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BIOSYNTHESIS OF ISOTETRANDRINE

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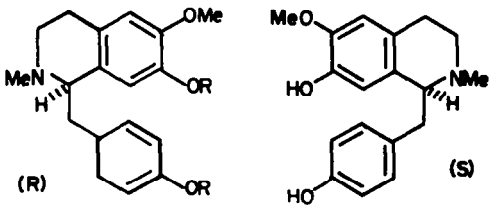
Abstract—The incorporation of (±)-coclaurine, (±)-N-methylcoclaurine, didehydro-N-methylcoclaurinium iodide, (+)-(*S*)-N-methylcoclaurine and (−)-(*R*)-N-methylcoclaurine into isotetrandrine in *Cocculus laurifolius* DC has been studied and specific utilization of (±)-, (+)-(*S*)- and (−)-(*R*)-N-methylcoclaurines and didehydro-N-methylcoclaurinium iodide demonstrated. The evidence supports intermolecular oxidative coupline of (+)-(*S*)- and (−)-(*R*)-N-methylcoclaurines to form isotetrandrine. Double labelling experiment with (±)-N-[^{14}C] methyl [1−^{3}H] coclaurine demonstrated that the hydrogen atom at the asymmetric centre in N-methylcoclaurine is retained in the bioconversion into isotetrandrine.

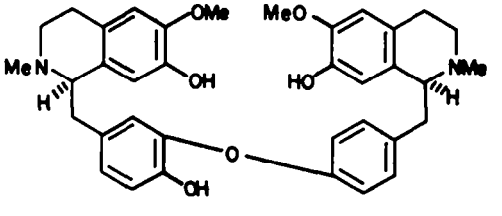
Isotetrandrine, the bisbenzylisoquinoline alkaloid first isolated from *Stephania cepharantha*[1] exhibits anti-inflammatory, analgesic and hypothermic effects[2] and has been assigned the structure and stereochemistry[3] as shown in **6**. Biogenetically isotetrandrine is a biscoclaurine derivative.

Isotetrandrine[3] (**6**) has two asymmetric centres C_1 and $C_{1'}$ having *R* and *S* configuration respectively. The biscoclaurine alkaloids[4] of this type can be formed in nature by the following plausible biosynthetic pathways. Intermolecular oxidative coupling of (+)-(*S*)-N-methylcoclaurine (**3**) and (−)-(*R*)-N-methylcoclaurine (**1**) can form a dimeric intermediate of the type **4** which in turn can undergo intramolecular oxidative coupling to generate **5**. O-Methylation of the phenolic OH groups in **5** can finally yield isotetrandrine (**6**). Alternately oxidative dimerization of (+)-(*S*)-N-methylcoclaurine (**3**) or (−)-(*R*)-N-methylcoclaurine (**1**) can form a biscoclaurine intermediate having "*S, S*" or "*R, R*" configuration at the asymmetric centres. Specific change in configuration possibly *via* a dehydro intermediate, at one of the asymmetric centres can then generate the desired configuration at the asymmetric centres as is present in isotetrandrine (**6**). In another possibility (+)-(*S*)- and (−)-(*R*)-N-methylcoclaurines can undergo interconversion *via* the dehydro intermediate (**10**) prior to inter oxidative coupling can occur. The fourth possibility for the formation of isotetrandrine (**6**) in nature can be that the didehydro-N-methylcoclaurinium salt (**10**) can undergo oxidative dimerisation to give the key dehydrobisbenzyl isoquinoline intermediate of the type **7**. Stereospecific reduction at C_1 and $C_{1'}$ in **7** can then generate "*R*" and "*S*" configuration at the respective asymmetric centres to provide isotetrandrine (**5**) nucleus. We have studied some of these aspects of biosynthesis of isotetrandrine (**6**) and we now present our results.



1: R = H
2: R = Me

3



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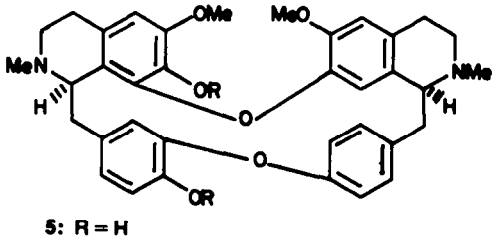
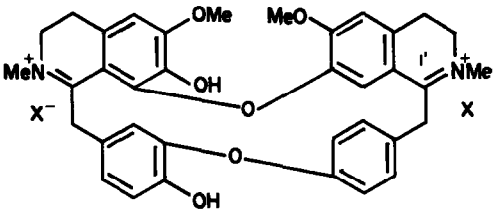


5: R = H
6: R = Me

Feeding of (±)-coclaurine **8** (experiment 1) and (±)-N-methylcoclaurine **9** (experiment 2) established that **8** and **9** are efficient precursors of isotetrandrine (**6**). Sodium–liquid ammonia fission of labelled isotetrandrine (**6**) derived from (±)-N-methyl [3′, 5′, 8−$^{3}H_3$] coclaurine (**9**) (experiment 2) gave (−)-(*R*)-NOO-trimethylcoclaurine (**2**) and (+)-(*S*)-N-methylcoclaurine (**3**). The former had essentially 1/4 and the latter 3/4 radio activity of the parent base.

Feeding of (±)-N [^{14}C] methyl [1−^{3}H] coclaurine (**9**) (experiment 3) gave isotetrandrine (**6**) labelled both with ^{14}C and ^{3}H. The $^{14}C:{}^{3}H$ ratios in the precursor and biosynthetic base was practically unchanged. The experiment demonstrated that the H atom at the C_1 position in N-methylcoclaurine is retained in the biosynthesis of isotetrandrine.

Feeding of didehydro N-[^{14}C] methylcoclaurinium iodide (**10**) ($X^- = I^-$) (experiment 4) gave isotetrandrine (**6**). The position of the label in the biosynthetic base was shown as follows: labelled **6** was treated with methyl



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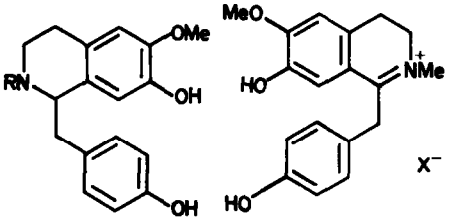
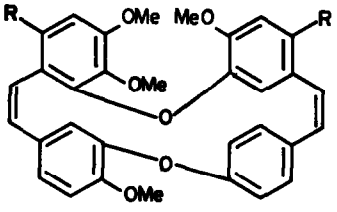
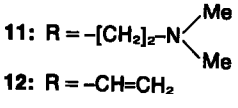


8: R = H
9: R = Me

10





11: R = $-[CH_2]_2-N(Me)_2$

12: R = $-CH=CH_2$

iodide to give the corresponding dimethiodide which was converted into the methohydroxide form by anion exchange resin with practically no loss of radio activity. Hofmann degradation of the methohydroxide furnished isotetrandrine methyl methine-I (**11**) which had essentially the same radio activity as the parent base. Treatment of **11** with dimethyl sulphate potassium hydroxide gave trimethylamine, trapped as its hydrochloride which had essentially one half the molar radio activity of the parent base.

Parallel feedings of (+)-(*S*)-N-methylcoclaurine (**3**) (experiment 5) and (−) - (*R*) - N - methylcoclaurine (**1**) (experiment 6) demonstrated that both the enantimoers were incorporated efficiently into isotetrandrine (**6**). Sodium liquid ammonia reduction of **6** derived from (+)-(*S*)-N-methyl [3′, 5′, 8-$^3H_3$]-coclaurine (**3**) feeding gave (−)-(*R*)-NOO-tri-methylcoclaurine (**2**) (radio inactive) and (+)-(*S*)-N-methyl- coclaurine (**3**) (essentially same molar radio activity as the parent base). **6** Derived from (−)-(*R*)-N-methyl- [3′,5′,8-$^3H_3$] coclaurine (**1**) feeding was similarly degraded to give (−)-(*R*)-NOO-trimethylcoclaurine (**2**) (essentially same molar radio activity as the parent base) and (+)-(*S*)-N-methyl-coclaurine (**3**) (radio inactive). These results thus established specific incorporation of (+)-(*S*)-N-methyl-coclaurine (**3**) and (−)-(*R*)-N-methylcoclaurine (**1**) into isotetrandrine (**6**) and ruled out the possibility that the enantiomers are interconvertible *via* the dehydro-N-methyl-coclaurinium (**10**) ion.

The foregoing results strongly support the following sequence for the biosynthesis of isotetrandrine (**6**) in *Cocculus laurifolius*: coclaurine (**8**)→(+)-(*S*)-N-methyl-coclaurine (**3**) and (−)-(*R*)-N-methylcoclaurine (**1**)→inter and intramolecular oxidative coupling→isotetrandrine (**6**).

EXPERIMENTAL

For general directions i.e. spectroscopy details, counting method, syntheses and labelling of precursors see earlier papers in this series.[5,6]

Feeding experiments. For feeding purposes **9** was dissolved in water (1 ml) containing tartaric acid (10 mg). **8**-hydrochloride and **10** iodide were dissolved in aqueous dimethyl sulphoxide (1 ml). Freshly cut young branches of *C. laurifolius* were dipped into the soln of the precursors. When uptake was complete the twigs were dipped in water, left for 6–7 days and then worked up for **6**.

Isolation and purification of isotetrandrine. The young branches (typically 140 g wet) of *C. laurifolius* fed with precursor were macerated in EtOH (250 ml) containing radio inactive **6** (90 mg) and left for 15 hr. The EtOH was decanted and the plant material was percolated with fresh EtOH (5 × 250 ml) containing 1% AcOH. The solvent from the combined extract was removed *in vacuo*. The green viscous mass, so obtained, was extracted with 2% HCl (5 × 10 ml), basified with Na_2CO_3 (pH9) and the liberated bases were extracted with $CHCl_3$ (5 × 20 ml), washed with water, dried (Na_2SO_4) and the solvent removed. The residue was subjected to preparative tlc, (plate; SiO_2 solvent; $CHCl_3$: MeOH, 92:8), to give isotetrandrine (65 mg), m.p. 182–183° (lit.[1] 182°). The radio active base in each case was crystallised from MeOH to constant activity.

Feeding of doubly labelled precursor. **9** hydrochloride (activity: ^{14}C, 2.23 μCi and ^3H, 67.8 μCi) was fed to freshly cut young branches of *C. laurifolius* and after 5 days the plants were harvested and worked up for **6** (molar ^{14}C activity 0.148 μCi mmol^{-1} and ^3H, 4.48 μCi mmol^{-1}).

Hofmann degradation of labelled isotetrandrine. Labelled **6** (280 mg; molar activity 0.036 μCi mmol^{-1} derived from **10** (X^- = I^-) in MeOH (10 ml) was refluxed with MeI (6 ml) to give ratio active isotetrandrine dimethiodide (360 mg), m.p. 241° (lit.[7] 242°) (molar activity 0.0356 μCi mmol^{-1}).

A soln of the preceding radio active dimethiodide (352 mg) in MeOH (40 ml) was passed through a column of freshly generated amberlite IR-410 anion exchange resin (OH form) (20 g) to afford the corresponding methohydroxide of the base which was heated in MeOH (10 ml) to give **11** (279 mg) m.p. 170–171° (lit.[8] 172°).

Compound **11** (260 mg) in water (10 ml) was adjusted to pH_{10} with KOH and stirred at 0° with Me_2SO_4 (1 ml) and 10N KOH

Table 1. Tracer experiments in *Cocculus laurifolius*

Expt.	Precursor Fed	Incorporation (%) into isotetrandrine(**6**)
1.	(±)-[3′, 5′, 8-$^3H_3$] coclaurine (**8**)	0.17
2.	(±)-N-Methyl [3′, 5′, 8-$^3H_3$] coclaurine (**9**)	0.19
3.	(±)-N-[^{14}C] Methyl [1 − ^3H] coclaurine (**9**)	0.28
4.	Didehydro-N-[^{14}C] methyl coclaurinium iodide (**10**)	0.34
5.	(+)-(S)-N-Methyl [3′, 5′, 8-$^3H_3$] coclaurine (**3**)	0.32
6.	(−)-(R)-N-Methyl [3′, 5′, 8-$^3H_3$] coclaurine (**1**)	0.47

Table 2. Activities of isotetrandrine degradation products

Compound	Molar activity (μCi mmol^{-1})
Isotetrandrine (6)	0.48
(*R*)-Noo-Trimethylcoclaurine (2)	inactive
(*S*)-N-Methylcoclaurine (3)	0.47

Isotetrandrine (6) (335 mg) derived from (-)-(*R*)-N-methyl [3,5,8-$^3H_3$] coclaurine (1) was degraded by sodium liquid ammonia.

The radioactivity of the degradation products is given in Table 3.

Table 3.

Compound	Molar activity (μCi mmol^{-1})
Isotetrandrine (6)	0.252
(*R*)-NOO-Trimethylcoclaurine (2)	0.25
(*S*)-N-Methylcoclaurine (3)	inactive

(0.8 ml) for 1 hr. At hourly intervals, three more portions of Me_2SO_4 (0.8 ml) and 10N KOH (0.25 ml) were added. After a total of 5 hr, KOH (10 g) was added and the resulting mixture was refluxed for 2 hr. The Et_3N so evolved was collected in 15% HCl as base hydrochloride (molar activity = 0.018 μCi mmol^{-1}).

Reductive fission of tritum labelled isotetrandrine. A soln of 6 (370 mg) (molar activity = 0.63 μCi mmol^{-1} derived from 9 (experiment 2) in dry toluene (25 ml) was added dropwise to liquid ammonia (200 ml) (δ over Na metal) containing Na (280 mg). More Na (480 mg) was added to it until a permanent blue colour persisted. The resulting mixture was stirred for 4 hr at −60° and allowed to stand overnight at room temp. Water was then added and the nonphenolic bases (A) were extracted with ether (5 × 250 ml). The aqueous alkaline soln was adjusted to pH 7 by adding ammonium chloride. The liberated phenolic bases (B) were extracted with $CHCl_3$ (4 × 25 ml), washed with water (B) (Na_2SO_4) and the solvent was removed.

The mixture of nonphenolic bases (A) and phenolic bases (B) was subjected to preparative tlc (Plate: SiO_2, GF 254; Solvent; $CHCl_3$: MeOH, 88: 12) to give NOO-methylcoclaurine[3] (2) (molar activity 0.153 μCi mmol^{-1}) and N-methylcoclaurine (molar activity 0.45 μCi mmol^{-1}). 6 (380 mg) derived from (+)-(*S*) 3 was degraded by sodium liquid ammonia as above. The radio activity of the degration products is given in Table 2.

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