Biosynthesis of dictamnine. The origin of the quinoline nucleus[1]

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The biosynthesis of dictamnine, a furoquinoline alkaloid, was studied in intact *Dictamnus albus* plants. The quinoline ring system is derived from anthranilic acid and acetate. Tryptophan is not a precursor. Anthranilic acid concentration appears to control the rate of dictamnine biosynthesis in intact *D. albus*.

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INTRODUCTION

Furoquinoline alkaloids are found in many of the genera of the family Rutaceae (2, 3). Dictamnine (II) is the prototype of this group of compounds. Several suggestions have been advanced to account for the biogenetic origin of its carbon skeleton (3–9). The structural correspondence to the ring system of dictamnine of a number of compounds which have been postulated as precursors of the furoquinoline nucleus is shown in Scheme 1.

We have investigated the biosynthesis of dictamnine in *Dictamnus albus* L. (*D. fraxinella alba* Pers.) by tracer methods. Our results demonstrate that, in this plant, the quinoline nucleus of dictamnine is specifically derived from anthranilic acid (III) and acetate, and that tryptophan (I) is not a significant precursor. Even though the origin of the two remaining carbon atoms of the furan nucleus remains unsettled, the incorporation data to be presented invalidate the hypotheses that the non-anthranilic fragment originates from succinate (V) (3), from ornithine (VI) (9), or from erythrose (VII) (8).

METHODS AND RESULTS

The major alkaloids of *Dictamnus albus* L. ("gas plant") are dictamnine (10, 11) and skimmianine (7,8-dimethoxydictamnine) (12). The former is localized in the roots and seeds of the plant, and the latter is found only in the leaves (12). Dictamnine content of the roots is maximal during the summer months, and that of the seeds increases rapidly during this period (12). The tracer work was therefore carried out during June and July (1965 and 1966). In a series of experiments, a number of suitably labelled putative precursors were administered to intact mature plants of *D. albus* by the wick technique (13). In each experiment dictamnine was extracted from the roots of the plants and was purified to constant radioactivity. The details of these experiments are recorded in Table I.

The labelled samples of dictamnine were degraded by the reaction sequences shown in Scheme 2. The specific activities of the degradation products so obtained are given in Table II. These degradation sequences permitted determination of the activity at each of C-2, C-3, C-4, C-10, and C-11 of the dictamnine nucleus and at the *O*-methyl group (Scheme 2).

The distribution patterns of the radioactivity derived from individual precursors within the labelled dictamnine samples which were degraded are summarized in Table III. Dictamnine derived from [methyl-^{14}C]-methionine contained all activity in the *O*-methyl group. Radioactivity from [carboxyl-^{14}C]-anthranilic acid was restricted to C-4. Approximately 80% of the activity derived from [1-^{14}C]- and from [2-^{14}C]-acetate was recovered in C-10 and in C-11, respectively. Label from [1-^{14}C]-glycolate and from [1-^{14}C]-ribose was spread non-randomly throughout the molecule. The similarity of the non-random distribution of activity in these two samples is noteworthy.

The results of a feeding experiment with doubly labelled anthranilic acid are presented in Table IV. The dictamnine which

[1]A preliminary account of part of this work has been published (1).

TABLE I

Incorporation of labelled compounds into dictamnine

Precursor	Specific activity (counts min^{-1} $mmole^{-1}$ $\times 10^{-9}$)	Total activity fed (counts $min^{-1} \times 10^{-7}$)	Product			
			Weight of dry *D. albus* roots (g)	Dictamnine		
				Weight (mg)	Specific activity (counts min^{-1} $mmole^{-1} \times 10^{-4}$)	Specific radiochemical yield* ($\times 10^4$)
[Carboxyl-^{14}C]-anthranilic acid†	0.0454±0.0009	2.95±0.06	222	274	6.21±0.20	1 275
[Carboxyl-^{14}C]-anthranilic acid‡	0.556±0.007	0.657±0.008	25	16	8.92±0.18	178
Sodium [1-^{14}C]-acetate§	2.25±0.05	11.23±0.27	200	310	0.255±0.007	1.13
Sodium [2-^{14}C]-acetate§	1.89±0.03	9.43±0.15	172	165	0.799±0.018	4.22
L-[Methyl-^{14}C]-methionine§	8.07±0.16	5.95±0.11	190	181	2.54±0.05	3.15
Sodium [1-^{14}C]-glycolate‖	2.13±0.02	4.57±0.05	145	103	1.30±0.03	6.10
D-[1-^{14}C]-Ribose§	1.36±0.02	5.79±0.09	220	230	1.68±0.04	12.4
DL-[β-^{14}C]-Tryptophan§	3.33±0.08	3.76±0.09	297	186	0.032±0.009	0.09
Sodium [5-^{14}C]-α-ketoglutarate‖	5.22±0.07	8.46±0.11	126	172	0.054±0.004	0.10

*Specific radiochemical yield = 100(specific activity of product)/(specific activity of precursor).
†Volk Radiochemical Co. (see Table IV).
‡Merck Sharp and Dohme of Canada, Ltd.
§New England Nuclear Corp.
‖Radiochemical Centre.

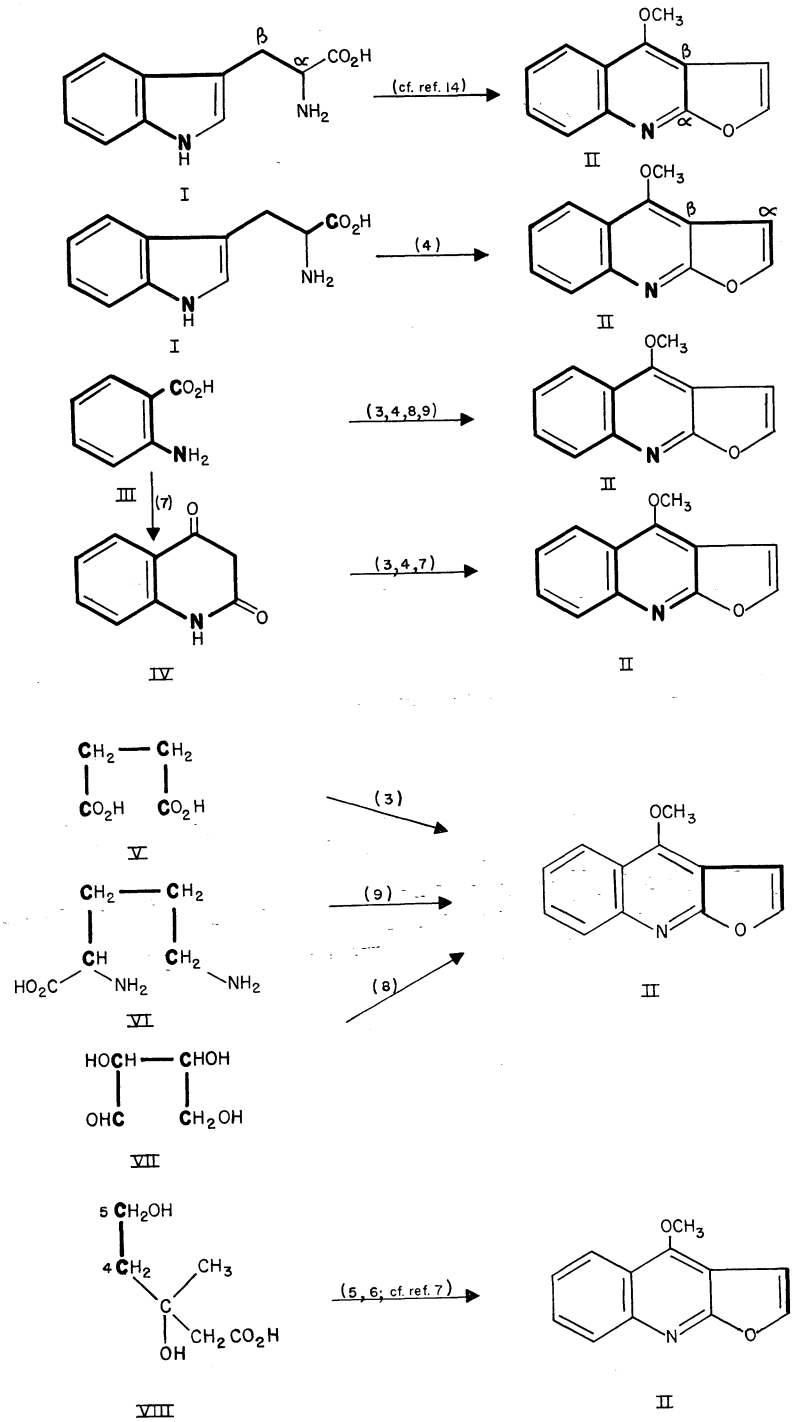
TABLE II

Degradation of dictamnine derived from labelled precursors

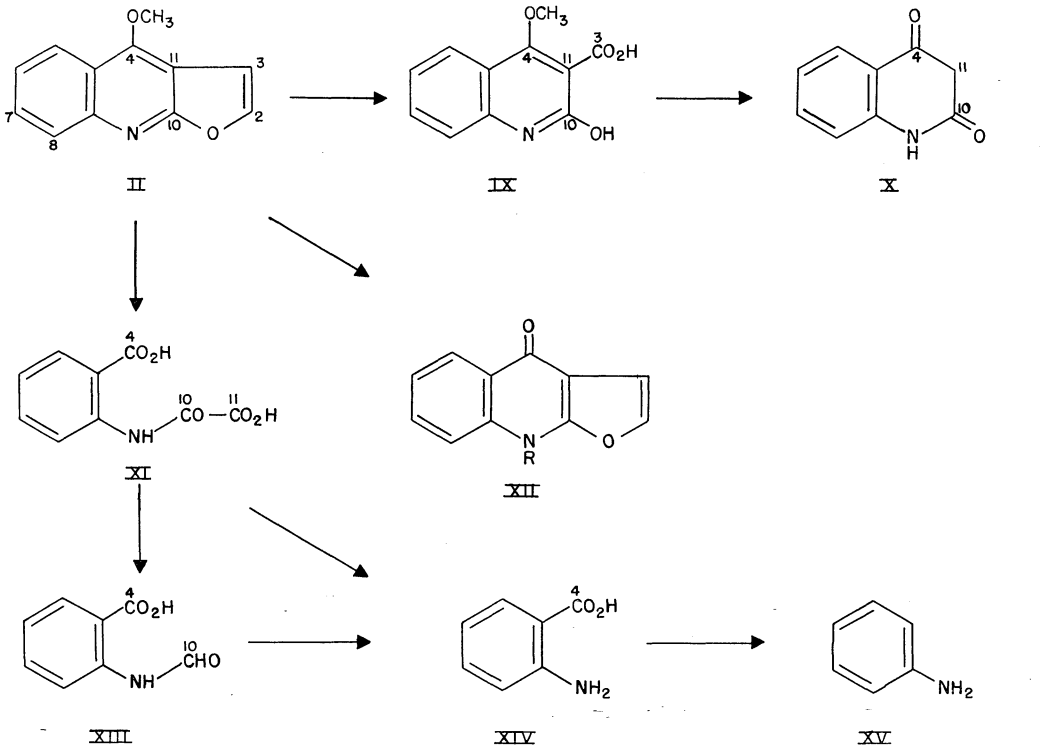
Compound	[Carboxyl-^{14}C]-anthranilic acid		[1-^{14}C]-Acetate		[2-^{14}C]-Acetate		L-[Methyl-^{14}C]-methionine		[1-^{14}C]-Glycolate		D-[1-^{14}C]-Ribose	
	Specific activity (counts min^{-1} mmole^{-1} $\times 10^{-4}$)	Relative specific activity* (%)	Specific activity (counts min^{-1} mmole^{-1} $\times 10^{-3}$)	Relative specific activity (%)	Specific activity (counts min^{-1} mmole^{-1} $\times 10^{-3}$)	Relative specific activity (%)	Specific activity (counts min^{-1} mmole^{-1} $\times 10^{-4}$)	Relative specific activity (%)	Specific activity (counts min^{-1} mmole^{-1} $\times 10^{-4}$)	Relative specific activity (%)	Specific activity (counts min^{-1} mmole^{-1} $\times 10^{-4}$)	Relative specific activity (%)
Dictamnine (II)	6.21 $\pm$ 0.20	100 $\pm$ 3	2.55 $\pm$ 0.07	100 $\pm$ 3	7.99 $\pm$ 0.18	100 $\pm$ 2	2.54 $\pm$ 0.05	100 $\pm$ 2	1.30 $\pm$ 0.03	100 $\pm$ 3	1.68 $\pm$ 0.04	100 $\pm$ 2
Isodictamnine (XII, R = CH_3)	—		2.40 $\pm$ 0.07	94 $\pm$ 4	7.73 $\pm$ 0.15	97 $\pm$ 3	0.086 $\pm$ 0.008	3 $\pm$ 0.3	1.03 $\pm$ 0.02	79 $\pm$ 3	1.40 $\pm$ 0.02	83 $\pm$ 2
Dictamnic acid (IX)	—		—		—		—		—		1.53 $\pm$ 0.09	91 $\pm$ 6
Oxalylanthranilic acid (XI)	6.25 $\pm$ 0.11	101 $\pm$ 4	2.33 $\pm$ 0.04	91 $\pm$ 3	7.76 $\pm$ 0.18† 3.72 $\pm$ 0.07	97 $\pm$ 3	—		0.95 $\pm$ 0.03	73 $\pm$ 3	1.18 $\pm$ 0.03	70 $\pm$ 2
Formylanthranilic acid (XIII)	—		2.26 $\pm$ 0.06	89 $\pm$ 3	0.61 $\pm$ 0.06	16 $\pm$ 2	—		0.75 $\pm$ 0.02	58 $\pm$ 2	0.86 $\pm$ 0.03	51 $\pm$ 2
Anthranilic acid (XIV)	6.30 $\pm$ 0.13	102 $\pm$ 4	0.27 $\pm$ 0.03	11 $\pm$ 1	0.18 $\pm$ 0.03	5 $\pm$ 1	—		0.57 $\pm$ 0.02	44 $\pm$ 2	0.62 $\pm$ 0.01	37 $\pm$ 1
Aniline (XV)	0.008 $\pm$ 0.002	0.1 $\pm$ 0.03	0.12 $\pm$ 0.02	5 $\pm$ 1	0.11 $\pm$ 0.02	3 $\pm$ 1	—		0.22 $\pm$ 0.01	17 $\pm$ 1	0.22 $\pm$ 0.01	13 $\pm$ 1

*Dictamnine = 100.

†For further degradation, this oxalylanthranilic acid (specific activity (7.76 $\pm$ 0.18) $\times$ 10^3 counts min^{-1} mmole^{-1}) was diluted with inactive carrier to yield, after recrystallization, oxalylanthranilic acid (specific activity (3.72 $\pm$ 0.07) $\times$ 10^3 counts min^{-1} mmole^{-1}). The relative specific activity of further intermediates in the degradation sequence, given in the table, is based on the original dictamnine.



SCHEME 1. Postulated precursors of dictamnine.



SCHEME 2. Degradation of dictamnine, permitting determination of activity at individual carbon atoms: OCH_3 = dictamnine (II) minus isodictamnine (XII, R = CH_3); C-2 = dictamnine (II) minus dictamnic acid (IX); C-2 plus C-3 = isodictamnine (XII, R = CH_3) minus oxalylanthranilic acid (XI); C-3 = C-2 plus C-3 minus C-2; C-4 = anthranilic acid (XIV) minus aniline (XV); C-10 = formylanthranilic acid (XIII) minus anthranilic acid (XIV); C-11 = oxalylanthranilic acid (XI) minus formylanthranilic acid (XIII); and C-5, C-6, C-7, C-8, C-12, and C-13 (benzene ring) = aniline (XV).

was obtained was found, within experimental error, to have a $^{14}C/^{15}N$ ratio identical with that of the doubly labelled precursor.

DISCUSSION

The quinoline nucleus is found in a variety of naturally occurring compounds. Studies of the biosynthesis of a number of these indicate the existence of several pathways leading to this ring system.

Only one of these pathways is fully understood. Kynurenic acid (XVI) and its relatives, which are found in mammalian tissues, in insects, and in certain microorganisms, have been shown, by incorporation studies, by recognition of intermediates, and by elucidation of the reaction sequence at the enzyme level, to be products of tryptophan (I) catabolism (14). The correspondence of the carbon skeleton of kynurenic acid (XVI) to that of tryptophan (I) is shown in Scheme 3*a*. The ommochrome pigments of insects are of related origin (15).

The fragmentary knowledge of the biosynthetic origin of other quinoline derivatives rests entirely on tracer studies.

Tryptophan (I) serves as a precursor of the quinoline ring of quinine (XIX), a plant product. Activity from [α-^{14}C]-tryptophan was recovered in C-2 of its quinoline nucleus (16). It is postulated that the skeletal correspondence of quinine and tryptophan is as shown in Scheme 3*b*. This mode of conversion, in which C-2 of the indole nucleus of the precursor is preserved in the product, thus differs from that of the kynurenic acid pathway, in which C-2 of the indole nucleus is extruded as formic acid. It is assumed that compounds of the type XVII and XVIII are intermediates in quinine biosynthesis. This

TABLE III
Distribution of radioactivity in dictamnine

Precursor	Relative specific activity (%) at:						
	OCH_3	C-2	C-3	C-10	C-11	C-4	C-5, C-6, C-7, C-8, C-12, C-13
[Carboxyl-^{14}C]-anthranilic acid	← 0±5 →			← 0±5 →		101±4	0
[1-^{14}C]-Acetic acid	6±5	← 3±5 →*		78±4	3±4	6±1	5±1
[2-^{14}C]-Acetic acid	3±4	← 0±4 →*		11±2	81±3	2±1	3±1
L-[Methyl-^{14}C]-methionine	97±2	← 3±0.3 →					
[1-^{14}C]-Glycolic acid	21±4	← 6±4 →*		14±3	15±3	27±2	17±1
D-[1-^{14}C]-Ribose	17±3	9±6	4±7	14±2	19±3	24±1	13±1

*The low level of incorporation precluded individual determination of activity at these two carbon atoms.

TABLE IV
Incorporation of [^{14}C,^{15}N]-anthranilic acid into dictamnine

	Specific activity (counts min^{-1} $mmole^{-1}$)	% excess ^{15}N above natural abundance*	$^{14}C/^{15}N$ ($\times 10^{-5}$)
Anthranilic acid†	$(4.54\pm0.09)\times10^7$	90.2±0.8	5.04±0.11
Dictamnine	$(6.21\pm0.20)\times10^4$	0.124±0.003	5.01±0.12

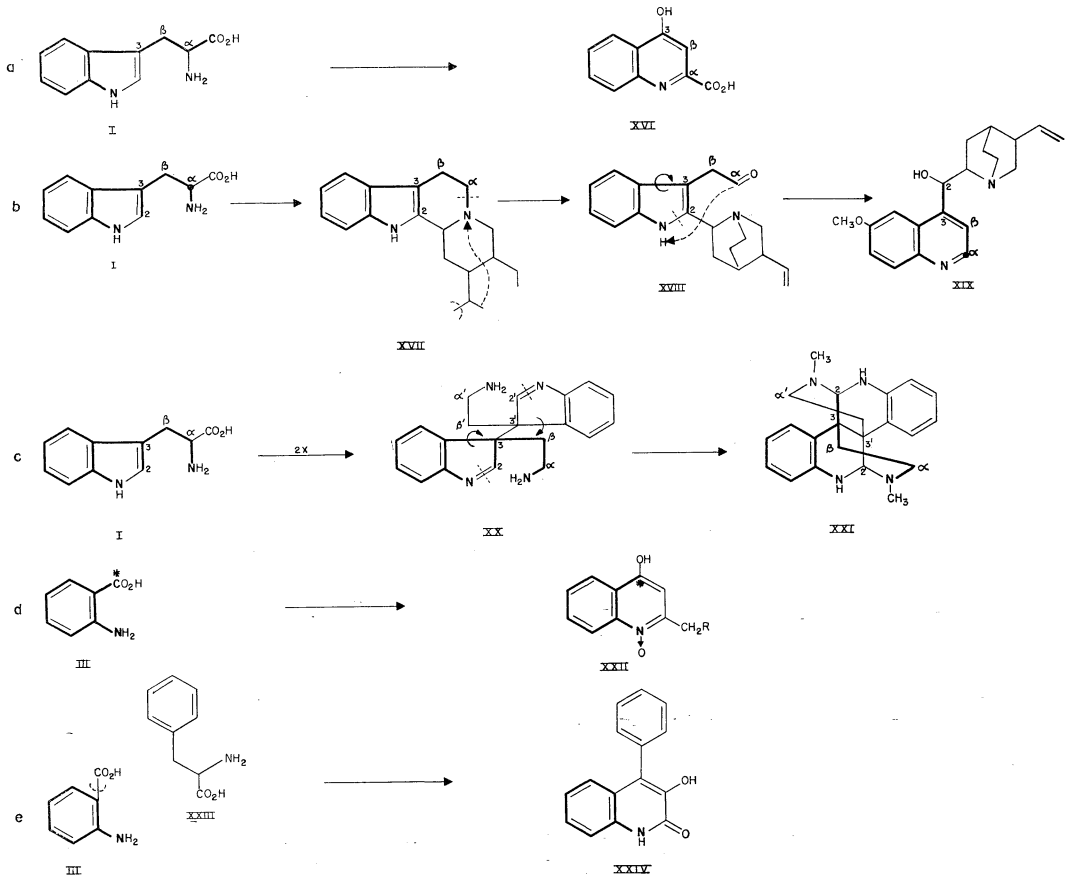
*Atmospheric nitrogen, used as a line standard, showed a value of 0.00733 ±0.00003 for the ratio of the ion of mass 29 to that of mass 28.
†The doubly labelled anthranilic acid was prepared by recrystallization of a mixture consisting of [carboxyl-^{14}C]-anthranilic acid (1.24 mg, specific activity (3.66 ± 0.07) × 10^9 counts min^{-1} $mmole^{-1}$ (Volk Radiochemical Co.)) and [^{15}N]-anthranilic acid (98.76 mg, 91.3 ± 0.8% excess ^{15}N above natural abundance). The latter was prepared from potassium [^{15}N]-phthalimide (Volk Radiochemical Co.) by Hofmann rearrangement (45).

hypothesis is supported by the finding that a monoterpenoid precursor is incorporated into the non-tryptophan derived fragment of several indole alkaloids related to XVII (17), as well as into the vinylquinuclidine moiety of quinine (XIX) (18, 19).

Activity from tryptophan enters calycanthine (XXI), an alkaloid containing two fused quinoline nuclei. Even though specificity of incorporation has not yet been demonstrated, it is likely that the alkaloid arises by rearrangement of a tryptamine dimer (XX) (Scheme 3*c*) (20).

Anthranilic acid (III) has been implicated in the biosynthesis of several quinoline derivatives of bacterial and fungal origin. A series of 2-alkyl-4-hydroxyquinoline *N*-oxides (XXII) occur in various strains of *Pseudomonas*. Activity from [carboxyl-^{14}C]-anthranilic acid was incorporated into these compounds. Partial degradation indicates that this activity is confined to C-4 of the nucleus. Acetate supplies the alkyl side chains (Scheme 3*d*) (21). The nucleus of anthranilic acid, but not its carboxyl group, is incorporated into viridicatin (XXIV), a quinoline derivative produced by a number of *Penicillium* species. Phenylalanine (XXIII) supplies the remaining carbon atoms of the product (Scheme 3*e*) (22). It is not known whether tryptophan, which serves as the precursor of anthranilic acid (14), and is also derived from it (14), is implicated in the biosynthesis of these two quinoline derivatives.

Tryptophan (I) and anthranilic acid (III) have both been suggested as precursors of dictamnine (Scheme 1). The first objective of the present study was an experimental examination of these postulates. It was found that dictamnine isolated from plants to which DL-[β-^{14}C]-tryptophan had been administered was essentially inactive (Table I), but that activity from [carboxyl-^{14}C]-anthranilic acid was incorporated in a high radiochemical yield (Table I), and was present solely at C-4 of the alkaloid, the predicted site (Table III). Furthermore, anthranilic acid, doubly labelled with ^{15}N in the amino group and with



SCHEME 3. Incorporation of tryptophan and anthranilic acid into quinoline derivatives.

^{14}C in the carboxyl group, was incorporated into dictamnine without change in the $^{14}C/^{15}N$ ratio (Table IV).

It can be inferred from these results that tryptophan is not an intermediate in the biosynthesis of dictamnine. Anthranilic acid serves as a precursor of tryptophan. This process is accompanied by decarboxylation, and only the aniline moiety of anthranilic acid is incorporated into the amino acid (14). Since activity from [carboxyl-^{14}C]-anthranilic acid entered C-4 of dictamnine specifically, tryptophan cannot be an obligatory intermediate. Tryptophan can serve as a precursor of anthranilic acid (14). Since doubly labelled anthranilic acid entered dictamnine without change in the isotope ratio, anthranilic acid is incorporated intact, and it is unlikely that tryptophan is involved in the pathway. Doubly labelled anthranilic acid would be expected to be converted into [indole-^{15}N]-labelled tryptophan which, in turn, would yield [^{15}N]-labelled anthranilic acid. If this tryptophan-derived, endogenously produced [^{15}N]-anthranilic acid, as well as the doubly labelled precursor, had been incorporated into dictamnine, the $^{14}C/^{15}N$ ratio in the isolated alkaloid would have been lower than, rather than identical with, that of the administered tracer.

It is evident that dictamnine does not arise from tryptophan in the manner shown in Scheme 1 (4), and that the biosynthesis of its quinoline nucleus does not correspond to that of kynurenic acid (XVI) (14) (Scheme 3*a*), but that anthranilic acid is a direct precursor.

Various proposals have been made (Scheme 1) concerning the source of the

TABLE V

Incorporation of acetate into dictamnine

Precursor	$CH_3{}^{14}CO_2H$				$^{14}CH_3CO_2H$			
Dictamnine carbons	C-10	C-11	C-3	C-2	C-10	C-11	C-3	C-2
Distribution of radioactivity (%) in C-2, C-3, C-10, and C-11 of dictamnine								
Found*	[93]	4	← 4 →		12	[88]	← 0 →	
Calculated								
Via succinate (3)	$HO_2C—CH_2—CH_2—CO_2H$				$HO_2C—CH_2—CH_2—CO_2H$			
Glyoxylate cycle	50	0	0	50	0	50	50	0
Tricarboxylic acid cycle	50	0	0	50	0	50	50	0
					↓			
					17	33	33	17 (steady-state distribution)
Via malate and oxaloacetate	$HO_2C—CH_2—\underset{\displaystyle OH}{\underset{\vert}{CH}}—CO_2H$				$HO_2C—CH_2—\underset{\displaystyle OH}{\underset{\vert}{CH}}—CO_2H$			
Glyoxylate cycle (steady-state distribution)	50	0	0	50	0	50	50	0
Via succinyl-CoA, α-ketoglutarate, or ornithine (9)	$CoAS—CO—CH_2—CH_2—CO_2H$ $HO_2C—CO—CH_2—CH_2—CO_2H$ $HO_2C—\underset{\displaystyle NH_2}{\underset{\vert}{CH}}—CH_2—CH_2—CH_2NH_2$				$CoAS—CO—CH_2—CH_2—CO_2H$ $HO_2C—CO—CH_2—CH_2—CO_2H$ $HO_2C—\underset{\displaystyle NH_2}{\underset{\vert}{CH}}—CH_2—CH_2—CH_2NH_2$			
Tricarboxylic acid cycle	0	0	0	100	0	0	100	0
					↓			
					34	34	32	0 (steady-state distribution)
	$HO_2C—CH_2—CH_2—CO—SCoA$ $HO_2C—CH_2—CH_2—CO—CO_2H$ $H_2NCH_2—CH_2—CH_2—\underset{\displaystyle NH_2}{\underset{\vert}{CH}}—CO_2H$				$HO_2C—CH_2—CH_2—CO—SCoA$ $HO_2C—CH_2—CH_2—CO—CO_2H$ $H_2NCH_2—CH_2—CH_2—\underset{\displaystyle NH_2}{\underset{\vert}{CH}}—CO_2H$			
	[100]	0	0	0	0	[100]	0	0
					↓			
					0	32	34	34 (steady-state distribution)

*Sum of the relative specific activities at C-2, C-3, C-10, and C-11 equals 100; calculated from the data in Table III.

four remaining carbon atoms (C-2, C-3, C-10, and C-11) of the dictamnine nucleus. Several feeding experiments were carried out (Table I) to examine their origin.

Acetate was found to serve as the precursor of C-10 and C-11, the non-anthranilic carbon atoms of the pyridine ring (Table III). Little, if any, activity from acetate entered C-2 and C-3 of the furan nucleus. Activity from [5-^{14}C]-α-ketoglutarate failed to enter dictamnine (Table I).

These results eliminate succinate (V) (3) and ornithine (VI) (9) (Scheme 1) as sources of the four-carbon unit. The distribution of activity in the non-anthranilic carbon atoms of dictamnine, which would have resulted from the incorporation of [1-^{14}C]- and [2-^{14}C]-acetate by way of succinate, oxaloacetate, or succinyl-coenzyme A, or by way of α-ketoglutarate and ornithine, can be predicted on the basis of the normal operation of the tricarboxylic acid cycle or the glyoxylate cycle. Only one of these calculated radioactivity patterns was consistent with that actually observed (Table V). If acetate had indeed been incorporated by way of α-ketoglutarate and of either succinyl-coenzyme A or ornithine, [5-^{14}C]-α-ketoglutarate should have yielded dictamnine with a labelling pattern similar to that obtained with [1-^{14}C]-acetate. Since activity from [5-^{14}C]-α-ketoglutarate was not incorporated, however (Table I), acetate did not enter dictamnine by this pathway.

A further four-carbon precursor, postulated (8) to account for the non-anthranilic carbon atoms of dictamnine, is erythrose (VII) (Scheme 1). Erythrose-4-phosphate is an intermediate of the photosynthetic carbon reduction cycle, of which ribose-5-phosphate is also a component. Entry of [1-^{14}C]-ribose into the events of the cycle would yield [4-^{14}C]-erythrose-4-phosphate, whose intact incorporation into dictamnine (8) would lead to labelling at C-2 of the non-anthranilic four-carbon moiety of the alkaloid. Another metabolic route from ribose-5-phosphate to erythrose-4-phosphate is the pentose phosphate pathway. By this route label from [1-^{14}C]-ribose can enter C-1 of the tetrose (cf. refs. 23–25), whose intact incorporation into dictamnine would then lead to activity at C-10 of the non-anthranilic acid fragment. The observed distribution of radioactivity in dictamnine derived from [1-^{14}C]-ribose (Table III) is not in accord with the erythrose hypothesis.

The foregoing discussion leads to the conclusion that the non-anthranilic fragment of dictamnine is not derived from a four-carbon unit, but from two different two-carbon precursors. One of these, acetate, yields C-10 and C-11.

In an attempt to account for the origin of the remaining two-carbon unit (C-2 and C-3) of dictamnine, incorporation of [1-^{14}C]-glycolate was tested. The distribution of activity in the dictamnine which was isolated (Table III) shows that this two-carbon compound is not a direct precursor. Label was non-randomly distributed throughout the molecule. Only a small fraction of this activity was present in C-2 and C-3 of dictamnine. Almost one-half of the label was found in the anthranilic acid derived entity. This was not unexpected. It has been shown (26) that [1-^{14}C]-glycolate yields 3,4-labelled hexoses. Activity from [3,4-^{14}C]-glucose is incorporated into the carboxyl group and into two carbon atoms of the nucleus of shikimic acid (24), and hence into the carboxyl group and the corresponding two nuclear carbons of anthranilic acid (27). More surprising was the result that one-fifth of the activity derived from [1-^{14}C]-glycolate was present in the *O*-methyl group of dictamnine. This carbon is specifically derived from the *S*-methyl group of methionine (Table III). C-2 of glycolic acid is known to serve as a methyl precursor in plants (28). The steps in this process are not understood. Incorporation of label from [1-^{14}C]-glycolate into a methyl group has not hitherto been observed. The most puzzling aspect of the incorporation of [1-^{14}C]-glycolate into dictamnine is the equal labelling of the two acetate-derived carbon atoms (C-10 and C-11). This would appear to indicate a pathway from glycolate to acetate by way of a symmetrical intermediate. No evidence for such a pathway is available.

The similarity of the distribution in

dictamnine of activity from [1-^{14}C]-glycolic acid and [1-^{14}C]-ribose (Table III) is understandable in the light of the finding (29) that, in tobacco plants, activity from [1-^{14}C]-ribose is incorporated into both carbon atoms of glycolate (distribution: carboxyl carbon, 33%; α carbon, 66%). The labelling pattern in the anthranilic acid moiety of dictamnine, derived from [1-^{14}C]-ribose, may also be accounted for in an alternative manner. By the pentose phosphate pathway [1-^{14}C]-ribose yields 1,3-labelled hexose (23), which would yield shikimic (24) and anthranilic acids (27) labelled in the carboxyl group as well as in the nucleus.

In a further attempt to establish the source of the elusive two-carbon unit of dictamnine, a feeding experiment with DL-[4-^{14}C]-mevalonic acid[2] was carried out. Furoquinolines are accompanied by alkaloids of the 3-isopentylcarbostyryl and the 2-isopropyl-2,3-dihydrofuroquinoline series in a number of plants. The suggestion has been made (5, 6; cf. ref. 7) that dictamnine may be derived from a compound of this type by loss of an isopropyl group, and that C-2 and C-3 of its furan nucleus are vestiges of an isoprene unit, corresponding to C-4 and C-5 of mevalonic acid[3] (VIII) (Scheme 1). If this were so, specific incorporation of acetate into these sites would be mandatory. Even though the observed incorporation of acetate into the dictamnine carbon atoms in question was marginal (Table III), the low activity at these sites may have been due to a slow rate of synthesis of mevalonate compared with a high rate of entry of acetate into the quinoline nucleus. Difficulty in observing incorporation of acetate into mevalonate-derived alkaloids has been encountered previously (cf. refs. 19 and 31).

Since the total activity of [4-^{14}C]-mevalonic acid at our disposal ($(0.43 \pm 0.01) \times 10^7$ counts min^{-1}) was much less than that generally used in our feeding experiments, the chances of obtaining conclusive evidence in this experiment were regarded as minimal from the outset.

To enhance the probability of success, a preliminary experiment was undertaken. It had been observed that the specific activity of dictamnine obtained from the feeding experiment with doubly labelled anthranilic acid (weight fed 100 mg, specific activity $(4.54 \pm 0.09) \times 10^7$ counts min^{-1} mmole^{-1} (Table I)) was of the same order as that of the dictamnine obtained from another anthranilic acid experiment (weight fed 1.6 mg, specific activity $(55.6 \pm 0.7) \times 10^7$ counts min^{-1} mmole^{-1} (Table I)). This suggested that the concentration of anthranilic acid, rather than its specific activity, determined the specific activity of dictamnine isolated after a metabolic period of 48 h. To test this concept, the efficiency of incorporation into dictamnine of [2-^{14}C]-acetate in the presence of different weights of cold anthranilic acid was investigated in three parallel experiments of 2 days' duration (Table VI).

The results show that the efficiency of incorporation of acetate into dictamnine was proportional to the weight of cold anthranilic acid. It can be inferred, therefore, that, in a 48 h metabolic period, anthranilic acid concentration determines the rate of biosynthesis of dictamnine.

Accordingly, the labelled mevalonic acid was administered to *Dictamnus* plants in the presence of cold anthranilic acid. Dictamnine was isolated and purified to constant activity, which amounted to little more than 1 count min^{-1} mg^{-1} above background. Thus the result of this attempt to test the mevalonic acid hypothesis of the origin of the two-carbon unit (C-2 and C-3) of dictamnine (5–7) (Scheme 1) was inconclusive.

The evidence which is presented establishes anthranilic acid and acetate as precursors of the pyridine nucleus of dictamnine in *Dictamnus albus* L. The mode of entry of these fragments, possibly by way of *N*-acetylanthranilate or *o*-aminobenzoylacetate (7), and the origin of the furan two-carbon unit of dictamnine remain to be investigated.

In a recent study (32) on the biosynthesis

[2]This sample of DL-[4-^{14}C]-mevalonic acid (dibenzylethylenediamine salt) was a gift from Dr. H.-G. Floss (30).

[3]The specific incorporation, in *Pimpinella magna*, of radioactivity from [4-^{14}C]-mevalonic acid into the α-furan carbon of the furocoumarin pimpinellin has been demonstrated (30).

TABLE VI

The effect of anthranilic acid on the incorporation of [2-^{14}C]-acetate into dictamnine

Composition of feeding solution		Product			
			Dictamnine		
Total activity of [2-^{14}C]-acetate* fed (counts min^{-1} × 10^{-7})	Anthranilic acid (mg)	Weight of dry *D. albus* roots (g)	Weight (mg)	Specific activity (counts min^{-1} mmole^{-1} × 10^{-3})	Specific radiochemical yield (×10^4)
0.28±0.01	0	28	19	1.09±0.07	4.8
0.28±0.01	15	46	35	3.03±0.11	13.4
0.28±0.01	50	30	20	12.32±0.36	54.4

*Volk Radiochemical Co.; specific activity (2.27 ± 0.06) × 10^8 counts min^{-1} mmole^{-1}.

of skimmianine (7,8-dimethoxydictamnine) in *Skimmia japonica* Thunb., the incorporation of [^{3}H$_4$]-anthranilic acid, [1-^{14}C]- and [2-^{14}C]-acetate, and DL-[β-^{14}C]-tryptophan was tested. All activity from [2-^{14}C]-acetate was found to reside at C-11 of skimmianine and activity from [1-^{14}C]-acetate was inferred to be localized at C-10, results similar to our own. Label from [^{3}H$_4$]-anthranilic acid was shown to be restricted to the benzenoid nucleus of skimmianine, a finding which complements our demonstration of intact incorporation of [^{14}C,^{15}N]-anthranilic acid into dictamnine.

Incorporation of [β-^{14}C]-tryptophan into skimmianine was very low (specific radiochemical yield, 0.01 × 10^{-4}% (32); cf. specific radiochemical yield in the incorporation of activity from [β-^{14}C]-tryptophan into dictamnine, 0.10 × 10^{-4}% (Table I)), but activity appeared to be localized at C-11 of skimmianine. This result was interpreted to show that an *o*-aminobenzoylacetate moiety, derived from tryptophan (cf. ref. 4), could serve as a precursor of skimmianine in *S. japonica*. Our own results exclude tryptophan as an intermediate in the biosynthesis of dictamnine in *D. albus*. It is possible that the rate-limiting process in the biosynthesis of skimmianine is the introduction of the methoxy groups into anthranilic acid. If this were slow, compared with the degradation of tryptophan to anthranilic acid, the divergent findings on the incorporation of tryptophan into the two alkaloids could be reconciled. It will be of interest to determine the ^{14}C/^{15}N ratio in the skimmianine obtained from the *D. albus* plants to which doubly labelled anthranilic acid had been administered. This work is in progress.

EXPERIMENTAL

Isolation of Dictamnine (II) (11)

Finely powdered dried roots of *Dictamnus albus* (200 g) were moistened with sodium carbonate solution (10%, 100 ml) and extracted with petroleum ether (b.p. 30–60°) for 24 h in a Soxhlet apparatus. A limonoid of unknown structure[4] (55 mg), m.p. 255°, mol. wt. 558, crystallized from the extract and was separated by filtration.

Anal. Found: C, 63.35; H, 6.87. $C_{30}H_{38}O_{10}\cdot\frac{1}{2}H_2O$ requires C, 63.46; H, 6.93.

The filtrate was extracted with dilute hydrochloric acid (5%, 3 × 20 ml), and the combined acid extracts were washed with ether (40 ml) and basified with sodium carbonate solution (10%). Dictamnine (195 mg), m.p. 132–133° (reported m.p. 132–133° (11)), precipitated and was purified by sublimation *in vacuo* at 95–100° and 2 × 10^{-3} mm and recrystallization from ethanol.

Degradation of Dictamnine

Isodictamnine (XII, R = CH_3) (11)

Dictamnine (15 mg) and methyl iodide (5 ml) were heated at 80° in a sealed tube for 2 h. Excess methyl iodide was distilled off and the product was crystallized from water, yielding isodictamnine (12 mg, 80%), m.p. 186–187° (reported m.p. 188° (11)).

N-Ethylnordictamnine (XII, R = C_2H_5) (11)

Dictamnine (18 mg) and ethyl iodide (1 ml) were heated at 100° in a sealed tube for 5 h. Excess solvent was distilled off, and the residue was dissolved in a small amount of petroleum ether (b.p. 30–60°) and applied to a short alumina column, which was then eluted with ether. Unreacted dictamnine emerged in the first 20 ml of eluate, followed in the next 30 ml by *N*-ethylnordictamnine (14 mg, 73%), which, after high-vacuum sublimation, melted at 149° (reported m.p. 150° (11)).

[4]This compound has now been identified (33) as α-nomilyl acetate, a substance not previously detected in *Dictamnus*.

Oxidation of Dictamnine (II) to Dictamnic Acid (XI) (11)

A solution of potassium permanganate (60 mg) in acetone (5 ml) was added to a solution of dictamnine (25 mg) in acetone (6 ml), and the mixture was refluxed for 1 h. The precipitate was filtered off and suspended in aqueous sodium carbonate (10%, 3 × 1.5 ml), and the manganese dioxide was separated from the solution by centrifugation. When the combined carbonate extracts were acidified with 6 *M* hydrochloric acid, dictamnic acid (15 mg, 65%), m.p. 263–265° (decomp.) (reported m.p. 260° (decomp.) (11)), separated.

Dictamnic acid was treated with diazomethane to yield the methyl ester, which, on vacuum sublimation, melted at 185–186° (reported m.p. 186° (34)).

Conversion of Dictamnic Acid (IX) into 2,4-Dihydroxyquinoline (X) (11)

Dictamnic acid (15 mg) was refluxed with concentrated hydrochloric acid (10.5 ml) for 30 min. The mixture was evaporated and the residue sublimed *in vacuo*, yielding 2,4-dihydroxyquinoline (6 mg, 56%), m.p. above 320°, identical with a commercial specimen.

Oxidation of Dictamnine (II) to N-Oxalylanthranilic Acid (XI)

Dictamnine (31 mg) was dissolved in dilute sulfuric acid (2 *M*, 3.5 ml). The solution was cooled in an ice bath and stirred, and potassium permanganate (68 mg in 3 ml of water) was added dropwise over 30 min. The reaction mixture was extracted with ether (5 × 10 ml), and the combined ether extracts were washed with a small amount of water, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was dissolved in dilute ammonia (1 *M*, 1 ml), and the solution was filtered and acidified with cold concentrated hydrochloric acid. The product which precipitated was recrystallized from water and sublimed at 135–140° and 2×10^{-3} mm to yield oxalylanthranilic acid (22 mg, 68%), m.p. 185° (decomp.) (reported m.p. 188–189° (35), 202–203° and 229–230°[5] (36)), identical with an authentic specimen prepared from anthranilic acid and oxalic acid (35).

Hydrolysis of N-Oxalylanthranilic Acid (XI)

A solution of oxalylanthranilic acid (20 mg) in hydrochloric acid (6 *M*, 2 ml) was refluxed for 5 min. After the reaction mixture was cooled, ammonium chloride (1.5 g) was added and the solution was extracted with ether (5 × 5 ml). The ether extract was washed with water and dried over anhydrous sodium sulfate. Removal of the ether and sublimation of the residue at 95° and 2×10^{-3} mm gave anthranilic acid (9.5 mg, 72.5%), m.p. 141–143°.

N-Formylanthranilic Acid (XIII)

Oxalylanthranilic acid (15 mg) and polyphosphoric acid (0.3 ml) were heated at 150–160° for 10 min and then briefly to 180°, when evolution of carbon dioxide had ceased. After the reaction mixture had cooled to room temperature, crushed ice (3 g) was added and the mixture was extracted with ether (4 × 10 ml). The ether extract was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated, yielding *N*-formylanthranilic acid (8 mg, 86%), m.p. 166–168° (reported m.p. 169° (38), 168° (39), 167° (37), 162° (40), and 127° (41)), identical with an authentic specimen prepared from anthranilic acid and formic acid (37–39).

Hydrolysis of N-Formylanthranilic Acid (XIII) (39)

Formylanthranilic acid (7 mg) was dissolved in hydrochloric acid (6 *M*, 1 ml) and kept for 2 days at room temperature. Ammonium chloride (0.8 g) was added and the solution was extracted with ether (4 × 5 ml). The ether extract was washed with water, dried over anhydrous sodium sulfate, and concentrated. Sublimation of the residue at 95° and 2×10^{-3} mm gave anthranilic acid (3.6 mg, 62%), m.p. 141–143°.

Decarboxylation of Anthranilic Acid (38, 42)

Anthranilic acid (6 mg) was heated at 205–210° for 50 min in a sealed, evacuated tube. After being cooled, the contents of the tube were dissolved in ether (4 ml), the ether solution was washed with aqueous sodium hydroxide (1 *M*, 2 × 1 ml) and with a small amount of water, and the product was then extracted into dilute hydrochloric acid (1 *M*, 2 × 1 ml). The acid extract was evaporated to dryness and the residue was sublimed at 90° and 2×10^{-3} mm, yielding aniline hydrochloride (4.4 mg, 78%), m.p. 192–193°.

Administration of Radioactive Tracers to D. albus Plants and Isolation and Degradation of Labelled Dictamnine

Six mature plants of *D. albus* (white-flowering variety) were used in each feeding experiment. The labelled compound was dissolved in distilled water (10 ml), and equal portions of the solution were administered to individual plants by aspiration into the stem through a cotton wick (13). The plants were allowed to grow in contact with tracer solution for 48 h. The labelled compounds which were administered to the plants in separate experiments are listed in Table I. Dictamnine was isolated from the roots of the plants in each case, and the samples so obtained were crystallized to constant activity. The chemical and radiochemical yields are shown in Table I.

The reactions described above were used in the degradation of the labelled dictamnine samples. The degradation products obtained in individual experiments and their specific activity are listed in Table II. Each product was crystallized to constant activity. Radioactivity was assayed on samples of finite thickness on aluminium planchets, using a Nuclear-Chicago Corp. gas-flow counter (model 1052) in a low-background sample changer (C 115). Counts were recorded on a printing timer (C 111B) in association with a scaler (181B). For plating, a

[5]This compound may have been the corresponding dehydration product, 3,1,4-benzoxazone-2-carboxylic acid (cf. ref. 37).

1% solution of collodion in dimethylformamide was used as the solvent.

The usual corrections for background and self-absorption were applied. Limits shown in the tables are standard deviation of the mean.

Assay of [^{15}N]-Labelled Compounds

The ^{15}N content of [^{15}N]-anthranilic acid and of the dictamnine sample obtained from plants to which [^{14}C,^{15}N]-labelled anthranilic acid had been administered (Table IV) was determined mass spectrometrically on nitrogen gas samples prepared by oxidation of the ammonia formed by Kjeldahl digestion of the compounds (43). The ^{15}N content of the dictamnine sample was measured by double collection and balancing of the signals from the ions of mass 28 and 29 by the null method (44). The ^{15}N content of the highly enriched sample of anthranilic acid was measured, conventionally, by repeated scanning of the signals from mass 28 and 29 and normalization to the line standard.

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