Biosynthesis of the Morphinandienone Alkaloid, Sebiferine

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The incorporation of (±)-nor-reticuline, (±)-reticuline, (±)-nor-orientaline, and (±)-laudanosine into sebiferine (*O*-methylflavinantine) in *Cocculus laurifolius* DC has been studied and the specific utilization of reticuline demonstrated. A double-labelling experiment involving the 4'-*O*-methyl group of (±)-nor-reticuline showed that the methoxy-function is retained in the bioconversion of the precursor into sebiferine and there was considerable loss of tritium at C-1. Parallel experiments with (+)- and (−)-reticulines showed that the stereospecificity is not maintained in the biosynthesis of sebiferine from 1-benzylisoquinoline precursors. Feeding experiments also demonstrated that the plants can also efficiently convert flavinantine into sebiferine.

SEBIFERINE (3), a morphinandienone alkaloid isolated recently from the stem bark of *Litsea sebifera*[1] (Lauraceae) and shown to be identical with *O*-methylflavinantine,[2,3] can be biosynthesised from suitable 1-benzyltetrahydroisoquinoline precursors through various biosynthetic routes. *para,para*-Oxidative coupling of reticuline (10) as suggested[4,5] can give isosalutaridine[6] (4). O-Methylation of the phenolic hydroxy-group in (4) would then afford sebiferine (3). Sebiferine (3) can also be formed from orientaline (12) *via* the bis-dienone (8).

It has been established that flavinantine (5) in *Croton flavens*[7] (Euphorbiaceae) is biosynthesised from reticuline (10), and that orientaline (12) is very poorly metabolised by the plants to form (5). We have studied the biosynthesis of sebiferine (3) by feeding labelled hypothetical 1-benzylisoquinoline precursors to *Cocculus laurifolius* DC (Menispermaceae) plants. The results are recorded in the Table. Parallel feedings of (±)-reticuline (10) (experiment 1), (±)-nor-reticuline (9) (experiment 2), and (±)-nor-orientaline (11) (experiment 3) demonstrated that (9) and (10) are efficient precursors of (3), and (11) is not incorporated into (3).

Miller and his co-workers[8] have synthesised (±)-*O*-methylflavinantine from (±)-laudanosine (13) by electrochemical oxidation in fairly good yield. Whether the plants also follow the same path was tested by feeding labelled (±)-laudanosine (13) to *C. laurifolius*: compound (13) (experiment 4) was not incorporated into (3).

Tracer experiments on *C. laurifolius*

Expt.	Precursor fed	Incorporation (%) into sebiferine (3)
1	(±)-[2',6',8-$^3H_3$]Reticuline (10)	0.42
2	(±)-[2',6',8-$^3H_3$]Nor-reticuline (9)	0.32
3	(±)-[5',8-$^3H_2$]Nor-orientaline (11)	0.000 3
4	(±)-[2',6',8-$^3H_3$]Laudanosine (13)	0.003
5	(±)-[1-^{3}H, 4-O-*methyl* ^{14}C]Nor-reticuline (9)	0.36
6	(−)-[4-^{3}H]Flavinantine (5)	0.6
7	(+)-[2',6',8-$^3H_3$]Reticuline (1)	0.46
8	(−)-[2',6',8-$^3H_3$]Reticuline (7)	0.58

The experiment described above established that reticuline (10) is a precursor of sebiferine (3) in *C. laurifolius*. Two possibilities can be considered for the bioconversion of (10) into (3). In one, oxidative coupling of (10) can give (4). *O*-Methylation of the phenolic group in (4) then affords (3). In the second the isosalutaridine (4) derived from reticuline (10) can be

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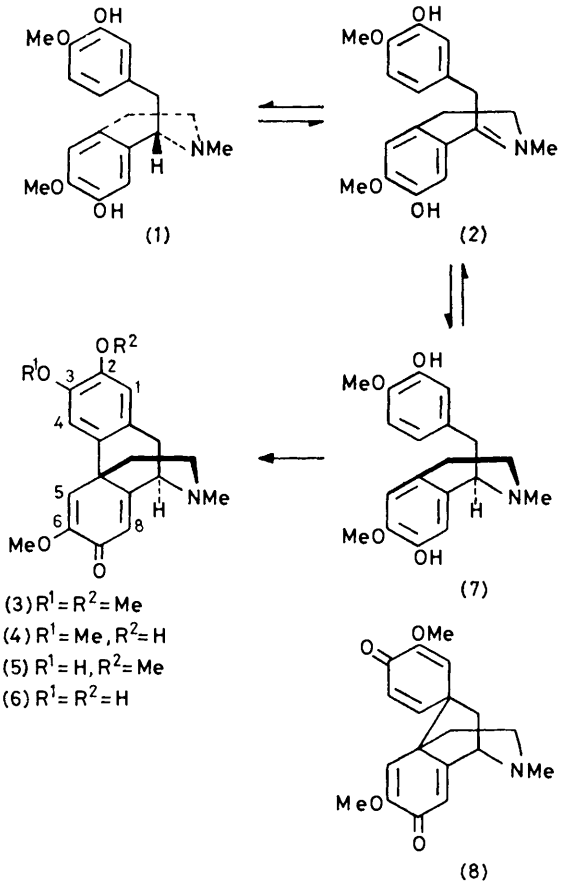
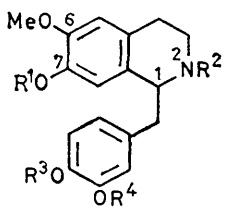
converted into flavinantine (5) either by demethylation–remethylation or by methyl migration, and finally *O*-methylation of the phenolic hydroxy-group in (5) can yield (3).



(1) (2) (7) (8)

(3) $R^1 = R^2 = Me$
(4) $R^1 = Me, R^2 = H$
(5) $R^1 = H, R^2 = Me$
(6) $R^1 = R^2 = H$

(±)-[1-^{3}H, 4-O-*methyl*-^{14}C]Nor-reticuline (9) (experiment 5) was fed to young cut branches of *C. laurifolius;* it was again efficiently metabolised by the plants to form (3). However, there was considerable loss of



(9) $R^1 = R^2 = R^4 = H, R^3 = Me$
(10) $R^1 = R^4 = H, R^2 = R^3 = Me$
(11) $R^1 = R^2 = R^3 = H, R^4 = Me$
(12) $R^1 = R^3 = H, R^2 = R^4 = Me$
(13) $R^1 = R^2 = R^3 = R^4 = Me$

tritium. A Ziesel determination of the methoxy-group of the radioactive base gave triethylmethylammonium iodide which had essentially one third of the activity of the parent base. The result established that the 4′-*O*-methyl group of nor-reticuline (9) is retained in the biotransformation of (9) into (3) and further demonstrated that an *O*-norflavinantine type of intermediate (6) is not involved. This result, however, does not exclude the possibility of complete methyl migration from the 3-*O*-methyl of (4) to the adjacent phenolic hydroxy-group. There appears to be no precedent for such a methyl migration. Partial methyl migrations are, however, recorded.[9,10]

Feeding of [4-^{3}H]flavinantine[11] (5) (experiment 6) demonstrated that the enzyme systems present in *C. laurifolius* are capable of converting (5) into (3) efficiently.

The foregoing experiments established that the nor-reticuline (9) and reticuline (10) are the specific precursors of sebiferine (3) in *C. laurifolius*. The precursors used, however, were racemic. The enzyme system which carries out the oxidative coupling step would be expected to be stereospecific, and only one of the two enantiomers should normally act as a direct substrate. Parallel feedings of (−)-reticuline (7) (experiment 8) and (+)-reticuline (experiment 7) demonstrated that (+)-reticuline was incorporated almost as efficiently as the (−)-enantiomer. These results are interpreted[12] as showing that (−)- and (+)-reticuline are undergoing interconversion in the plant by oxidation and reduction presumably *via* the 1,2-didehydro-derivative (2). (+)-Reticuline (1) is incorporated into sebiferine (3) by way of the intermediate (2). The evidence establishes the presence of a highly active oxidation–reduction system in *C. laurifolius*, as has been observed in poppy.[12] Sebiferine (3) has been isolated in (−)-form from the bark of *Nemuaron virillardii*[3] (Monimiaceae) and in (±)-form and (−)-form from the roots of *Rhigiocarya racemifera* Miers[13,14] (Menispermaceae). The occurrence of the racemic form in the plants is most probably due to the presence of an active oxidation–reduction system.

The foregoing results strongly support the following sequence for the biosynthesis of sebiferine in *C. laurifolius* DC. Nor-reticuline (9) ⟶ (+)-reticuline (1) ⇌ didehydroreticuline (2) ⇌ (−)-reticuline (7) ⟶ sebiferine (3).

EXPERIMENTAL

T.l.c. was carried out, unless specified to the contrary, on silica G.F. 254.

Counting Methods.—Liquid scintillation counting was used for measurement of ^{3}H and ^{14}C activities (Packard 314 EX instrument). Samples were counted in 7 ml of scintillator after dissolution in methanol or dimethylformamide

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(0.2 ml) and values are corrected for self absorption. Relative efficiencies were obtained by counting [1,2-$^3H_2$]- and [2-^{14}C]-hexadecane standards.

Synthesis of 1-*Benzylisoquinoline Precursors*.—Racemic reticuline,[15] nor-reticuline,[16] and nor-orientaline,[17] were prepared by standard methods. (±)-*OO*-dibenzoylreticuline was resolved [16] by treatment with (+)- and (−)-*OO*-dibenzyltartaric acids. Hydrogenolysis of the products with hydrochloric acid furnished (−)- and (+)-reticuline, respectively.

Labelling of Precursors.—*Tritiation*. Tritium in the phenolic 1-benzylisoquinoline precursors was introduced according to the method of ref. 10. (±)-Reticuline (110 mg) in tritiated water (0.5 ml; activity 80 mCi) containing potassium t-butoxide (230 mg) was heated under nitrogen (sealed tube) for 120 h at 100 °C. The resulting mixture was worked up as earlier to give the crude product, which was chromatographed on a column of alumina. Elution with chloroform–methanol (49 : 1) afforded material which was further purified through its perchlorate to give (±)-[2′,6′,8-$^3H_3$]reticuline (10). (±)-[2′,6′,8-$^3H_3$]Nor-reticuline (9), (±)-[5′,8-$^3H_2$]nor-orientaline (11), (−)-[2′,6′,8-$^3H_3$]-reticuline (7), and (+)-[2′,6′,8-$^3H_3$]reticuline (1) were prepared similarly. (±)-[2′,6′,8-$^3H_3$]Laudanosine (13) was prepared from (±)-[2′6′,8-$^3H_3$]reticuline with diazomethane.

(±)-[1-^{3}H]Nor-reticuline was prepared by reduction of 7-benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydroisoquinoline in dry dimethyl sulphoxide with sodium [^{3}H]-borohydride followed by acid-catalysed debenzylation. (±)-[4′-O-*methyl*-^{14}C]Nor-reticuline was prepared by a standard procedure. (±)-[1-^{3}H,4′-O-*methyl*-^{14}C]Nor-reticuline was prepared by mixing (±)-[4′-O-*methyl*-^{14}C]-nor-reticuline and (±)-[1-^{3}H]nor-reticuline.

(−)-[4-^{3}H]*Flavinantine*. Tritium at C-4 in (5) was introduced according to the method [11] used for deuterium exchange. Flavinantine (5) (40 mg), ^3H_2O (0.5 ml, 50 mCi), and dimethylformamide (0.25 ml) were heated under reflux in a sealed tube (under nitrogen) at 100 °C for 82 h and the product was worked up in the usual manner. The residue (18 mg) was chromatographed over a column of neutral alumina (4 g). Elution with chloroform yielded [4-^{3}H]-flavinantine.

Feeding Experiments.—For feeding purposes reticuline and laudanosine hydrochlorides were dissolved in water. Nor-reticuline was dissolved in water (1 ml) containing tartaric acid (10 mg). Flavinantine and nor-orientaline were dissolved in aqueous dimethyl sulphoxide (1 ml). Into the solution of precursor, freshly cut young branches of *C. laurifolius* plants were dipped and allowed to take up the precursor. When uptake was complete the plants were dipped in water, left for 5–6 days, and then worked up for sebiferine.

Isolation and Purification.—Stems and leaves (typically 120 g wet wt.) of the plants were macerated in ethanol (250 ml) with inactive sebiferine (75 mg) and left for 24 h. The ethanol was then decanted and the plant material was percolated with fresh ethanol (10 × 100 ml). The combined ethanolic extract was concentrated *in vacuo*. The green viscous mass so obtained was treated with 5% hydrochloric acid (6 × 20 ml). The acidic solution was defatted with hexane (5 × 25 ml) and then basified with sodium hydrogen carbonate. The liberated bases were extracted with chloroform (5 × 25 ml); the extracts were washed with water, dried (Na_2SO_4), and evaporated to afford crude sebiferine (70 mg), which was chromatographed over a column of basic alumina (8 g). Elution was carried out with chloroform and chloroform–methanol (49 : 1). The fractions containing sebiferine (t.l.c.) were mixed and evaporated. The purified base was further subjected to preparative t.l.c. (solvent chloroform–methanol 19 : 1) and the region containing sebiferine was cut out and eluted with chloroform–methanol (4 : 1). The solvent was removed from the eluate and the residue was crystallised from methanol to give sebiferine (3) (30 mg), m.p. 110—111° (lit.,[1] 112—113°); methiodide m.p. 250—252° (lit.,[1] 252—253°).

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