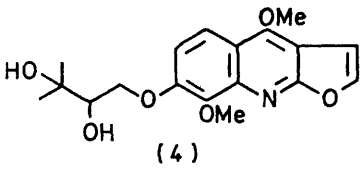
The observation that no exchange occurs when [2,3-$^3H_2$]-dictamnine is treated with dilute aqueous acid encouraged us to investigate other biosynthetic problems employing doubly labelled quinolines. The 4-hydroxy-3-prenyl-2-quinolone derivatives (**1a**—**c**) are each good precursors of dictamnine in *Skimmia japonica* (2·0—3·8% incorporation); labelled 4-hydroxy- and 4-methoxy-2-quinolone were also incorporated (1·3—1·5%), but not 2,4-dimethoxyquinoline.[1] The enzyme system apparently can effect *N*-demethylation, and the results pose the question of whether the C-4 methoxy-group of (**1b**) is retained or whether *O*-demethylation-remethylation occurs at one or more points in the pathway. Accordingly, we fed to *Choisya ternata* the [1′-^{14}C] 4-methoxy-3-prenylquinolone (**1b**) mixed with a sample in which the 4-methoxy-group was labelled with tritium. The ^{3}H:^{14}C ratio was maintained in the skimmianine isolated from this experiment, and degradation of the product showed that randomisation of ^{14}C had not occurred. Thus, the 4-methoxyquinolone (**1b**) is a specific precursor of skimmianine, and in this route the methyl group remains intact in the intermediates (**3**) and (**2a**). The tritiated precursor was prepared conveniently by exchange of the acidic 4-hydroxy-proton of the prenyl derivative (**1a**) with ^3H_2O, and subsequent reaction with unlabelled diazomethane. We expected from the accepted mechanism of the diazomethane reaction to obtain a singly labelled product, ArOCH$_2$^{3}H, but concurrent studies with the deuteriated quinolone and with model compounds, indicate that a mixture of mono-, di-, and tri-deuteriated species is present.

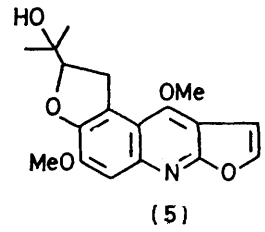
Although platydesmine (**3**) is a highly efficient specific precursor of dictamnine in *S. japonica*,[1] the biomechanism of the process, (**3**) → (**2**), is unknown. Benzylic oxidation leading to a ketone or to an alcohol has been proposed,[6] and we sought to distinguish between these alternatives by feeding precursors appropriately labelled with tritium. The 4-methoxyquinolone (**1b**) in which both benzylic hydrogen atoms were labelled with tritium was mixed with the ^{14}C-quinolone (^{3}H:^{14}C atomic ratio, 2:1) and administered to *C. ternata*. Doubly labelled skimmianine (**2b**) (6·2% incorporation) was isolated and had an isotope ratio (1·1:1 and 1·2:1 in two experiments) showing that approximately half of the tritium label was retained; degradation showed that the product (**2b**) was specifically labelled with ^{14}C at position 3. The results eliminate the hypothesis that the furan rings of dictamnine and of skimmianine arise



SCHEME 2

from platydesmine (**3**) *via* a ketone; the most probable 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). Since there is no evidence to show that a carbon–oxygen bond is formed, our results are also consistent with direct hydride ion abstraction from C-1′ and subsequent fragmentation of the resultant species. It is likely that these conclusions apply equally to the biosynthesis of furocoumarins.





The furoquinoline alkaloids evoxine (**4**) and choisyine (**5**) are also constituents of *Choisya ternata*,[7] and we took the opportunity to study their biosynthesis. Incorporations of the doubly labelled precursors (**2a**), and (**1b**; ^{3}H and ^{14}C at C-1′) into the two alkaloids were substantially lower than into skimmianine. However, the ^{3}H:^{14}C ratios were similar to those for skimmianine in the same experiments, suggesting that introduction of oxygen functions into the benzene ring is a late step in these cases also.

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