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BIOSYNTHESIS OF TETRAHYDROPALMATINE AND PALMATINE

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Abstract—The incorporation of (±)-norlaudanosoline, norprotosinomenine, nororientaline, norlaudanidine, reticuline and laudanosine into tetrahydropalmatine and palmatine has been studied, and specific utilization of reticuline demonstrated. Feeding of (±)-[N-methyl-^{14}C] reticuline showed that C atom 8 of tetrahydropalmatine and palmatine are formed by oxidative cyclisation of the N-Me group of reticuline. Parallel experiments with (*R*)-, and (*S*)-, reticulines demonstrated specific incorporation of (*R*)-isomer into these bases. Feeding experiments also revealed that the plants can convert tetrahydropalmatine into palmatine with high efficiency.

Tetrahydropalmatine (**18**) and palmatine (**20**) are representatives of protoberberine alkaloids.[1,2] These bases occur in nature either as tetrahydroprotoberberines or quaternary protoberberine salts. Tetrahydroprotoberberines are important intermediates in the biosynthesis of a large number of 1-benzyltetrahydroisoquinoline derived alkaloids.[3] Recent tracer experiments have shown that tetrahydroprotoberberine alkaloids give rise in nature to benzophenanthridine, spirobenzylisoquinoline, protopine, phthalideisoquinoline, rhoeadine and retroprotoberberine alkaloids.

A biogenetic connection between the benzylisoquinoline and berberine group of alkaloids[4] recognised quite early has been firmly confirmed by tracer experiments.[5-8] It has been demonstrated that the C atom 8 of berberine group of alkaloids is derived from the N-Me group of 1-benzyl-isoquinoline precursors.[9-12] Negligible incorporation of reticuline into tetrahydropalmatine in *Papaver somniferum*[13] is recorded.

According to classical theory[4] tetrahydropalmatine[14] (**18**) and palmatine[15] (**20**) can be formed in nature from 1-benzyltetrahydroisoquinoline precursors by condensation with one carbon unit. The current view,[9,10] however, suggests that the C atom 8 of these alkaloids can be derived from N-Me group of 1-benzyltetrahydroisoquinoline precursors. Tetrahydropalmatine (**18**) and palmatine (**10**) can thus be formed from these precursors by alternate biosynthetic pathways as follows:

(+)-Reticuline (**1**) can oxidise to iminium salt **6** which can cyclise to form tetrahydroprotoberberine nucleus of scoularine (**10**) type. Tetrahydropalmatine (**18**) can then form by O-methylation via columbamine (**11**) or schefferine (**12**). Dehydrogenation of **18** can finally yield palmatine (**20**). In the second possibility protosinomenine (**2**) can oxidise to iminium salt **7** which can cyclise to give tetrahydroprotoberberine system of aequaline (**14**) type. Compound **18** can form from **14** by O-methylation via schefferine (**12**) or corypalmine (**13**). In the third possibility orientaline (**3**) can oxidise to iminium salt **19** which can cyclise to form the dienone **9**. Dienone-phenol rearrangement as shown in **9** can then afford tetrahydroprotoberberine nucleus of stepholidine (**15**) type. Compound **18** can then form from **15** by O-methylation via columbamine (**11**) or cycemanine (**17**).

Tetrahydropalmatine (**18**) and palmatine (**19**) can also form in plants from laudanidine (**4**) via **8** and **12** and from laudanosine (**5**). N-Nor bases of these 1-benzyltetrahydroisoquinoline precursors can also serve as precursors of **18** and **20**.

(L)-Tyrosine (experiment 1) was initially fed to young cut branches of *Cocculus laurifolius* (Menispermaceae) and to young plants of *Cissampelos pariera* (Menispermaceae) and it was found that the plants in both cases were biosynthesising tetrahydropalmatine (**18**) and palmatine (**20**). Incorporation of tyrosine into protoberberine alkaloids was, however, slightly higher in *C. laurifolius*. In subsequent experiments labelled hypothetical precursors were, therefore, fed to young cut branches of *C. laurifolius* plants. The results of several feedings are recorded in the Table 1.

Feeding (±)-tyrosine in parallel with (±)-, nororintaline (**25**; experiment 7), norprotosinomenine (**24**; experiment 6), and nor-laudanidine (**26**; experiment 8) revealed that these 1-benzyltetrahydroisoquinoline derivatives are very poorly metabolised by the plants. Feeding with (±)-, norlaudanosoline (**24**; experiment 9) and reticuline (**23**; experiment 3) showed that **24** and **23** are efficient precursors of tetrahydropalmatine (**18**) and palmatine (**20**). The completely methylated 1-benzyltetrahydroisoquinoline, (±)-laudanosine (**27**; experiment 11) was not incorporated.

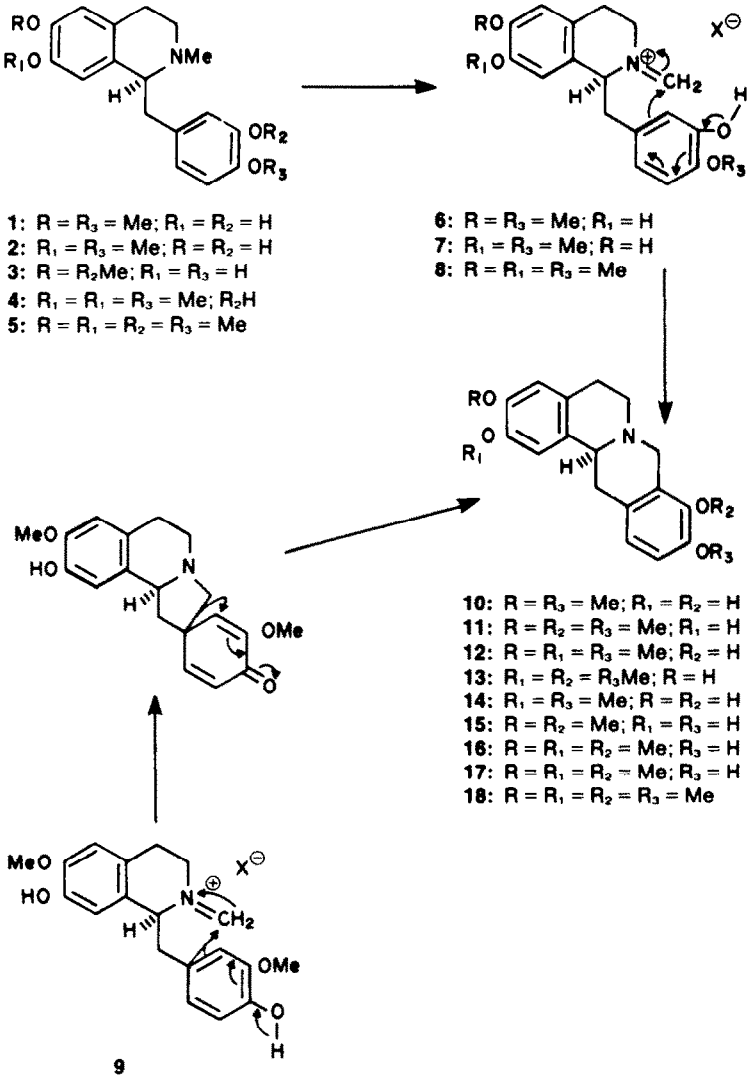
Biosynthetic tetrahydropalmatine (**18**) derived from (±)-[3-^{14}C] reticuline (**23**, experiment 2) was treated with methyl iodide to give tetrahydropalmatine methiodide (**28**) which had essentially the same radioactivity as the parent base. Compound **28** was converted into its methohydroxide (**29**) by IR-410 anion exchange resin. Hofmann degradation of **29** gave the methine **30** with essentially no loss of radioactivity. Ozonolysis of **30** gave radio-active formaldehyde (dimedone derivative, 98% of original activity).

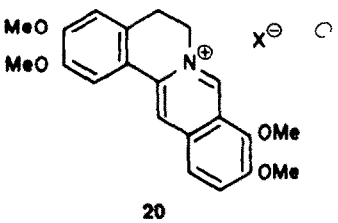
Biosynthetic palmatine (**20**) derived from (±)-[3-^{14}C] reticuline (**23**; experiment 2) was reduced with

Table 1. Tracer experiments on *C. laurifolius*

Expt.	Precursor	Incorporation(%) into alkaloids (18)	(20)
1	(L)-[U-^{14}C] Tyrosine	0.028, 0.013†	0.032, 0.018†
2	[2-^{14}C] Dopamine	0.018‡	0.02‡
		0.18	0.20
3	(±)-[2′,6′,8-$^{3}H_3$] Reticuline (**23**)	0.34	0.48
4	(±)-[2′,6′,8-$^{3}H_3$] Reticuline methiodide (**21**)	0.00376	0.006
5	(±)-[N-methyl-^{14}C] Reticuline (**23**)	0.90	0.82
6	(±)-[Aryl-^{3}H] Norprotosinomenine (**24**)	0.00147	0.0026
7	(±)-[Aryl-^{3}H] Nororientaline (**25**)	0.0026	0.0032
8	(±)-[2′,6′,$^{3}H_2$] Norlaudanidine (**26**)	0.0033	0.0042
9	(±)-[1-^{3}H] Norlaudanosoline (**22**)	0.24	0.32
10	(±)-[1-^{3}H, 4′-methoxy-^{14}C] Reticuline (**23**)	0.80	—
11	(±)-[2′,6′,8-$^{3}H_3$] Laudanosine (**27**)	0.0045	0.0052
12	(+)-[2′,6′,8-$^{3}H_3$] Reticuline (**1**)	1.22	1.26
13	(−)-[2′,6′,8-$^{3}H_3$] Reticuline	0.0126	0.02
14	(±)-[3-^{14}C] Nor-reticuline	0.72	0.70
15	[6-^{14}C] Palmatine (**20**)	0.0006	—
16	(−)-[6-^{14}C] Tetrahydropalmatine (**18**)	—	7.52

†Feeding in *Cissampelos pariera*.
‡Feeding in *Stephania glabra*.





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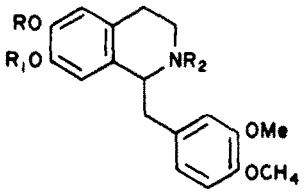
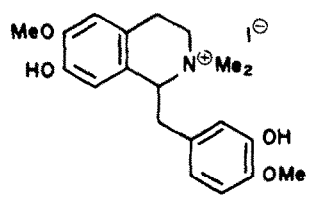


22: R = R_1 = R_2 = R_3 = R_4 = H
23: R = R_2 = R_4 = Me; R_1 = R_3 = H
24: R = R_2 = R_3 = H; R_1 = R_4 = Me
25: R = R_3 = Me; R_1 = R_2 = R_4 = H
26: R_2 = R_3 = H; R = R_1 = R_4 = Me
27: R = R_1 = R_2 = R_3 = R_4 = Me



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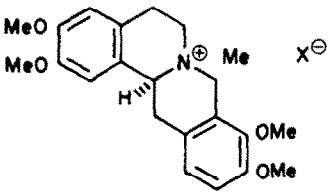
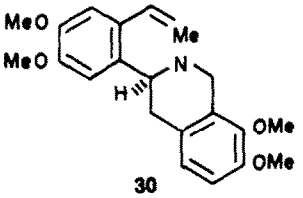


28: X = 1
29: X = OH



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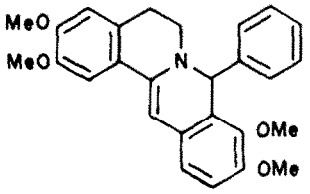

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Sn/HCl to give DL-tetrahydropalmatine which had essentially the same radio-activity as the parent base. It was then subjected to Hofmann degradation as above to give the corresponding methine which on ozonolysis afforded radio-active formaldehyde (dimedone derivative, 97% of the original activity). The results thus established specific incorporation of reticuline (**23**) into tetrahydropalmatine (**18**) and palmatine (**20**) in *C. laurifolius*. Reticuline (**23**) is incorporated intact into **18** was shown by double labelling experiment as follows:

(±)-[1-^{3}H, 4'-methoxy-^{14}C] Reticuline (**23**; experiment 10) was fed to young cut branches of *C. laurifolius* plants and biosynthetic tetrahydropalmatine (**18**) was isolated. The ratios of ^{14}C:^{3}H in the precursor was 1:38 and in the biosynthetic base 1:37.

The C atoms 8 in tetrahydropalmatine (18) and palmatine (20) are formed by oxidative cyclisation of N-Me group of reticuline and shown as follows: (±)-[N-methyl-^{14}C] Reticuline (**23**; experiment 5) was fed to young cut branches of *C. laurifolius* plants and biosynthetic **18** and **20** were isolated. Biosynthetic palmatine (**20**) was treated with phenylmagnesium bromide to give 8-phenyldihydropalmatine (**31**). Chromic acid oxidation of **31** in the usual way (Kuhn–Roth) gave radio-active benzoic acid (102% original activity.

Biosynthetic tetrahydropalmatine (**18**) derived from (±)-[N-methyl-^{14}C] reticuline feeding was dehydrogenated to give radio-active palmatine (**20**) which was degraded as above to give radio active benzoic acid (98% original activity).

The foregoing experiments established that reticuline (**23**) is a specific precursor of tetrahydropalmatine (**18**) and palmatine (**20**) in *C. laurifolius*. The precursors used, however, were racemic It would be expected that in the biotransformation only one of the two optical isomers should act as a direct substrate. Parallel feedings with (+)-reticuline (**1**) and (−)-reticuline demonstrated that stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursors into tetrahydropalmatine (**18**) and palmatine (**20**). (+)-Reticuline (**1**) was incorporated about 70 times more efficiently than the (−)-enantiomer.

Feeding of labelled tetrahydropalmatine (**18**; experiment 15) and palmatine (**20**; experiment 14) showed that **18** was very efficiently incorporated into

20 whereas the incorporation of **20** into **18** was practically negligible.

(+)-Reticuline (**1**) has been isolated from *C. laurifolius*. Its presence in the plant was again confirmed by feeding (−) [U-^{14}C] tyrosine (incorporation 0.26%). (+)-Reticuline (**1**) is, thus, a true precursor of tetrahydropalmatine (**18**) and palmatine (**2**). The foregoing experiments strongly support the following sequence for the biosynthesis of tetrahydropalmatine (**18**) and palmatine (**20**) in *C. laurifolius*.

Tyrosine → norlaudanosoline (**22**) → (+)-reticuline (**1**) → tetrahydropalmatine (**18**) → palmatine (**20**).

EXPERIMENTAL

For general directions (spectroscopy details, counting method, synthesis and labelling of precursors) see earlier paper in the series.[16,17]

Feeding experiments. Labelled reticuline and norprotosinomenine were fed as their hydrochlorides nororientaline, nor-reticuline, norlaudanidine and tetrahydropalmatine were fed as their tartrates by stem cut method to young branches of *Cocculus laurifolius* DC and by cotton wick method to *Cissampelos pariera* and *Stephania glabra* plants. Palmatine and nor-laudanosoline in H_2O (1 ml H_2O containing 0.2 ml of DMSO) were fed to young *Stephania glabra* plants.

Isolation of tetrahydropalmatine. Young branches with leaves (typically 160 g wet wt.) of *C. laurifolius* were macerated in EtOH (300 ml) with radio-inactive tetrahydropalmatine (100 mg) and left overnight. The alcoholic extract was decanted and the plant material was extracted with alcohol (5 × 250 ml). The combined ethanolic extract was concentrated *in vacuo* to afford a greenish viscous mass which was extracted with 2% HCl (4 × 25 ml). The aqueous acidic soln was defatted with hexane (4 × 15 ml), basified (pH 8–9) with Na_2CO_3-aq and the liberated bases were extracted with $CHCl_3$ (6 × 30 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4) and concentrated to give a crude alkaloidal mixture which was subjected to preparative tlc (*plates*: SiO_2; *solvent*: $CHCl_3$:MeOH 97:3) to give *tetrahydropalmatine* (74 mg) m.p. 141° (lit.[14] 142°). In each case isolated tetrahydropalmatine was crystallised from MeOH to constant activity. The radio chemical purity of the sample was established by dilution technique.

Isolation of palmatine. Young branches with leaves of *C. laurifolius* DC. (typically 180 g wet wt.) were macerated in EtOH (300 ml) with radio-inactive palmatine (130 mg) and worked up as above to give aqueous acidic extract. The acidic extract was basified with Na_2CO_3 (pH 10) and extracted with $CHCl_3$:MeOH (90:10) and n-BuOH (6 × 30 ml) to give a mixture of bases from which palmatine chloride (85 mg) m.p. 203–205° (lit.[15] 205°) was isolated by preparative tlc (*plate*: SiO_2; *solvent*: $CHCl_3$:MeOH 80:20). The radioactive base (10 mg) was diluted with inactive material (90 mg) and the mixture was recrystallised from EtOH-H_2O until constant activity (80 mg).

Feeding of (±)-[1-^{3}H, 4′-*methoxy*-^{14}C] *reticuline.* Young cut branches of *C. laurifolius* plants were fed with (±)-[1-^{3}H; 4′-methoxy-^{14}C] reticuline (activity: ^{3}H, 0.133 mCi; ^{14}C, 0.0035 mCi; ^{3}H: ^{14}C 38:1). The plants were kept alive for 8 days and harvested. Tetrahydropalmatine (100 mg) was added and reisolated in the usual way. The biosynthetic base was crystallised from MeOH to constant activity and counted for ^{3}H and ^{14}C activities. The ratio of ^{3}H and ^{14}C in the biosynthetic base was found to be 37:1.

Degradation of (−)-6-^{14}C-*tetrahydropalmatine.* Biosynthetic tetrahydropalmatine (290 mg) (molar activity 1.22 × 10 disint min^{-1} mmol^{-1}) in MeOH (10 ml) was refluxed with MeI (3 ml) to give radioactive **28** (292 mg) m.p. 248–250° (lit.[18] 248–251°) (molar activity 1.187 × 10^5 disint min.$^{-1}$ mmol^{-1}).

A soln of the preceding radioactive methiodide (280 mg) in MeOH (100 ml) was passed through a column of freshly generated amberlite IR-400 anion exchange resin (OH form) (8.0 g) and the soln recycled five times. The resin was finally eluted with MeOH (150 ml). The solvent from the combined eluate was removed to afford radio-active **29**. Labelled **29** in MeOH (10 ml) was refluxed with KOH (4.4 g) in H_2O (5 ml) for 5 hr. The solvent was removed, H_2O (20 ml) added and the product was extracted with $CHCl_3$ (5 × 20 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4) and the solvent removed *in vacuo* to give radioactive **30** (167 mg) m.p. 116–117° (ether–pet ether) (lit.[19] 115–116°) (molar activity 1.02 × 10^5 disint. min.$^{-1}$ m. mol^{-1}).

Ozonized O_2 was passed through a soln of the radioactive **30** (128 mg) in EtOAc (8 ml) at −78° for 10 min. The solvent from the mixture was removed under reduced pressure and to the residue H_2O (35 ml), Zn dust (320 mg) and $AgNO_3$ (15 mg) were added. The mixture was refluxed for 20 min and the formaldehyde, thus formed, was distilled. The distillate was collected in a soln of dimedone (300 mg) in aqueous EtOH (80 ml). It was then worked up in the usual manner to give formaldehyde dimedone derivative, m.p. 193–194°, as needles from EtOH (molar activity 1.12 × 10^5 disint min^{-1} m. mol^{-1}; 98% original).

Degradation of [6-^{14}C] *palmatine.* Labelled **20** (415 mg) (molar activity 8.45 × 10^4 disint min^{-1} m. mol^{-1}) in MeOH (280 ml) was reduced with $NaBH_4$ to give (±) tetrahydropalmatine (300 mg) m.p. 145–146° (lit.[19] 148–149°) (molar activity 8.34 × 10^4 disint min^{-1} m. mol^{-1}). The radio-active base in benzene was treated with MeI to give the methiodide (302 mg; molar activity 8.35 × 10^4 disint. min.$^{-1}$ m. mol^{-1}) which was subjected to Hofman degradation as above to give the methine (128 mg) m.p. 114–115° (lit.[1] 115–116°) (molar activity 8.30 × 10^4 disint min^{-1} m. mol^{-1}). Ozonolysis of the radio-active methine gave formaldehyde dimedone derivative (molar activity 8.29 × 10^4 disint. min^{-1} m. mol^{-1}) (97% original).

Degradation of [8-^{14}C] *palmatine.* Anhydrous labelled palmatine chloride (160 mg; molar activity 3.51 × 10^4 disint min^{-1} m. mol^{-1}) was suspended in dry ether (10 ml). Excess of an ethereal soln of PhMgBr (prepared from Mg turning (0.12 g) and bromobenzene 0.4 ml) was slowly added to it. The mixture was stirred and heated under reflux (N_2 atmos) for 7 hr and then at room temp for 24 hr. It was then worked up in the usual manner to give **31** (92 mg) m.p. 157–159° (lit. 158–160°) (molar activity 3.24 × 10^4 disint min^{-1} m. mol^{-1}). Radio-active **31** (84 mg) was treated with CrO_3 (4 g) in 10% H_2SO_4 (12 ml) in the usual way to give radio-active benzoic acid (molar activity 3.58 × 10^4 disint min^{-1} m. mol^{-1}) (102% original).

Degradation of [8-^{14}C] *tetrahydropalmatine.* Labelled tetrahydropalmatine (310 mg; molar activity 8.34 × 10 disint min^{-1} m. mol^{-1}) in EtOH (4 ml) was refluxed with MeI (300 mg) to give palmatine (180 mg) m.p. 238–40° activity 8.20 × 10^4 disint min^{-1} m. mol^{-1}). Radio-active palmatine (120 mg) was treated with PhMgBr to give **31** (65 mg; molar activity 8.28 × 10^4 disint min^{-1} m. mol^{-1}). Kun–Rot oxidation of **31** (65 mg) (molar activity 8.28 × 10^4 disint min^{-1} m.mol^{-1}) gave radio-active benzoic acid (molar activity 8.01 × 10^4 disint min^{-1} m. mol^{-1}) (96% original

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