Biosynthesis of Oxyacanthine

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The incorporation of (±)-norcoclaurine, (±)-coclaurine, (±)-*N*-methylcoclaurine, didehydro-*N*-methylcoclaurinium iodide, (+)-(*S*)-*N*-methylcoclaurine and (−)-(*R*)-*N*-methylcoclaurine into oxyacanthine in *Cocculus laurifolius* DC has been studied and specific utilization of (±)-*N*-methylcoclaurine, (+)-(*S*)-*N*-methylcoclaurine, (−)-(*R*)-*N*-methylcoclaurine, and didehydro-*N*-methylcoclaurinium iodide demonstrated. The evidence supports intermolecular oxidative coupling of (+)-(*S*)-*N*-methylcoclaurine and (−)-(*R*)-*N*-methylcoclaurine to give oxyacanthine. A double labelling experiment with (±)-[1-^{3}H,*N*-$^{14}CH_3$]-*N*-methylcoclaurine demonstrated that the hydrogen atom at the asymmetric centre in the 1-benzylisoquinoline precursor is retained in the bioconversion into oxyacanthine.

OXYACANTHINE, the hypotensive principle of *Berberis vulgaris* Linn,[1] represents a group of bisbenzylisoquinoline alkaloids containing two phenyl ether linkages.[2,3] The base occurs in many species of the genus *Berberis* and *Mahonia*.[4] Structure (7) (undefined stereochemistry) assigned to oxyacanthine [5] was confirmed by the synthesis of its *O*-methyl ether.[6] The absolute configuration at the asymmetric centres C-1 and -1′ was determined as *S* and *R* respectively by the sodium–liquid ammonia method.[7] Repandine,[8] a stereoisomer of oxyacanthine,[9] has the *S* configuration at both asymmetric centres.

Oxyacanthine (7) can be formed in nature from coclaurine derivatives.[3] Intermolecular oxidative coupling of *N*-methylcoclaurine (1) and (3) units forms an ether linkage between the benzylic ' halves ' giving a dauricine type intermediate (5). This in turn can undergo intramolecular oxidative coupling to generate (6). Selective *O*-methylation of the phenolic hydroxy group at C-6 in (6) finally gives rise to oxyacanthine (7).

Biosynthetic studies on the bisbenzylisoquinoline alkaloid epistephanine [10] which has only one asymmetric centre and cocsulin [11] and cocsulinin [12] which have the same configuration at both the asymmetric centres have

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[2] M. Kulpa in ' The Alkaloids,' ed. R. H. F. Manske, Academic Press, New York, 1971, vol. 13, p. 303.
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[5] F. U. Bruchausen and P. H. Gericke, *Arch. Pharm.*, 1931, **269**, 115.
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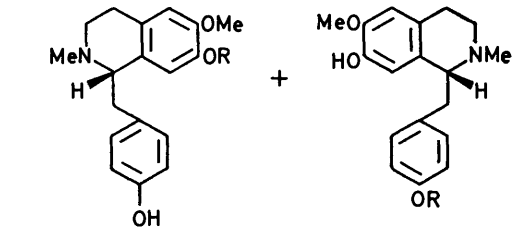
[12] D. S. Bhakuni, S. Tewari, and A. N. Singh, *J.C.S. Perkin I*, 1978, 380.

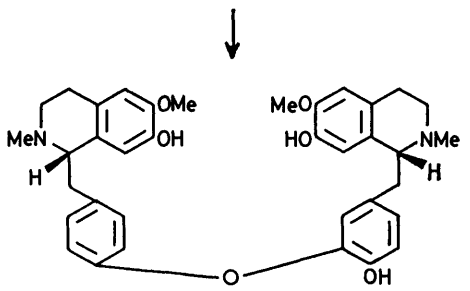
been reported. We now present the first report on the biosynthesis of a bisbenzylisoquinoline alkaloid which has opposite configurations at the two asymmetric centres.

(±)-Tyrosine was initially fed to young cut branches of *Cocculus laurifolius* DC (Menispermaceae) and it was found that oxyacanthine (7) was actively biosynthesised by the plants. In subsequent experiments labelled hypothetical 1-benzylisoquinoline precursors were fed to young cut branches of *C. laurifolius*. The results of several feedings are recorded in Table 1. Feeding of (±)-norcoclaurine (9) (experiment 2), (±)-coclaurine

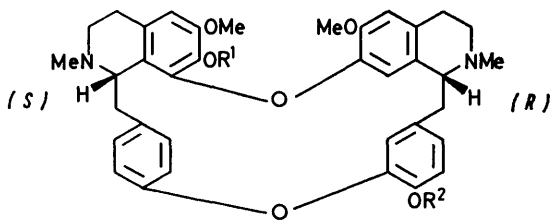


(1) R = H
(2) R = Me

(3) R = H
(4) R = Me



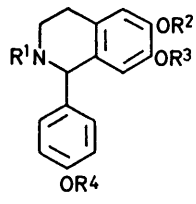
(5)



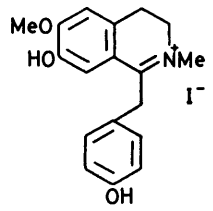
(6) $R^1 = R^2 = H$
(7) R^1 = Me, R^2 = H
(8) $R^1 = R^2$ = Me

(10) (experiment 3), (±)-*N*-methylcoclaurine (11) (experiment 4), and didehydro-*N*-methylcoclaurinium iodide (13) (experiment 6) established that (9)—(11) and (13) were efficient precursors of oxyacanthine (7). (±)-*NOO*-Trimethylcoclaurine (12) (experiment 5), as expected, was not incorporated into (7).

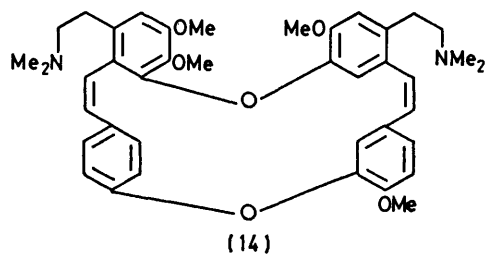
Labelled oxyacanthine (7) derived from (±)-[3′,5′,8-$^3H_3$]-*N*-methylcoclaurine (experiment 4) feeding was treated with diazomethane to give *O*-methyloxyacanthine (8) with no loss of radioactivity. Reductive fission [7] of (8) with sodium–liquid ammonia gave armepavine (2) and *NO*-dimethylcoclaurine (4). Each of these 1-benzyltetrahydroisoquinoline derivatives had essentially half the radioactivity of the parent base.



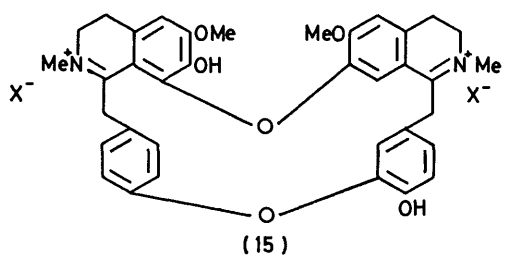
(9) $R^1 = R^2 = R^3 = R^4 = H$
(10) $R^1 = R^3 = R^4 = H, R^2$ = Me
(11) $R^1 = R^2$ = Me, $R^3 = R^4 = H$
(12) $R^1 = R^2 = R^3 = R^4$ = Me



(13)



(14)



(15)

Feeding of (±)-[1-^{3}H,*N*-$^{14}CH_3$]-*N*-methylcoclaurine (experiment 7) gave oxyacanthine (7) labelled both with

TABLE 1

Tracer experiments on *C. laurifolius*

Experiment	Precursor fed	Incorporation (%) into oxyacanthine (7)
1	(±)-[*U*-^{14}C]Tyrosine	0.09
2	(±)-[1-^{3}H]Norcoclaurine (9)	0.12
3	(±)-[3′,5′,8-$^3H_3$]Coclaurine (10)	0.15
4	(±)-[3′,5′,8-$^3H_3$]-*N*-Methylcoclaurine (11)	0.25
5	(±)-[3′,5′,8-$^3H_3$]-*NOO*-Trimethylcoclaurine (12)	0.0024
6	[*N*-$^{14}CH_3$]-Didehydro-*N*-methylcoclaurine (13)	0.45
7	(±)-[1-^{3}H,*N*-$^{14}CH_3$]-*N*-Methylcoclaurine (11)	0.28
8	(−)-(*R*)-[3′,5′,8-$^3H_3$]-*N*-Methylcoclaurine (3)	0.22
9	(+)-(*S*)-[3′,5′,8-$^3H_3$]-*N*-Methylcoclaurine (1)	0.27

^{14}C and ^{3}H. The ^{14}C : ^{3}H ratio in the precursor and the biosynthetic base was practically unchanged. This experiment demonstrated that the hydrogen atom at the

asymmetric centre in the l-benzylisoquinoline precursor is retained in the biosynthesis of (7) from (1) and (3).

Feeding of [N-$^{14}CH_3$]-didehydro-N-methylcoclaurinium iodide (13) (experiment 6) gave labelled oxyacanthine (7). The regiospecificity of the ^{14}C labelling in biosynthetic oxyacanthine (7) was demonstrated as follows: labelled oxyacanthine (7) was treated with diazomethane to give O-methyloxyacanthine (8) which was converted into its dimethiodide and then to the hydroxide form with essentially no loss of radioactivity. Hofmann degradation of this compound furnished compound (14) which had essentially the same radioactivity as the parent base. Treatment of (14) with dimethyl sulphate followed by potassium hydroxide gave trimethylamine trapped as its hydrochloride with essentially half the molar activity of the parent base. The result thus established that didehydro-N-methylcoclaurinium iodide (13) is specifically incorporated into oxyacanthine (7). There exist two possibilities for the incorporation of (13) into (7). In one, stereospecific reduction of (13) can give rise to (+)-(*S*)-N-methylcoclaurine (1) and (−)-(*R*)-N-methylcoclaurine (3) which can then undergo oxidative coupling to give oxyacanthine (7) with the *S* and *R* configurations at the asymmetric centres C-1 and -1′ respectively. In the other, didehydro-N-methylcoclaurinium iodide (13) can dimerise and a didehydrobisbenzylisoquinoline of the type (15) can be formed. Stereospecific reduction at C-1 and -1′ in (15) can then generate *S* and *R* configurations respectively to form oxyacanthine (7). The relatively higher incorporation (Table 1) of the didehydro-N-methylcoclaurinium iodide (13) (experiment 6) compared with (±)-N-methylcoclaurine (11) (experiment 4) may be interpreted as lending support to the second possibility.

Parallel feedings of (+)-(*S*)-N-methylcoclaurine (1) (experiment 9) and (−)-(*R*)-N-methylcoclaurine (3) (experiment 8) demonstrated that both the enantiomers were incorporated efficiently into oxyacanthine (7). Base (7) derived from both the enantiomers was separately degraded [7] to (+)-(*S*)-armepavine (2) and (−)-(*R*)-*NO*-dimethylcoclaurine (4). Armepavine (2) obtained from the degradation of (7) derived from the feeding of (+)-(*S*)-N-methylcoclaurine (1) had essentially the same radioactivity as the parent base whereas (−)-(*R*)-*NO*-dimethylcoclaurine (4) so obtained was practically radioinactive. On the other hand (2) obtained from the degradation of (7) derived from the feeding of (−)-(*R*)-N-methylcoclaurine (3) was essentially radioinactive whereas (4) obtained in the same degradation had practically the same radioactivity as the parent base. The specific incorporation of (+)-(*S*)-N-methylcoclaurine (1) and (−)-(*R*)-N-methylcoclaurine (3) into oxyacanthine (7) and the finding that the hydrogen atom at the asymmetric centre in N-methylcoclaurine is retained in the biosynthesis of (7) from (1), ruled out the possibility that the enantiomers are interconvertible *via* the didehydro-N-methylcoclaurinium ion (13) in the plants.

The foregoing results strongly support the following sequence for the biosynthesis of oxyacanthine (7) in *C. laurifolius*: tyrosine ⟶ norcoclaurine (9) ⟶ coclaurine (10) ⟶ (+)-(*S*)-N-methylcoclaurine (1) + (−)-(*R*)-N-methylcoclaurine (3) ⟶ (inter- and intramolecular oxidative couplings) ⟶ oxyacanthine (7).

EXPERIMENTAL

For general directions see refs. 11 and 12.

Feeding Experiments.—For feeding purposes N-methylcoclaurine (11) and *NOO*-trimethylcoclaurine (12) were dissolved in water (1 ml) containing tartaric acid (10 mg). Coclaurine (10) hydrochloride and norcoclaurine (9) hydrochloride were dissolved in aqueous dimethyl sulphoxide (1 ml). Freshly cut young branches of *C. laurifolius* were dipped into the solution of the precursors. When uptake was complete the twigs were dipped in water, left for 6—7 days, and then worked up for oxyacanthine (7).

Isolation and Purification of Oxyacanthine (7).—The young stems and leaves (typically 135 g wet) of *C. laurifolius* fed with precursor were macerated in ethanol (300 ml) containing inactive oxyacanthine (7) (100 mg) and left for 18 h. The ethanol was decanted and the plant material was percolated with fresh ethanol (5 × 200 ml) containing 1% acetic acid. The green viscous mass so obtained was extracted with 5% hydrochloric acid (5 × 10 ml). The acidic solution was defatted with light petroleum (4 × 10 ml), basified with sodium carbonate (pH 9), and the precipitated bases were filtered. The precipitate and aqueous basic filtrate were extracted with chloroform–methanol (3 : 1; 5 × 40 ml), washed with water, dried (Na_2SO_4), and the solvent was removed. The residue was chromatographed on a column of neutral alumina. Elution (t.l.c. control) with chloroform–methanol (99 : 1) gave oxyacanthine (7) (70 mg), m.p. 217—218° (lit.,[13] 216—217°). It was crystallised from acetone to constant activity.

Feeding of Doubly Labelled Precursor.—(±)-[1-^{3}H, N-$^{14}CH_3$]-N-Methylcoclaurine (11) hydrochloride (^{14}C activity, 11.32; ^3H, 259.7 μCi mmol^{-1}; ^{14}C : 3H 1 : 22.9) was fed to freshly cut young branches of *C. laurifolius* and after five days the plants were harvested and worked up for oxyacanthine (7) (^{14}C activity 30.13; ^3H 662.86 μCi mmol^{-1}, ^{14}C : ^3H 1 : 22).

Hofmann Degradation of Labelled Oxyacanthine.—Labelled oxyacanthine (7) (300 mg; activity 36.7 × 10^{-2} μCi mmol^{-1}), derived from [N-$^{14}CH_3$]-didehydro-N-methylcoclaurinum iodide (13) (experiment 6), in methanol (15 ml) was treated with ethereal diazomethane (from 2 g of nitrosomethylurea) to afford O-methyloxyacanthine (8) (294 mg), m.p. 138—139° (lit.,[14] 139—140°) (^{14}C activity 36.4 × 10^{-2} μCi mmol^{-1}).

The preceding O-methyl ether (8) (290 mg) in methanol (10 ml) was heated gently to reflux with methyl iodide (6 ml) to give radioactive O-*methyloxyacanthine dimethiodide* (295 mg), m.p. 259—262° (lit.,[14] 260—262°) (^{14}C activity 36.2 × 10^{-2} μCi mmol^{-1}).

A solution of the above radioactive dimethiodide (290 mg) in methanol (40 ml) was passed through a column of freshly generated Amberlite IR-410 anion exchange resin (OH^-

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[14] T. Kugo, M. Tanaka, and T. Sagae, *J. Pharm. Soc. Japan*, 1960, **80**, 1425.

form) (8 g) to afford the corresponding methohydroxide of the base which was heated in methanol (8 ml) with potassium hydroxide (6 g) in water (22 ml) to give the ring opened compound (14) (158 mg), m.p. 97—98° (lit.,[14] 97—99°) (^{14}C activity 35.58×10^{-2} μCi mmol^{-1}).

Compound (14) (150 mg) in water (10 ml) was adjusted to pH 10 with potassium hydroxide and then stirred at 0 °C with dimethyl sulphate (1 ml) and 10N-potassium hydroxide (0.5 ml) for 1 h. At hourly intervals, three more portions of dimethyl sulphate (0.5 ml) and 10N-potassium hydroxide (0.25 ml) were added. After a total of 5 h, potassium hydroxide (10 g) was added to it and the resulting mixture heated to reflux for 2 h. The trimethylamine so evolved was collected in 15% hydrochloric acid (^{14}C activity 17.56×10^{-2} μCi mmol^{-1}).

Reductive Fission of Tritium Labelled Oxyacanthines.—(1). Oxyacanthine (7) (290 mg) (^{3}H activity 8.78×10^{-2} μCi mmol^{-1}) derived from (±)-[3′,5′,8-$^{3}H_3$]-*N*-methylcoclaurine (11) (experiment 4) in methanol (20 ml) was treated with ethereal diazomethane (from 2 g of nitrosomethylurea) to afford labelled *O-methyloxyacanthine* (8) (280 mg), m.p. 138—139° (lit.,[14] 139—140°) (^{3}H activity 8.8×10^{-2} μCi mmol^{-1}).

A solution of the preceding *O*-methyloxyacanthine (8) (275 mg) in dry toluene (30 ml) was added dropwise with stirring to liquid ammonia (200 ml) (dried over sodium metal) containing sodium (180 mg). More sodium metal (600 mg) was added until a permanent blue colour persisted. The resulting mixture was stirred for 3 h at −60 °C and then left overnight at room temperature. Water was then added and the non-phenolic products were extracted with ether (5 × 30 ml). The aqueous layer was adjusted to pH 7 by adding ammonium chloride, the liberated phenolic bases were extracted with chloroform (4 × 30 ml), washed with water, and dried (Na_2SO_4). Removal of the solvent from the chloroform extract gave an oily residue (150 mg) which on t.l.c. [SiO_2; chloroform–methanol 9 : 1] showed two spots.

The mixture of phenolic bases was subjected to preparative t.l.c. [SiO_2 GF_{254}; chloroform–methanol (9 : 1)] to give armepavine (2), m.p. 145—147° (lit.,[15] 145—146°) (^{3}H activity 4.1×10^{-2} μCi mmol^{-1}) and *NO*-dimethylcoclaurine [16] (4) (^{3}H activity 4.45×10^{-2} μCi mmol^{-1}).

(2). Oxyacanthine (7) (290 mg) derived from (+)-(*S*)-[3′,5′,8-$^{3}H_3$]-*N*-methylcoclaurine (1) (experiment 9) was treated with ethereal diazomethane to give the radioactive *O*-methyloxyacanthine (8) which was subjected to sodium–liquid ammonia fission as above. The radioactivities of the degradation products are given in Table 2.

TABLE 2

Activities of oxyacanthine degradation products

Compound	Molar activity (μCi mmol^{-1})
Oxyacanthine (7)	5.99×10^{-2}
O-Methyloxyacanthine (8)	6.00×10^{-2}
Armepavine (2)	5.92×10^{-2}
NO-Dimethylcoclaurine (4)	Inactive

(3). Oxyacanthine (7) (300 mg) derived from (−)-(*R*)-[3′,5′,8-$^{3}H_3$]-*N*-methylcoclaurine (3) (experiment 8) was converted to *O*-methyloxyacanthine (8) and then subjected to sodium–liquid ammonia fission as above. The radioactivities of the degradation products are given in Table 3.

TABLE 3

Activities of oxyacanthine degradation products

Compound	Molar activity (μCi mmol^{-1})
Oxyacanthine (7)	24.8×10^{-2}
O-Methyloxyacanthine (8)	24.6×10^{-2}
NO-Dimethylcoclaurine (4)	24.0×10^{-2}
Armepavine (2)	0.1×10^{-2}

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[16] M. Tomita and Y. Sasaki, *Chem. and Pharm. Bull.* (*Japan*), 1952, **2**, 375.