

THE BIOSYNTHESIS OF THE ALKALOIDS OF *CISSAMPELOS PAREIRA* LINN

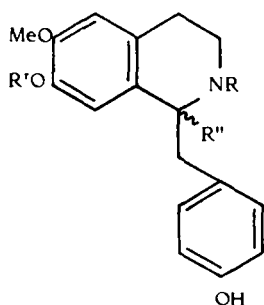
DEWAN S. BHAKUNI*, SUDHA JAIN AND REKHA CHATURVEDI

Central Drug Research Institute
 Lucknow 226 001, India

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Abstract - Tracer experiments show that the bisbenzylisoquinoline alkaloid, (S,R)-hayatidin (10) is stereospecifically biosynthesized in young *Cissampelos pareira* Linn plants by intermolecular oxidative coupling of (S)-(5)- and (R)-(3), N-methylcoclaurines whereas (R,R)-isochondrodendrine (14) and (R,R)-bebeerine (12) are formed in the plants by oxidative dimerization of (R)-N-methylcoclaurine (3).

Cissampelos pareira Linn (Menispermaceae), a perennial climbing shrub found in many parts of the world, is a rich source of 1-benzylisoquinoline derived alkaloids. The bisbenzylisoquinoline alkaloids^{1,2} isolated from the plant are (S,S)-4"-O-methylbebeerine (9), (S,R)-hayatidin (10), (R,R)-bebeerine (12), (R,R)-isochondrodendrine (14), (R,R)-cycleanine (15), (dl)-hayatin (11), (dl)-hayatinin, cissampareine, insularine and sepeerine. The aporphine and protoberberine alkaloids that have been isolated from the plants are cyclanoline chloride³ (cissamine chloride), dicentrine and dehydrodicentrine.¹ The structure and stereochemistry of these alkaloids have been established. Of the isolated bases hayatin methiodide is a potent



1 R = R' = H, R'' = H

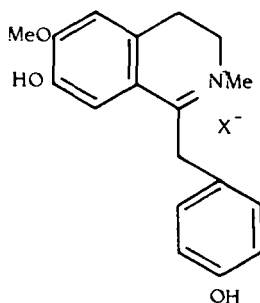
2 R = Me, R' = H, R'' = H

3 R = Me, R' = H, R'' = H

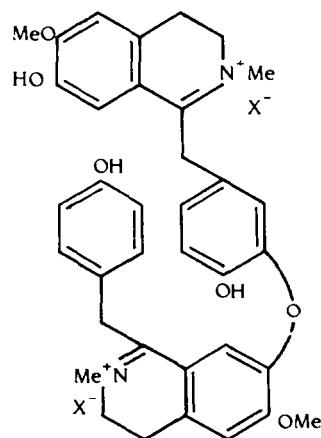
4 R = R' = Me, R'' = H

5 R = Me, R' = H, R'' = H

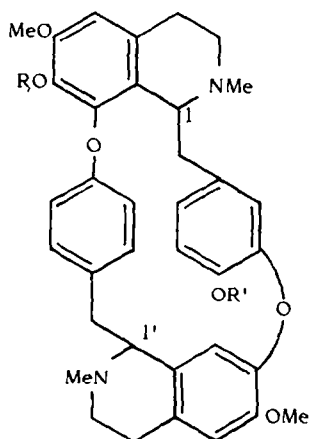
6 R = R' = Me, R'' = H



7



8



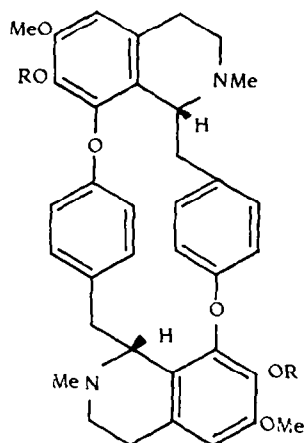
9 $R = H, R' = Me$ ($1 = 1' = S$)

10 $R = H, R' = Me$ ($1 = S, 1' = R$)

11 $R = R' = H$ (*dl*)

12 $R = R' = H$ ($1 = 1' = R$)

13 $R = R' = Me$



14 $R = H$

15 $R = Me$

neuromuscular blocking agent.⁴ *l*-Bebeerine, *d*-isochondrodendrine and *dl*-hayatinin are reported to exhibit curare like activity.⁵ Cissampareine, *l*-bebeerine and *d*-isochondrodendrine are found to possess cytotoxic activity.²

Biogenetically bisbenzylisoquinoline alkaloids, 4''-O-methylbebeerine (9), hayatinin (10), hayatin (11), bebeerine (12) and isochondrodendrine (14) could be derived in *Cissampelos pareira* Linn plant from coclaurine derivatives.⁶ (R,R)-Isochondrodendrine (14) and (R,R)-bebeerine (12) could be formed by oxidative dimerization of (R)-(-)-N-methylcoclaurine (3) whereas oxidative dimerization of (S)-(+)-N-methylcoclaurine (5) would yield (S,S)-4''-O-methylbebeerine (9). (S,R)-Hayatinin (10) having the 'S' and 'R' configuration at the asymmetric centres C_1 and $C_{1'}$, respectively, could be formed either by intermolecular oxidative coupling of (S)-(+)-N-methylcoclaurine (5) and (R)-(-)-N-methylcoclaurine (3) or by oxidative dimerization of dihydro-N-methylcoclaurine (7) via the dimeric intermediate 8. Stereospecific reduction at C_1 and $C_{1'}$ in 8 could then generate the desired stereochemistry at the two centres.

(L)-[U-¹⁴C]Tyrosine (experiment 1) (Table) was initially fed to young *C. pareira* Linn plants and it was found that hayatinin (10), bebeerine (12), isochondrodendrine (14) and cycleanine (15) were being actively biosynthesized. In subsequent experiments, labeled hypothetical precursors were fed to young *C. pareira* plants. The results of several feedings are recorded in Table.

Feeding of labeled coclaurine (1) (experiment 2), N-methylcoclaurine (2) (experiment 3) and dihydro-N-methylcoclaurine (7) (experiment 5) established that 1, 2 and 7 were efficient precursors of hayatinin (10), bebeerine (12), isochondrodendrine (14) and cycleanine (15). As expected (±)-NOO-trimethylcoclaurine (experiment 4) was not incorporated into these bisbenzylisoquinoline alkaloids. The intermediate level of incorporation of dihydro-N-[¹⁴C]methylcoclaurine (7) (experiment 5) suggested that probably 7 gets reduced to (S)-N-methylcoclaurine (5) and (R)-N-methylcoclaurine (3).

Biosynthetic hayatinin (10) derived from the feeding of (±)-[3',5',8-³H₃]N-methylcoclaurine (2) (experiment 3) was treated with CH₂N₂ to give radioactive O-methylhayatinin (13) with essentially the same radioactivity as the parent base. Sodium-liquid ammonia reductive cleavage of 13 furnished (S)-NOO-trimethylcoclaurine (6) (25% activity of original) and (R)-N-methylcoclaurine (3) (73% activity of original).

Biosynthetic isochondrodendrine (14) derived from (±)-[3',5',8-³H₃]N-methylcoclaurine (2) (experiment 3) was converted into radioactive O-methylisochondrodendrine (15) by treatment of the base with CH₂N₂. Sodium-liquid ammonia reductive fission of 15 afforded (R)-armepavine (4) with essentially the same

TABLE: Tracer experiments on *Cissampelos pareira* Linn

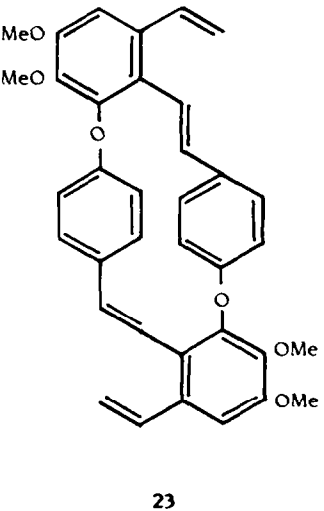
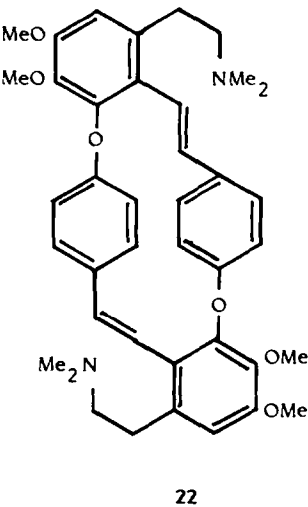
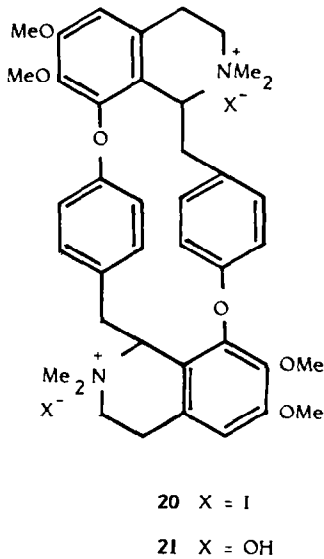
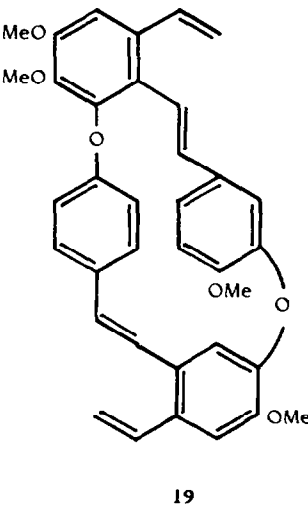
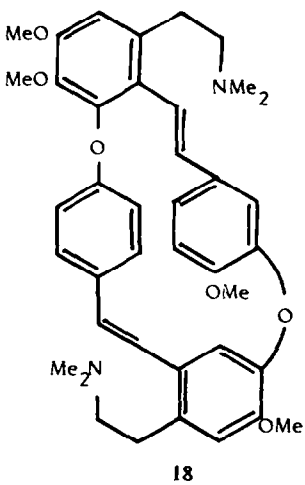
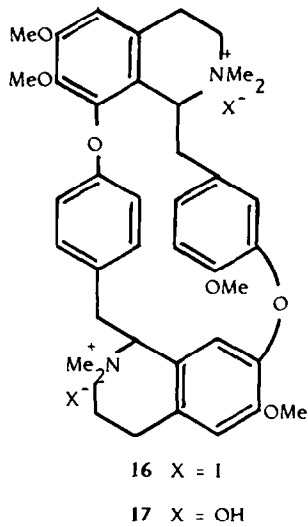
Expt.	Precursor fed	% Incorporation into			
		Hayatidin (10)	Bebeerine (12)	Isochondrodendrine (14)	Cycleanine (15)
1	(L)-[U- ^{14}C]Tyrosine	0.1	0.12	0.11	0.09
2	($\pm$)-[3',5',8- $^3\text{H}_3$]-Coclaurine (1)	0.16	0.17	0.20	0.10
3	($\pm$)-[3',5',8- $^3\text{H}_3$]N-Methyl- coclaurine (2)	0.18	0.19	0.22	0.15
4	($\pm$)-[3',5',8- $^3\text{H}_3$]NOO- Trimethylcoclaurine	0.0004	0.0003	0.0002	0.0005
5	Didehydro-N-[^{14}C]methyl coclaurine (7)	0.14	-	0.20	0.12
6	(S)-(+)-[3',5',8- $^3\text{H}_3$]- N-methylcoclaurine (5)	0.56	0.024	0.0025	.001
7	(R)-(-)-[3',5',8- $^3\text{H}_3$]- N-Methylcoclaurine (3)	0.48	0.86	0.97	0.99
8	($\pm$)-N-[^{14}C]Methyl- [1- ^3H]coclaurine (2)	0.32	0.28	0.63	0.42
9	[Aryl- ^3H]isochondrodendrine (14)	-	-	-	1.2

molar radioactivity as that of parent base. Biosynthetic cycleanine (15) derived from ($\pm$)-[3',5',8- $^3\text{H}_3$]-N-methylcoclaurine (2) (experiment 3) was cleaved with sodium-liquid ammonia to afford radioactive (R)-armepavine (4) (86% of original activity).

The experiments carried out so far, although have established that both the halves of the bisbenzyl-isoquinoline alkaloids hayatidin (10), bebeerine (12), isochondrodendrine (14) and cycleanine (15) are derived from ($\pm$)-N-methylcoclaurine (2), however the precursor used was labeled with tritium only. The doubly labeled ($\pm$)-N-[^{14}C]methyl[1- ^3H]coclaurine (2) (experiment 8) was, therefore, fed to young *C. pareira* plants. The doubly labeled precursor 2 was again incorporated efficiently into hayatidin (10), bebeerine (12), isochondrodendrine (14) and cycleanine (15). Moreover, $^{14}\text{C}/^3\text{H}$ ratios in the precursor and biosynthetic bases were essentially unchanged, suggesting thus that the hydrogen atom at the asymmetric centre C_1 in N-methylcoclaurine (2) remains intact during biotransformation of 2 into 10, 12, 14 and 15. The experiments thus suggest that 3 and 5 are not interconverted to form racemic bisbenzylisoquinoline bases via didehydro-N-methylcoclaurine (7). Efficient incorporation of ($\pm$)-[aryl- ^3H]isochondrodendrine (14) (experiment 2) into cycleanine (15) demonstrated that O-methylation is the terminal step in the biosynthesis of cycleanine (15).

Biosynthetic hayatidin (10) derived from didehydro-N-[^{14}C]methylcoclaurine (7) (experiment 5) was treated with CH_2N_2 to give the radioactive O-methylhayatidin (13). The base (13) was converted into its dimethiodide (16) (with practically no loss of radioactivity) and then into the corresponding methoxyhydroxide (17). Hofmann degradation of 17 gave the radioactive methine-I (18) (essentially no loss of radioactivity). Treatment of 18 with dimethyl sulphate and potassium hydroxide furnished trimethylamine (trapped as its hydrochloride) having essentially half the ^{14}C molar activity as that of parent base, while the methine-II (19) was essentially radioinactive.

Biosynthetic isochondrodendrine (14) derived from didehydro-N-[^{14}C]methylcoclaurine (7) (experiment 5) was converted into the O-methyl derivative (15) by treatment with CH_2N_2 and degraded as described above to give radioactive trimethylamine (trapped as its hydrochloride; 47% of original activity). The methine-II (23) was found essentially radioinactive. Biosynthetic cycleanine (15) derived from didehydro-N-[^{14}C]methylcoclaurine (7) (experiment 5) was similarly degraded to give radioactive trimethylamine (base hydrochloride, 44% of original activity) and the methine-II (23) was found essentially radioinactive. The results, thus, established that practically all the ^{14}C activity of didehydro-N-[^{14}C]methylcoclaurine (7) resided in the N-methyl functions of the biosynthetic hayatidin (10), isochondrodendrine (14) and cycleanine (15).



Although the foregoing results have demonstrated that N-methylcoclaurine is incorporated without degradation into the bisbenzylisoquinoline alkaloids (10, 12, 14 and 15) in *C. pareira*, the precursor used, however, was racemic. It would be expected that the enzyme system involved in the oxidative coupling process in the biosynthesis of these bases would be stereospecific. Parallel feedings with (S)-(-)- and (R)-(+), N-methylcoclaurines (experiments 6 and 7 respectively) demonstrated that stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline into the bisbenzylisoquinoline alkaloids in the plants. (R)-N-Methylcoclaurine (3) (experiment 7) was incorporated more efficiently into bebeerine (12), isochondrodendrine (14) and cycleanine (15). Sodium-liquid ammonia reductive cleavage separately of biosynthetic bases 14 and 15 derived from feeding of (R)-(-)-[3',5',8-³H₃]N-methylcoclaurine (3) (experiment 7) afforded only radioactive (R)-(-)-armepavine (4) with essentially the same molar radioactivity as that of parent base. The foregoing results thus confirmed that in *C. pareira* plants, the bisbenzylisoquinoline alkaloids, (R,R)-bebeerine (12), (R,R)-isochondrodendrine (14) and (R,R)-cycleanine (15) are formed by oxidative dimerization of (R)-N-methylcoclaurine (3).

Parallel feedings of (S)-N-methylcoclaurine (5) (experiment 6) and (R)-N-methylcoclaurine (3) (experiment 7) showed that both the labeled precursors (3 and 5) were incorporated with almost equal efficiency into hayatidin (10). Biosynthetic hayatidin (10) derived from (S)-[3',5',8-³H₃]N-methylcoclaurine was converted into radioactive O-methylhayatidin (13) by treatment with CH₂N₂. The compound 13 was subjected to sodium-liquid ammonia reductive fission. (S)-NOO-Trimethylcoclaurine (6), thus obtained, had essentially the same molar radioactivity as the parent base, whereas (R)-N-methylcoclaurine (3) was found essentially radioinactive. Biosynthetic hayatidin (10) derived from (R)-[3',5',8-³H₃]N-methylcoclaurine was converted into radioactive O-methylhayatidin (13) and similarly subjected to sodium-liquid ammonia reductive fission. (S)-NOO-Trimethylcoclaurine (6) obtained in this case was found essentially radioinactive whereas (R)-N-methylcoclaurine (3) had essentially the same molar activity as the parent base. These results, thus, confirmed the absolute configuration at the asymmetric centres C₁ and C₁' as 'S' and 'R' respectively in hayatidin (10) and established that hayatidin (10) in *C. pareira* plants is biosynthesized stereospecifically by inter molecular oxidative coupling of 3 and 5.

The biosynthesis of the bisbenzylisoquinoline alkaloid cycleanine (15) has been studied by us earlier in *Stephania glabra* (Roxb.) Miers (Menispermaceae) plant.⁶ Since this alkaloid has also been isolated from *C. pareira* Linn, we re-examined the biosynthesis of 15 in this plant and found that (R,R)cycleanine (15) is stereospecifically biosynthesized in *C. pareira* Linn plant also by oxidative dimerization of (R)-N-methylcoclaurine (3).

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see ref. 7.

Synthesis of Precursors - The racemates of coclaurine (1), N-methylcoclaurine (2) were prepared by standard procedures.⁸

Resolution - (±)-Di-O-benzyl-N-methylcoclaurine was resolved by treatment with (-)- and (+)-, di-*p*-toluoyl-tartaric acid.⁹ Hydrogenolysis of the benzyl ethers with HCl furnished (-)-(R)-N-methylcoclaurine (3) and (+)-(S)-N-methylcoclaurine (5) of known absolute configuration.

Labeling of Precursors - Tritiation⁸: A mixture of (±)-coclaurine (1) hydrochloride (120 mg) KOBu^t (100 mg) and tritiated water (0.2 ml, 150 mCi) was heated under N₂ (sealed tube) for 110 hr at 100° and worked up as earlier to give (±)-[3',5',8-³H₃]coclaurine (1) hydrochloride (86 mg). The other 1-benzyltetrahydroisoquinoline precursors were tritiated in the same way.

(±)-N-Methyl[1-³H]coclaurine was prepared by reduction of the corresponding dihydroisoquinoline with potassium boro[³H]hydride in dry dimethylformamide.

(±)-N-[¹⁴C]Methylcoclaurine was prepared by treating the corresponding dihydroisoquinoline with [¹⁴C]MeI and subsequent reduction of the methiodide with NaBH₄.

(±)-N-[¹⁴C]Methyl[1-³H]coclaurine was prepared by mixing (±)-N-[¹⁴C]methylcoclaurine and (±)-N-methyl[1-³H]coclaurine.

(±)-[3',5',8-³H₃]NOO-Trimethylcoclaurine was prepared by treating (±)-[3',5',8-³H₃]N-methylcoclaurine with CH₂N₂.

[Aryl-³H]isochondrodendrine (14): Tritiated water (0.2 ml, 150 mCi) and SOCl₂ (0.1 ml) were heated. The base (14) (110 mg) was added to it. The mixture under N₂ (sealed tube) was heated for 110 h at 100°.

The resulting mixture was worked up in the usual way to give [aryl-³H]-isochondrodendrine (**14**) (70 mg).

Feeding Experiments - For feeding purposes, tyrosine, N-methylcoclaurine and NOO-trimethylcoclaurine were dissolved in H₂O (1 ml) containing tartaric acid (12 mg). Coclaurine hydrochloride, didehydro-N-methylcoclaurinium iodide and isochondrodendrine were dissolved in H₂O (1 ml) and dimethylsulphoxide (0.2 ml). The solution of the precursor was then fed to young *C. pareira* Linn plants by wick feeding technique. When uptake was complete H₂O was added for washing. The plants were left for 6 to 8 days and then worked up for alkaloids of interest.

Isolation of Hayatidin (10) - The young plants (typically 125 g, wet wt.) of *C. pareira* were macerated in EtOH (250 ml) with radioactive hayatidin (**10**) (120 mg) and left for 10 hr. The EtOH was decanted and the plant material was percolated with fresh EtOH (6 x 200 ml). The combined extract was concentrated under reduced pressure to give a greenish viscous mass which was extracted with 5% hydrochloric acid (5 x 15 ml). The aqueous acidic extract was defatted with pet. ether (5 x 15 ml) and basified with Na₂CO₃. The liberated bases were extracted with CHCl₃-MeOH (85:15) (5 x 50 ml). The combined extract was washed with H₂O, dried (anhyd. Na₂SO₄) and the solvent removed under reduced pressure. The crude base was subjected to preparative tlc on silica gel GF₂₅₄ (solvent: CHCl₃-MeOH, 9:1). The region containing the desired alkaloid was cut and eluted with CHCl₃-MeOH (80:20). The solvent from the eluate was removed and the base crystallized from MeOH to give radioactive hayatidin (**10**) (72 mg), m.p. 175° (lit.¹⁰ 179-80°).

Isolation of Isochondrodendrine (14) - The young plants (110 g, wet wt.) of *C. pareira* were macerated in EtOH (250 ml) with radioactive isochondrodendrine (90 mg). The plant material was extracted with EtOH and the extract worked up as above to give radioactive isochondrodendrine (**14**) (52 mg), m.p. 317-18° (decomp.) (MeOH) [Lit.⁵ 318-19° (decomp.)].

Isolation of Cycleanine (15) - The young plants (120 g, wet wt.) of *C. pareira* were macerated in EtOH (250 ml) with radioactive cycleanine (**15**) (85 mg). The plant material was extracted with EtOH and the extract worked up as earlier to give radioactive cycleanine (**15**) (48 mg), m.p. 272° (MeOH) (lit.¹¹ 273°).

Isolation of Bebeerine (12) - The young plants (130 g, wet wt.) of *C. pareira* were macerated in EtOH (250 ml) with radioactive bebeerine (**12**) (90 mg). The plant material was extracted with EtOH and the extract worked up as above to give radioactive (**12**) (58 mg), m.p. 230-31° (lit.¹¹ 232°).

Degradation Experiments

1. Degradation of the Biosynthetic Hayatidin (10) derived from didehydro-N-[¹⁴C]methylcoclaurine - Labeled hayatidin (**10**) (experiment 5) was diluted with inactive material. A mixture of diluted labeled hayatidin (303 mg; molar activity 2.08×10^4 disint. min⁻¹ mmol⁻¹) and MeOH (10 ml) was treated with an excess of ethereal CH₂N₂ to give labeled O-methylhayatidin¹² (**13**) (molar activity 2.12×10^4 disint. min⁻¹ mmol⁻¹). A solution of **13** in Me OH (10 ml) was refluxed with MeI to yield O-methylhayatidin dimethiodide (**16**) (290 mg) (molar activity 2.10×10^4 disint. min⁻¹ mmol⁻¹). **16** In MeOH (100 ml) was passed through a column of freshly generated amberlite IR-400 anion exchange resin (OH[⊖] form)(6 g) to afford the corresponding radioactive methohydroxide (**17**). Labeled **17** in MeOH (10 ml) was refluxed with KOH (4.4 g in 5 ml H₂O) for 4 hr. The solvent from the resulting mixture was removed, H₂O (10 ml) added and the product extracted with CHCl₃ (5 x 50 ml). The combined CHCl₃ extract was washed with H₂O, dried (anhyd. Na₂SO₄) and concentrated *in vacuo* to furnish radioactive O-methylhayatidin methine-I¹³ (**18**) (molar activity 2.09×10^4 disint. min⁻¹ mmol⁻¹).

A mixture of the labeled methine-I (**18**) (240 mg), H₂O (8 ml), dimethyl sulphate (1 ml) and 10 N KOH (0.5 ml) at 0° was stirred for 1 hr. At hourly interval, three more portions of dimethyl sulphate (0.5 ml) and 10 N KOH (0.25 ml) were added. After a total of 5 hr, KOH (5 g) was again added to it and the mixture was refluxed for 2 hr. Trimethylamine, so evolved, was collected in hydrochloric acid (15%) and counted as trimethylamine hydrochloride (molar activity 1.08×10^4 disint. min⁻¹ mmol⁻¹). The remaining basic solution was extracted with CHCl₃ (4 x 15 ml), washed with H₂O, dried (anhyd. Na₂SO₄) and the solvent removed *in vacuo* to give the methine-II (**19**) (essentially radioinactive).

2. Degradation of the Biosynthetic Isochondrodendrine (14) derived from didehydro-N-[¹⁴C]methylcoclaurine - Labeled isochondrodendrine (**14**) (experiment 5) was diluted with inactive material. A mixture of diluted labeled isochondrodendrine (300 g) (molar activity 9.00×10^3 disint. min⁻¹ mmol⁻¹) and MeOH was treated with an excess of ethereal CH₂N₂ to afford labeled O-methyl-isochondrodendrine (**15**), m.p. 269° (acetone) (lit.¹⁴ 269-70°) (identical with cycleanine) (molar activity 8.95×10^3 disint. min⁻¹ mmol⁻¹).

The radioactive **15** in MeOH was treated with MeI to give the labeled demethiodide (**20**), m.p. 300° (decomp.) (H₂O) [lit.¹⁴ 302° (decomp.)] (molar activity 8.88×10^3 disint. min⁻¹ mmol⁻¹). **20** Was converted into the corresponding methohydroxide (**21**). Hofmann degradation of **21** as described above gave methine-I (**22**) (molar activity 8.71×10^3 disint. min⁻¹ mmol⁻¹). Labeled methine-I (**22**) was treated with dimethyl sulphate and KOH as above. The trimethylamine, so formed, was trapped as its hydrochloride (molar activity 4.20×10^3 disint. min⁻¹ mmol⁻¹). The methine-II (**23**), thus, formed was found to be radioinactive.

3. Degradation of the Biosynthetic Cycleanine (15) derived from Didehydro-N-[¹⁴C]methylcocclaurine - Labeled cycleanine (**15**) (experiment 5) was diluted with inactive material. A mixture of diluted labeled cycleanine (295 mg) (molar activity 1.08×10^4 disint. min⁻¹ mmol⁻¹) and MeOH (5 ml) was refluxed with MeI to give the radioactive dimethiodide (**20**) (molar activity 0.98×10^4 disint. min⁻¹ mmol⁻¹). **20** Was degraded as above to give the radioactive **22** (molar activity 0.92×10^4 disint. min⁻¹ mmol⁻¹) and then into trimethylamine (trapped as its hydrochloride) (molar activity 0.47×10^4 disint. min⁻¹ mmol⁻¹) and the methine-II (**23**) which was found to be radioinactive.

4. Degradation of the Biosynthetic Hayatidin (10) derived from (±)-[3',5',8-³H₃N-methylcocclaurine - Labeled hayatidin (**10**) (experiment 3) was diluted with inactive material. A mixture of diluted labeled hayatidin (295 mg) (molar activity 3.44×10^5 disint. min⁻¹ mmol⁻¹) and MeOH (10 ml) was treated with ethereal CH₂N₂ to afford radioactive O-methyl hayatidin¹¹ (**13**) (280 mg) (molar activity 3.42×10^5 disint. min⁻¹ mmol⁻¹).

A solution of **13** (275 mg) in dry toluene (5 ml) was added dropwise to liquid ammonia (150 ml) (dried over Na metal) containing Na (50 mg) and the mixture stirred at -60°. Na (80 mg) was added again to it till a permanent blue colour persisted. The mixture was stirred at -60° for 3 hr, allowed to stand for 20 hr at room temperature and then H₂O (10 ml) added to it. The product was extracted with ether (5 x 20 ml), washed with H₂O, dried (anhyd. Na₂SO₄) and the solvent removed. The residue was purified by preparative tlc on silica gel GF₂₅₄ (solvent: CHCl₃-MeOH; 99:1) to give radioactive (S)-NOO-trimethylcocclaurine (**6**), m.p. 61° (lit.¹⁵ 61-62°) (molar activity 0.86×10^5 disint. min⁻¹ mmol⁻¹). The aqueous basic solution after the removal of **6** was treated with NH₄Cl, extracted with CHCl₃ (5 x 25 ml), washed with H₂O, dried (anhyd. Na₂SO₄) and the solvent removed. The crude product was purified by preparative silica gel GF₂₅₄ tlc plates (solvent: CHCl₃-MeOH, 9:1) to afford radioactive (R)-N-methylcocclaurine (**3**) (84 mg), m.p. 177-78° (lit.¹⁶ 178°) (molar activity 2.49×10^5 disint. min⁻¹ mmol⁻¹).

5. Degradation of the Biosynthetic Isochondrodendrine (14) derived from (±)-[3',5',8-³H₃N-methylcocclaurine - Labeled isochondrodendrine (**14**) (experiment 5) was diluted with inactive material. A solution of diluted isochondrodendrine (**14**) (298 mg) (molar activity 4.66×10^5 disint. min⁻¹ mmol⁻¹) in MeOH (10 ml) was treated with an excess of ethereal CH₂N₂ to give O-methylisochondrodendrine (**15**) (285 mg) (molar activity 4.55×10^5 disint. min⁻¹ mmol⁻¹). The labeled **15** was subjected to sodium-liquid ammonia reductive fission as described earlier to afford labeled (R)-armepavine (**4**), m.p. 145° (lit.¹⁷ 145-46°) (molar activity 4.40×10^5 disint. min⁻¹ mmol⁻¹).

6. Degradation of the Biosynthetic cycleanine (15) derived from (±)-[3',5',8-³H₃N-methylcocclaurine - Labeled cycleanine (**15**) (experiment 3) was diluted with inactive material. Diluted labeled cycleanine (295 mg) (molar activity 1.78×10^5 disint. min⁻¹ mmol⁻¹) was subjected to sodium-liquid ammonia reductive fission as described earlier to furnish labeled (R)-armepavine (**4**) (molar activity 1.52×10^5 disint. min⁻¹ mmol⁻¹).

7. Degradation of the Biosynthetic Hayatidin (10) derived from (S)-[3',5',8-³H₃N-methylcocclaurine - Labeled hayatidin (**10**) (experiment 6) was diluted with inactive material. A solution of diluted labeled hayatidin (290 mg) (molar activity 5.06×10^4 disint. min⁻¹ mmol⁻¹) in MeOH (5 ml) was treated with CH₂N₂ to give labeled O-methylhayatidin (**13**) (molar activity 5.04×10^4 disint. min⁻¹ mmol⁻¹). **13** Was subjected to sodium-liquid ammonia reductive fission to furnish radioactive (S)-NOO-trimethylcocclaurine (**6**) (molar activity 5.00×10^4 disint. min⁻¹ mmol⁻¹) and radioinactive (R)-N-methylcocclaurine (**3**).

8. Degradation of the Biosynthetic hayatidin (10) derived from (R)-[3',5',8-³H₃N-methylcocclaurine - Labeled hayatidin (**10**) (experiment 7) was diluted with inactive material. A mixture of diluted hayatidin (190 mg) (molar activity 3.65×10^4 disint. min⁻¹ mmol⁻¹) and MeOH (5 ml) was treated with CH₂N₂ to give labeled O-methylhayatidin (**13**) (molar activity 3.63×10^4 disint. min⁻¹ mmol⁻¹). **13** Was subjected to sodium-liquid ammonia reductive fission as described earlier to afford the radio-active (R)-N-methylcocclaurine (**3**) (molar activity 3.25×10^4 disint. min⁻¹ mmol⁻¹) & radioinactive (S)-NOO-trimethylcocclaurine(**6**).

9. **Degradation of the Biosynthetic Isochondrodendrine (14) derived from (R)-[3',5',8-³H₃]N-methylcocclaurine** - Labeled isochondrodendrine (14) (experiment 7) was diluted with radioinactive isochondrodendrine. A mixture of diluted labeled isochondrodendrine (300 mg) (molar activity 2.57×10^5 disint. min⁻¹ mmol⁻¹) and MeOH (10 ml) was treated with ethereal CH₂N₂ to give radioactive O-methylisochondrodendrine (15) (molar activity 2.50×10^5 disint. min⁻¹ mmol⁻¹). Sodium-liquid ammonia reductive fission of 15 afforded labeled (R)-armepavine (4), m.p. 145° (lit.¹⁷ 145-46°) (molar activity 2.38×10^5 disint. min⁻¹ mmol⁻¹).

10. **Degradation of the Biosynthetic cycleanine (15) derived from (R)-[3',5',8-³H₃]N-methylcocclaurine** - Labeled cycleanine (15) (experiment 7) was diluted with inactive material. The diluted labeled cycleanine (292 mg) (molar activity 7.15×10^4 disint. min⁻¹ mmol⁻¹) was subjected to sodium-liquid ammonia reductive cleavage as described earlier to give (R)-armepavine (4) (molar activity 7.10×10^4 disint. min⁻¹ mmol⁻¹).

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