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BIOSYNTHESIS OF SKIMMIANINE IN *FAGARA COCO**

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Abstract—*Fagara coco* plants were inoculated in separate experiments with sodium acetate 1-^{14}C, sodium formate-^{14}C, mevalonic acid-4-^{14}C, mevalonic acid-5-^{14}C, 3,3-dimethylallyl alcohol-l-%, and 3,3-dimethylacrylic acid-1-^{14}C. The furanoquinoline alkaloid skimmianine was isolated from each plant and degraded to determine the activity at C-2 and C-3. The result from the acetate experiment confirmed previous findings for labelling only in the quinoline moiety; the same result was encountered with labelled dimethylacrylic acid. Sodium formate labelled mainly the methoxy groups, while the results from both mevalonic acids and dimethylallyl alcohol clearly indicated that C-2 and C-3 of the furan ring are derived from C-4 and C-S of mevalonic acid respectively.

INTRODUCTION

THE BIOSYNTHETIC pathway to furanoquinoline alkaloids has been the subject of considerable speculation.' It is presently accepted that the quinoline moiety is derived from a condensation between anthranilic acid and acetic acid,[2-5] leading to a 2,4-dihydroxyquinoline derivative which could condense with a suitable intermediate to form the furan ring. The identity of this intermediate was suspected to be an 'isoprene' unit after the structures of alkaloids such as lunacrine and balfourodine had been established.[6] Recently,[5] labelled 2,4-dihydroxy-3-dimethylallylquinoline was proven to be an excellent precursor of platydesminium salt and dictammine in S. *japonica* plants providing an indirect confirmation of the isoprenoid origin of carbons 2 and 3 of the furan ring. The conversion of platydesminium salt into dictammine must involve the fission of a 3-carbon fragment from the cyclized isoprene unit; a similar transformation has been obtained *in vitro.*'

We wish now to report a direct proof that C-2 and C-3 of the furan ring of skimmianine are derived from C-4 and C-5 of mevalonic acid respectively.

RESULTS

Mevalonic acid-4-^{14}C and -5-^{14}C were fed to intact *Fagara coco* (Rutaceae) plants in separate experiments; the plants were harvested after 21 days and radioactive skimmianine was isolated in both cases by a reported method.* Degradative results strongly confirmed

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TABLE 1. DEGRADATION OF SKIMMIANINE DERIVED FROM LABELLED PRECURSORS

Products	Sodium acetate-1-^{14}C 2.22 x 10^9 dis/min /mM		Sodium formate-^{14}C 9·25 x 10^{10} dis/min /mM		Mevalonic acid-4-^{14}C 1·20 x 10^8 dis/min /mM		Mevalonic acid-5-^{14}C 2.62 x 10^{10} dis/min /mM		3,3-Dimethylallyl alcohol-1-^{14}C 3·10 x 10 dis/min /mM		3,3-Dimethylacrylic acid-1-^{14}C 3·10 x 10^8 dis/min /mM	
	Specific activity dis/min /mM	Relative specific activity %	Specific activity dis/min /mM	Relative specific activity %	Specific activity dis/min /mM	Relative specific activity %	Specific activity dis/min /mM	Relative specific activity %	Specific activity dis/min /mM	Relative specific activity %	Specific activity dis/min /mM	Relative specific activity %
Skimmianine (I)	9.10 x 10^5	100	5·93 x 10^5	100	1.48 x 10^3	100	1·01 x 10^5	100	5·20 x 10^4	100	1·17 x 10^5	100
Skimmianic acid (II)	9.10 x 10^5	100	5·72 x 10^5	96	0.28 x 10^2	2	0·95 x 10^5	94	4.88 x 10^4	94	1.16 x 10^5	99
2,4-Dihydroxy-7,8-dimethoxy-quinoline (III)	9.03 x 10^5	99	4·00 x 10^5	67	—	—	2·40 x 10^3	2	3·90 x 10^3	7	1·12 x 10^5	95
$BaCO_3$ (from C-3)	4.83 x 10^3	0·5	3·20 x 10^3	0·5	—	—	0·83 x 10^5	82	4.41 x 10^4	85	1·40 x 10^2	0·1
4,7,8-Trimethoxy-2-hydroxy-3-ethyl-quinoline (IV)	—	—	5.84 x 10^5	100	—	—	—	—	—	—	—	—
Acetic acid (from C-2 and C-3)	—	—	3·90 x 10^3	1	—	—	—	—	—	—	—	—
Propionic acid (from C-2, C-3 and C-l 1)	—	—	6.97 x 10^3	1	—	—	—	—	—	—	—	—
Triethylmethyl ammonium iodide		—	1.85		x 10^5	31	—	—	—	—	—	—

the precursor role of mevalonic acid (see Table 1). At the same time, in order to test a metabolite of mevalonic acid as a precursor of the furan ring, 3,3-dimethylallyl alcohol-1-^{14}C[9] was administered to another plant and the radioactive alkaloid was isolated in the same manner; the label was localized at a position that indicated a non-randomized incorporation of the tracer and confirmed the results found with mevalonic acid.

Birch and Smith[10] had previously postulated that the isopentenyl group entering in condensation with the quinoline moiety to form the furanoquinoline structure could be a carboxylic intermediate; accordingly, we fed *F. coco* plant this with 3,3-dimethylacrylic acid-1-^{14}C;[9] degradation of the radioactive alkaloid showed that dimethylacrylic acid was a poor precursor of C-2 and C-3 of the furan ring. Feeding experiments with sodium acetate-1-^{14}C and sodium formate-^{14}C were carried out at the same time.

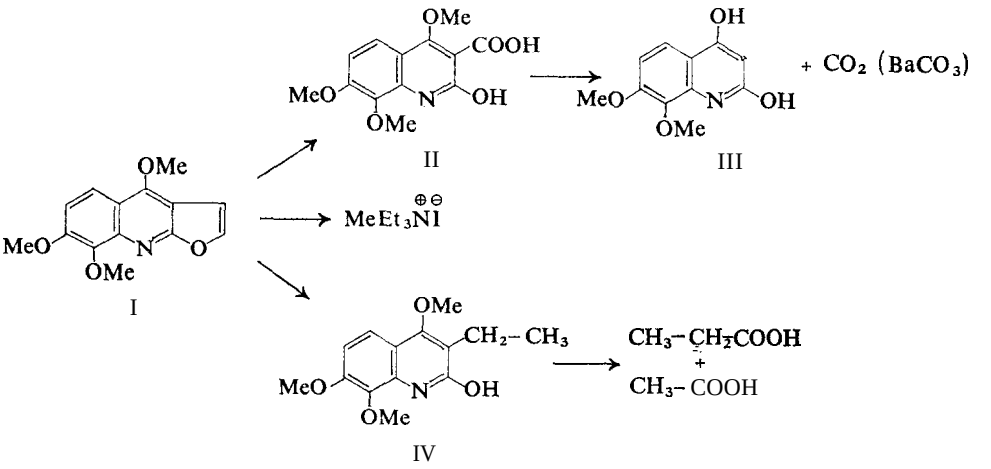

FIG. 1. DEGRADATION OF SKIMMIANINE.

In all cases, labelled samples of skimmianine were degraded (see Fig. 1) by oxidation to skimmianic acid[8] which was in turn decarboxylated to 2,4-dihydroxy-7,8-dimethoxyquinoline and CO, (collected as $BaCO_3$). In the experiment with labelled formate, the alkaloid was hydrogenated to 4,7,8-trimethoxy-2-hydroxy-3-ethyl quinoline which was submitted to the Kuhn-Roth degradation reaction;[2] acetic and propionic acid were separated by chromatographic procedures and purified by preparation of their 1-naphthylamino derivatives. Skimmianine from the formate experiment was also demethylated, and the liberated methyl iodide was collected as methyltriethylammonium iodide. The results of feeding experiments and the specific activities of the degradation products are shown in Table 1. The present experiment with labelled sodium acetate confirms previous findings[3] for no activity was detected at C-2 and C-3 of skimmianine. Sodium formate-^{14}C did not appear to be a direct precursor of those carbons since they were inactive and almost all the activity was found to be non-randomly distributed among the three methoxy groups.

In the experiments with labelled products known as steroid precursors, β-sitosterol was isolated from the extract and purified as the acetate by chromatographic methods. The specific activities of β-sitosterol acetate from the different experiments are recorded in Table 2.

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TABLE 2. ACTIVITY OF β-SITOSTEROL ACETATE DERIVED FROM LABELLED PRECURSORS

Product	Sodium acetate-1-W 2.22 x 10^9 dis/min/mM		Mevalonic acid-4-^{14}C 1·20 x 10^8 dis/min/mM		Mevalonic acid-5-W 2.62 x 10^{10} dis/min/mM		3,3-Dimethylallyl alcohol-1-^{14}C 3·10 x 10^8 dis/min/mM		3,3-Dimethylacrylic acid-1-^{14}C 3·10 x 10^8 dis/min/mM	
	Specific activity dis/min/mM	Specific incorp. %	Specific activity dis/min/mM	Specific incorp. %	Specific activity dis/min/mM	Specific incorp. %	Specific activity dis/min/mM	Specific incorp. %	Specific activity dis/min/mM	Specific incorp. %
β-Sitosterol acetate	7.66 x 10^5	0·03	1.11 x 10^5	0·09	4.42 x 10^6	0.02	5·50 x 10^5	0·18	2.40 x 10^5	0.06

DISCUSSION

The present results indicate that mevalonic acid, but not acetate, is a good precursor of the furan ring of skimmianine. This does not accord with the results obtained with β-sitosterol isolated from the same plant (Table 2). Known precursors of steroids,[11] e.g. acetate, mevalonic acid, dimethylallyl alcohol, and dimethylacrylic acid, yielded, as expected, radioactive β-sitosterol. The values of specific incorporation of these labelled products are in fair agreement with their position in the known pathway from acetate to cholesterol; dimethylacrylic acid, through hydroxymethylglutaric acid, could be degraded to acetate or to mevalonic acid, both precursors of sterols.[12] In addition to the failure of acetate to label C-2 and C-3, first reported by Monković et *al.*[2] on the biosynthesis of dictammine, dimethylacrylic acid was not incorporated into the alkaloid at the mentioned carbon-atoms. The result encountered in this experiment could be explained assuming that the hydroxymethylglutarate was only degraded to acetate'[13] which in turn would follow the pathway found for this intermediate.

More difficult to understand is the fact that acetate, incorporated into the sterols probably *aia* mevalonate, was not incorporated at the carbons of skimmianine now known to be derived from mevalonic acid. It is possible to postulate, in agreement with the Canadian authors,[2] that at the site of alkaloid biosynthesis the formation of mevalonate from acetate is slow compared with the rate of incorporation of acetate into the quinoline nucleus; however, when labelled mevalonate is available, the plant is able to use the fragment to form the furan ring.

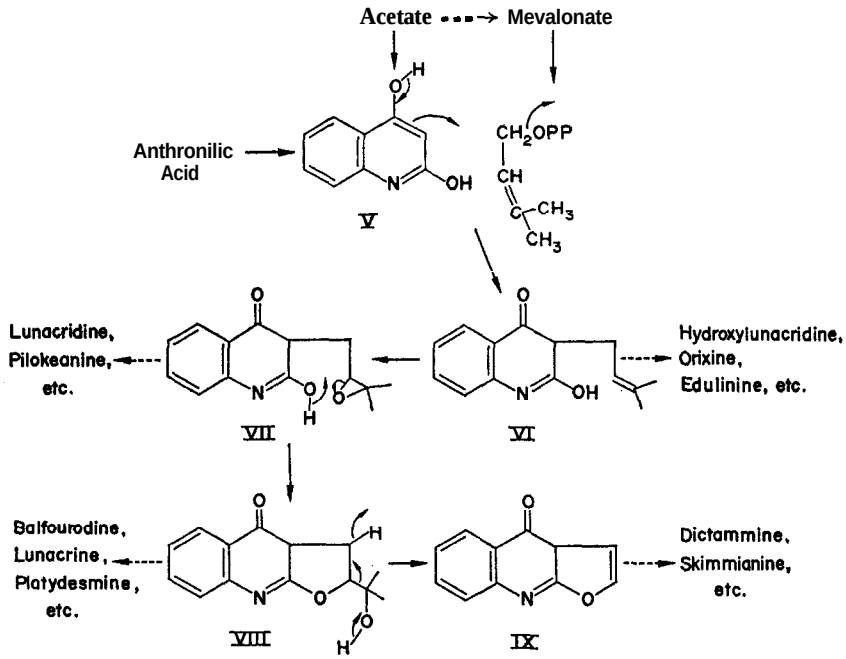

FIG. 2. HYPOTHETICAL BIOSYNTHETIC PATHWAY TO SKIMMIANINE AND OTHER RELATED ALKALOIDS.

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[12] K. BLOCH, L. C. CLARK and I. HARARY, *J. Biol. Chem.* 211,687 (1954).
[13] A. J. BIRCH, R. J. ENGLISH, R. A. MASSY WESTROPP and H. SMITH, *J. Chem. Soc.* 369 (1958).

From previous[2–5] and present results an hypothetical biosynthetic pathway to isopropyldihydrofuranoquinoline and furanoquinoline alkaloids can be postulated (see Fig. 2).

2,4-Dihydroxyquinoline (V) condenses with dimethylallylpyrophosphate to produce intermediate VI. A product with this type of structure has been isolated from a rutaceous plant;[14] furthermore, this kind of compound has been shown to be an efficient precursor of furanoquinoline alkaloids.[5] Intermediate VI would be closely related to alkaloids such as hydroxylunacridine,[15] edulinine,[16] orixine, and nororixine.[17] Compound VI could be oxidized to the epoxide VII which appears as the direct precursor of lunacridine, pilokeanine, etc.' On the other hand, intermediate VII could cyclize, as indicated by the arrows, to VIII, a close precursor of platydesmine, balfourodine, etc.' Elimination of the side chain as indicated in the scheme would afford intermediate IX which would lead to dictammine and skimmianine by unexceptional steps.

EXPERIMENTAL

M.ps were determined with a Fisher-Johns hot-plate and are uncorrected. Radioactive samples were counted with a Packard Tri-Carb model 3305 liquid scintillation spectrometer in the usual scintillation solutions. Preparative TLC was conducted on Al_2O_3 (Merck, PF_{254}, type E). Solvents were removed under diminished pressure below 50". Sodium acetate-1-^{14}C was purchased from the Comisión Nacional de Energia Atómica, Argentina; sodium formate-^{14}C from Amersham; mevalonic acid-5-^{14}C from Schwarz Bio-Research, Inc., Orangeburg, N.Y., U.S.A.

Administration of the Tracers and Isolation of Skimmianine and β-Sitosterol

One intact medium size ***F. coco*** plant (about 2-m in height) was utilized in each experiment. The plants were growing out-of-doors in La Calera mountains in the province of Córdoba, Argentina. The labelled compound was dissolved in water and the solution was administered to the plant either by the wick arrangement in three different branches (Experiments 1 and 2) or by injection into the tender trunk with a modified glass funnel (Experiments 3-6). The plants were harvested after 12 days, dried in a ventilated oven and grounded in a mill. The dried powder was moistened with 10% Na_2CO_3 solution and continuously extracted with petroleum (60–80) in a modified soxhlet apparatus for 96 hr. The extract was concentrated to 0·1 of its volumen and it was shaken with 6N HCl. The acid solution was neutralized with solid Na_2CO_3 and extracted with $CHCl_3$; this extract was evaporated and the residue crystallized from methanol. Skimmianine had m.p. 175-177" and its i.r. and NMR spectra were identical to those from an authentic sample. Radioactivity values are registered in Table 1.

The remained ethereal extract was evaporated and the residue was dissolved in 40 % ethanolic KOH and refluxed for 3 hr. The cooled basic solution was diluted with twice its volume of water and extracted with ether. The ethereal extract was washed with water and dried ($MgSO_4$). The residue obtained for evaporation of the ether was washed with methanol yielding a yellow solid product. The solid was chromatographed on Al_2O_3 (Woelm, neutral, grade I) eluting with petroleum and with benzene. The fractions containing β-sitosterol (monitored by TLC) were combined and evaporated. The residue was acetylated with Ac_2O in pyridine in the usual way. The acetate was purified by preparative TLC. β-Sitosterol acetate had m.p. 134-136" and its i.r. spectrum was identical to that of an authentic sample. Radioactivity values are recorded in Table 2.

Oxidation of Skimmianine to Skimmianic Acid (II): Typical Procedure

Skimmianine (I) (120 mg) was dissolved in acetone (16 ml), $KMnO_4$ (240 mg) was added in small portions, and the solution was refluxed for 30 min. The precipitate was filtered off. The dried solid was extracted with 10 % Na_2CO_3 (3 x 2 ml), separating the MnO_2 by centrifugation after each extraction. The combined basic extracts were cooled to 0" and acidified with cold concentrated HCl. The precipitate was filtered, washed with cold water, and dried. Recrystallization (twice) from acetic acid afforded pure skimmianic acid (20 mg), m.p. 247-248".

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Decarboxylation of Skimmianic Acid: Typical Procedure

Skimmianic acid (20 mg) was suspended in 6N HCl (15 ml) and the mixture was refluxed for 3 hr while a stream of dry N_2 was bubbled through the solution. The liberated CO_2 was collected as $BaCO_3$. The clear solution remained at the end of the reaction was concentrated to 0·1 of its volume, and 2,4-dihydroxy-7,8-dimethoxy-quinoline (III) (12 mg) was obtained on cooling. It was recrystallized from methanol-water to pure III (9 mg) of m.p. 248-249".

Reduction of Skimmianine to 4,7,8-Trimethoxy-2,4-dihydroxy-3-ethyl-quinoline (IV)

Skimmianine (63 mg) in acetic acid (10 ml) was hydrogenated at room temperature and atmospheric pressure over PtO_2 (30 mg) for 2 hr. The catalyst was filtered off, and the filtrate was evaporated. The crystalline residue was recrystallized from ethanol yielding 46 mg of IV of m.p. 184-185".

Kuhn-Roth Oxidation of 4,7,8-Trimethoxy-2,4-dihydroxy-3-ethyl-quinoline

The previous compound (36 mg) in 30% H_2SO_4 (12 ml) was treated with CrO_3 (4 g) and the mixture was boiled until 70 ml of distillate were collected. Following a reported method'* sodium acetate (10 mg) and sodium propionate (6 mg) were obtained. These were transformed into the respective l-naphthylamine derivatives[19] for radioactivity measures.

Demethylation of Skimmianine

Skimmianine (50 mg) in 50% HI (12 ml) was refluxed[20] for 2 hr in dry N_2. The evolved gas stream was purified by bubbling it through saturated $CdSO_4$ solution and saturated $Na_2S_2O_3$ solution and received into a cold 5% ethanolic triethylamine solution. The ethanolic solution was evaporated, and the residue was recrystallized from absolute ethanol–ethyl acetate. Methyltriethylammonium iodide had an i.r. spectrum identical to one from authentic sample.

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