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BIOSYNTHESIS OF THE BISBENZYLISOQUINOLINE ALKALOID, TETRANDRINE

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Key Word Index—*Cocculus laurifolius*; Menispermaceae; biosynthesis; tetrandrine; (±)-*N*-methylcoclaurine.

Abstract—The incorporation of (±)-coclaurine, (±)-norcoclaurine, (±)-*N*-methylcoclaurine and didehydro-*N*-methylcoclaurinium iodide into tetrandrine in *Cocculus laurifolius* has been studied and specific utilization of (±)-*N*-methylcoclaurine demonstrated. The evidence indicates that tetrandrine is formed in the plants by oxidative dimerization of *N*-methylcoclaurine. Double labelling experiment with (±)-*N*-[^{14}C]-methyl-[1-^{3}H]-coclaurine demonstrated that the hydrogen atom at the asymmetric centre in the 1-benzylisoquinoline precursor is retained in the bioconversion into tetrandrine. Parallel feedings of (+)-(*S*)- and (−)-(*R*)-*N*-methylcoclaurines showed that the stereospecificity is maintained in the biosynthesis of tetrandrine from the 1-benzylisoquinoline precursor.

INTRODUCTION

Tetrandrine, the bisbenzylisoquinoline alkaloid first isolated from *Stephania tetrandra* S. Moore [1] in 1928, has since been isolated from many species of *Stephania* [2, 3] and *Cyclea* [4–6]. Tetrandrine affects the central nervous system, respiratory and skeletal muscles [7, 8] and is also an effective tumor inhibitor [9].

Tetrandrine has been assigned the structure and stereochemistry [10, 11] shown in **5**. It is the optical antipode of phaeanthine [12] and is diastereoisomeric with isotetrandrine [13]; syntheses of **5** are also reported [14, 15]. The crystal structure of **5** has recently been determined by X-ray crystallography [16].

Biogenetically tetrandrine is a *bis*-coclaurine derivative [17]. Intermolecular oxidative coupling of *N*-methylcoclaurine (**1**) can form **3** which in turn can undergo intramolecular oxidative coupling to generate **4**. *O*-Methylation of the phenolic hydroxyl groups in **4** can finally yield tetrandrine (**5**).

The *bis*-benzylisoquinoline alkaloids epistephanine [18], cocsulin [19] and cocsulinin [20] have been shown to be derived in nature from coclaurine and *N*-methylcoclaurine. We now present the results of tracer experiments on the biosynthesis of tetrandrine.

RESULTS AND DISCUSSION

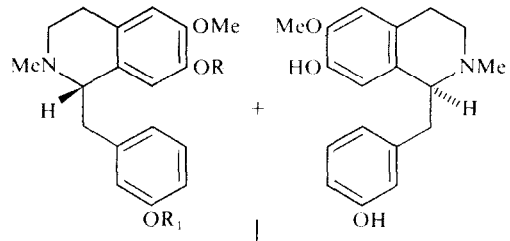
(±)-Tyrosine was initially fed to young cut branches of *Cocculus laurifolius* and it was found that tetrandrine (**5**) is being actively biosynthesized by the plants. In subsequent experiments, labelled hypothetical 1-benzylisoquinoline precursors were fed to young cut branches of *C. laurifolius*. The results of several feeding experiments are recorded in Table 1. Feeding of (±)-norcoclaurine (**6**) (expt 2), (±)-coclaurine (**7**) (expt 3) and (±)-*N*-methylcoclaurine (**8**) (expt 4) established that **6**–**8** were efficient precursors of **5**. The intermediate level of incorporation of the didehydro-*N*-methylcoclaurinium iodide (**10**) (expt 6) is probably due to prior reduction *in vivo* to *N*-methylcoclaurine (**8**). (±)-*N*,*O*,*O*-Trimethylcoclaurine (**9**) (expt 5), as expected, was not incorporated into **5**.

Labelled **5** derived from (±)-*N*-methyl-[3′,5′,8-^{3}H$_3$]-coclaurine feeding (expt 4) when cleaved with sodium–liquid ammonia [21] gave *N*,*O*,*O*-trimethylcoclaurine (**2**) and *N*-methylcoclaurine (**1**). The former had essentially 25% and the latter 75% of the radioactivity of the parent base.

Feeding of (±)-*N*-[^{14}C]-methyl-[1-^{3}H]-coclaurine (expt 7) gave **5**, labelled both with ^{14}C and ^{3}H. The ^{14}C:^{3}H ratios in the precursor and biosynthetic base were essentially unchanged. The experiment demonstrated that the H atom at the asymmetric centre in the 1-benzylisoquinoline precursor is retained in the biosynthesis of **5** from **1**.

Feeding of didehydro-*N*-[^{14}C]-methylcoclaurinium iodide (**10**) (expt 6) gave **5**. The regiospecificity of the labelling in biosynthetic **5** was shown as follows: labelled **5** was converted into its dimethiodide and then to its methohydroxide form with essentially no loss of radioactivity. Hofmann degradation of the methohydroxide furnished tetrandrine methyl methine-I (**11**) which had essentially the same radioactivity as the parent base. Treatment of **11** with dimethyl sulphate followed by potassium hydroxide gave trimethylamine trapped as its hydrochloride which had essentially one half the molar activity of the parent base.

Although the foregoing experiments established that *N*-methylcoclaurine is a specific precursor of tetrandrine in *C. laurifolius*, the precursors used were racemic. It would be expected that the enzyme system involved in the relevant biotransformation would be stereospecific, and that only one of the two optical isomers would normally act as a direct substrate. Parallel feedings with (+)-(*S*)- and (−)-(*R*)-*N*-methylcoclaurines showed that the stereospecificity is maintained in the oxidative dimerization of *N*-methylcoclaurine into tetrandrine. The *S*-form (**1**) (experiment 8) was incorporated into tetrandrine about



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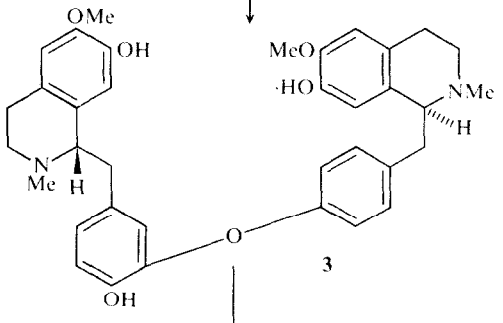

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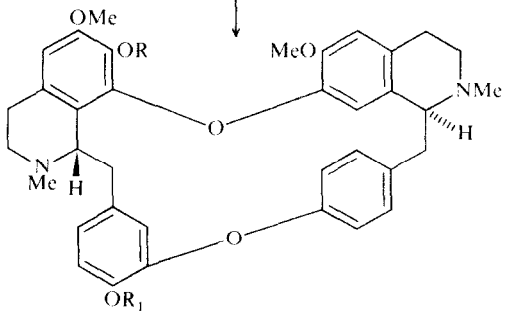

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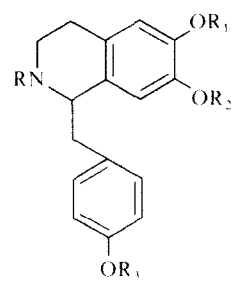
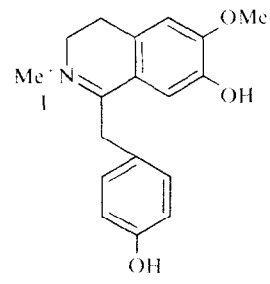


6 R = R_1 = R_2 = R_3 = H
7 R = R_2 = R_3 = H, R_1 = Me
8 R_2 = R_3 = H, R = R_1 = Me
9 R = R_1 = R_2 = R_3 = Me



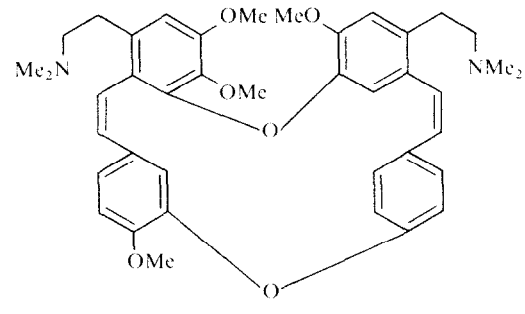


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Table 1. Tracer experiments on *Cocculus laurifolius*

Experiment	Precursor fed	% Incorporation into tetrandrine (**5**)
1	(L)-[U-^{14}C]-Tyrosine	0.07
2	(±)-[1-^{3}H]-Norcoclaurine (**6**)	0.10
3	(±)-[3′,5′,8-$^{3}H_3$]-Coclaurine (**7**)	0.15
4	(±)-*N*-Methyl-[3′,5′,8-$^{3}H_3$]-coclaurine (**8**)	0.21
5	(±)-*N*,*O*,*O*-Trimethyl-[3′,5′,8-$^{3}H_3$]-coclaurine (**9**)	0.002
6	Didehydro-*N*-[^{14}C]-methylcoclaurinium iodide (**10**)	0.17
7	(±)-*N*-[^{14}C]-Methyl-[1-^{3}H]-coclaurine (**8**)	0.26
8	(+)-*N*-Methyl-[3′,5′,8-$^{3}H_3$]-coclaurine (**1**)	0.24
9	(−)-*N*-Methyl-[3′,5′,8-$^{3}H_3$]-coclaurine	0.0023

100 times more efficiently than the *R*-form (experiment 9). Labelled **5** derived from (+)-(*S*)-*N*-methylcoclaurine (**1**) (experiment 8) feeding was subjected to sodium–liquid ammonia fission to give *N,O,O*-trimethylcoclaurine (**2**) and *N*-methylcoclaurine (**1**). The former had essentially 25% and the latter 75% of the radioactivity of the parent base. The result thus established that (+)-(*S*)-*N*-methylcoclaurine (**1**) is specifically incorporated into both halves of **5**.

(+)-(*S*)-*N*-Methylcoclaurine (**1**) has been found to be specifically incorporated into **5** and it has also been isolated from *C. laurifolius* [22] DC. Thus **1** is a true precursor of **5**. The foregoing results strongly support the following sequence for the biosynthesis of tetrandrine in *C. laurifolius* DC: tyrosine → norcoclaurine (**6**) → coclaurine (**7**) → (+)-(*S*)-*N*-methylcoclaurine (**1**) → (dimerization) → tetrandrine (**5**).

EXPERIMENTAL

For general methods, spectroscopy, radioactive counting, synthesis and labelling of precursors see refs. [19, 23].

Feeding experiments. For feeding purposes *N*-methylcoclaurine (**8**) and *N,O,O*-trimethylcoclaurine (**9**) were dissolved in H_2O (1 ml) containing tartaric acid (10 mg). Coclaurine (**7**) HCl, norcoclaurine (**6**) HCl and didehydro-*N*-methylcoclaurinium iodide (**10**) were dissolved in aq. DMSO (1 ml). Freshly cut young branches of *C. laurifolius* were dipped into the soln of precursors. When uptake was complete the twigs were dipped in H_2O, left for 6–7 days and then worked up for tetrandrine (**5**).

Isolation and purification of **5**. The young stems and leaves (typically 130 g fr. wt) of *C. laurifolius* DC fed with precursor were macerated in EtOH (250 ml) containing unlabelled **5** (110 mg) and left for 20 hr. The EtOH was decanted and the plant material was percolated with fresh EtOH (5 × 200 ml) containing 1% HOAc. The solvent from the combined extract was removed *in vacuo*. The green viscous mass thus obtained was extracted with 5% HCl (5 × 10 ml). The acidic soln was defatted with petrol (4 × 12 ml), basified with Na_2CO_3 (pH 9) and the liberated bases extracted with $CHCl_3$ (5 × 40 ml), washed with H_2O, dried (Na_2SO_4) and the solvent removed. The residue was chromatographed over a column of neutral Al_2O_3. Elution (TLC control) with C_6H_6 gave **5** (85 mg), mp 217–218° (lit. [3] 216–217°). It was crystallized from Et_2O to constant activity.

Feeding of doubly labelled precursor. (±)-*N*-[^{14}C]-Methyl-[1-^{3}H]-coclaurine (**8**) HCl (^{14}C, 11.3243 μCi/mmol and ^{3}H, 259.7 μCi/mmol, $^{14}C/^{3}H = 1:22.9$) was fed to freshly cut young branches of *C. laurifolius* and after 6 days the plants were harvested and worked up for **5** (molar ^{14}C activity 0.448 μCi/mmol and ^{3}H activity 9.6 μCi/mmol, $^{14}C/^{3}H = 1:21.4$).

Hofmann degradation of labelled **5**. Labelled **5** (300 mg; molar activity 2.26×10^{-2} μCi/mmol) derived from didehydro-*N*-[^{14}C]-methylcoclaurinium iodide (**10**), in MeOH (10 ml) was heated gently to reflux with MeI (6 ml) to give radioactive tetrandrine dimethiodide (310 mg), mp 271° (decomp.) (lit. [24] 269°, decomp.) (molar activity 2.28×10^{-2} μCi/mmol). A soln of the radioactive dimethiodide (305 mg) in MeOH (40 ml) was passed through a column of freshly generated Amberlite IR-410 anion exchange resin (OH form) (8 g) to afford the corresponding methohydroxide of the base which was heated in MeOH (8 ml) with KOH (6 g) in H_2O (22 ml) to give the tetrandrine methyl methine (**11**) (240 mg), mp 171–172° (lit. [24] 172°) (molar activity 2.25×10^{-2} μCi/mmol).

11 (230 mg) in H_2O (10 ml) was adjusted to pH 10 with KOH and then stirred at 0° with Me_2SO_4 (1 ml) and 10 N KOH (0.6 ml) for 1 hr. At 1 hr intervals, 3 more portions of Me_2SO_4 (0.6 ml) and 10 N KOH (0.25 ml) were added. After a total of 5 hr, KOH (10 g) was added and the resulting mixture refluxed for 2 hr. The trimethylamine so evolved was collected in 15% HCl (molar ^{14}C activity 1.05×10^{-2} μCi/mmol).

Reductive fission of tritium labelled tetrandrine. A soln of tetrandrine (**5**) (380 mg) (molar activity 9.97×10^{-2} μCi/mmol) derived from (±)-*N*-methyl-[3′,5′,8-$^{3}H_3$]-coclaurine (**8**) (expt 4) in dry toluene (30 ml) was added drop-wise to stirred liquid NH_3 (200 ml) containing Na (250 mg). More Na (400 mg) was added until a permanent blue colour persisted. The resulting mixture was stirred for 3 hr at −60° and then allowed to stand 18 hr at room temp. H_2O was then added and the non-phenolic bases (A) extracted with Et_2O (5 × 30 ml). The alkaline aq. layer was adjusted to pH 7 by adding NH_4Cl. The liberated phenolic bases (B) were extracted with $CHCl_3$ (4 × 30 ml), washed with H_2O, dried (Na_2SO_4) and the solvent removed. The mixture of A and B was subjected to prep. TLC (Si gel GF_{254}) ($CHCl_3$–MeOH, 22:3) to give *N,O,O*-trimethylcoclaurine [21] (**2**) (molar activity 2.2×10^{-2} μCi/mmol) and *N*-methylcoclaurine [21] (**8**) (molar activity 7.25×10^{-2} μCi/mmol).

Tetrandrine (**5**) (350 mg) derived from (+)-*N*-methyl-[3′,5′,8-$^{3}H_3$]-coclaurine (**1**) was degraded by Na–liq. NH_3 as above. The radioactivities of the degradation products were **5** 7.8×10^{-2}, *N,O,O*-trimethylcoclaurine (**2**) 1.84×10^{-2} and *N*-methylcoclaurine (**1**) 5.18×10^{-2} μCi/mmol.

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