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BIOSYNTHESIS OF (+)-, (−)- AND (±)-TETRAHYDROPALMATINES

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Abstract—Specific incorporation of didehydroreticuline and reticuline into (±)-, (+)-, and (−)-tetrahydropalmatines in *Cocculus laurifolius* and of (*R*)- and (*S*)-reticulines into (*R*)- and (*S*)-tetrahydropalmatines respectively has been demonstrated. Feeding of [1-^{3}H, 4′-methoxy-^{14}C] reticuline suggested that reticuline was not converted in the plants into didehydroreticuline and racemisation of optically active forms of tetrahydropalmatine did not take place *via* dehydrotetrahydropalmatine.

Reticuline, the 1-benzyltetrahydroisoquinoline derivative, has been shown to be a biological precursor of a large variety of alkaloids.[1] Further it has been demonstrated in many cases that stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursor into these alkaloids and only one of the enantiomers which has the same configuration at the asymmetric centre as is present in the derived alkaloid, is specifically incorporated. Using reticuline labelled with ^{3}H at position 1 and ^{14}C at one of the methoxy groups or in position 3, it has been shown that hydrogen atom at position 1 of the precursor remains untouched during biotransformation.[2] Exceptions to this are, biosynthesis of morphine[3] and morphinandienone alkaloid, sebiferine[4] where both the enantiomers of reticuline are incorporated into these alkaloids and thus stereospecificity is not maintained in the biotransformation. It has been suggested[3,4] that the enantiomer which has opposite configuration at the asymmetric centre is incorporated into the derived alkaloid *via* the iminium intermediate (**8**). Protoberberine alkaloids such as berberine[5] and palmatine[2] although do not have any asymmetric centre but are derived stereospecifically from *S*-reticuline.

Tetrahydropalmatine[6] is a representative of tetrahydroprotoberberine alkaloids[6,7] which are important intermediates in the biosynthesis of a large number of 1-benzyltetrahydroisoquinoline derived alkaloids.[1] The occurrence of the base in both the enantiomeric[8,9] and racemic[10] forms is of biosynthetic interest.

It has been demonstrated earlier that (−)-(*S*)-tetrahydropalmatine (**4**) in *Cocculus laurifolius*[2] is stereospecifically biosynthesized from (+)-(*S*)-reticuline (**1**). Further it has been shown that C-8 in (−)-tetrahydropalmatine (**4**) is formed by oxidative cyclisation of N-Me group of reticuline. (±)-(**6**), (+)-(**5**) And (−)-(**4**)-tetrahydropalmatines could be formed in the plants by the following alternate processes. (*S*)- And (*R*)-reticulines independently and specifically could form (*S*)- and (*R*)-tetrahydropalmatines respectively probably at different biosynthetic sites. The racemic tetrahydropalmatine (**6**) could be formed by mixing of the enantiomers at the time of isolation of the bases. Alternately reticuline could be oxidised to didehydroreticuline (**7**) which could be isomerized to the iminium salt (**8**) and then recyclised to form racemic tetrahydropalmatine (**6**). In the third possibility racemisation of the enantiomers could take place by oxidation-reduction processes *via* the intermediacy of dehydrotetrahydropalmatine (**9**).

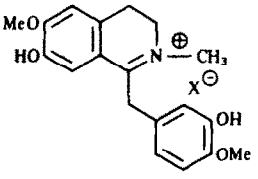
L-Tyrosine (experiment 1) was initially fed to young cut branches of *Cocculus laurifolius* D.C. (Menispermaceae) and it was found that all the three forms of tetrahydropalmatines are being formed. Feeding of tyrosine in parallel with (±)-reticuline (experiment 2) and didehydro-N-[$^{14}CH_3$] reticuline (experiment 3) showed that **3** and **7** are efficient precursors of **4**, **5** and **6** (Table 1). Regioselectivity of label in biosynthetic **4**, **5** and **6** derived from **7** was shown as follows: Radioactive **6** was dehydrogenated to give radioactive palmatine (**10**) which was treated with phenylmagnesium bromide to give radioactive 8-phenyldihydropalmatine (**11**). Chromic acid oxidation of **11** in the usual way (Kuhn-Roth) gave radioactive benzoic acid (94% original activity). Biosynthetic **5** was degraded as above. Radioactive benzoic acid, thus obtained, had 93% activity of the parent base. Degradation of biosynthetic **4** as above afforded radioactive benzoic acid (101% original activity). The foregoing experiments thus demonstrated that the enzyme system present in *C. laurifolius* is capable of converting didehydroreticuline (**7**) into **4**, **5** and **6** tetrahydropalmatines.

Table 1. Tracer experiments on *C. laurifolius*

Expt. No.	Precursor	% Incorporation into (±)-6	(+)-5	(−)-4
1	[U-^{14}C]-L-Tyrosine	0.016	0.015	0.012
2	(±)-[2′,6′,8-$^3H_3$] Reticuline (3)	0.24	0.12	0.18
3	Didehydro-N-[$^{14}CH_3$] reticuline (7)	0.30	0.17	0.22
4	(*S*)-(+)-[2′,6′,8-$^3H_3$] Reticuline (1)	0.12	0.003	0.12
5	(*R*)-(−)-[2′,6′,8-$^3H_3$] Reticuline (2)	0.10	0.10	0.003
6	(±)-[1-^{3}H,4′-Methoxy-^{14}C] reticuline (3)	0.14 (^{3}H)	0.12 (^{3}H)	0.11 (^{3}H)

1: R = H (S) Reticuline
2: R = H (R) Reticuline
3: R = H

4: R = H
5: R = H
6: R = H



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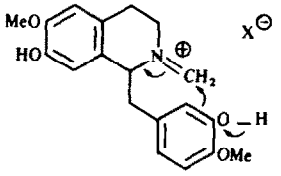

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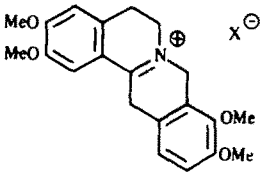

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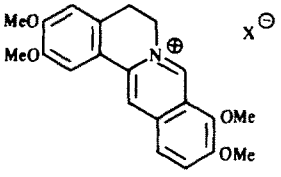

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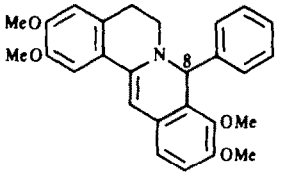

11

Parallel feedings of (*S*)-(+)-*reticuline* (**1**) (experiment 4) and (*R*)-(−)-reticuline (**2**) (experiment 5) to young cut branches of *C. laurifolius* demonstrated that **1** was incorporated into **4** about 40 times more efficiently than into **5** while **2** was incorporated into **5** about 33 times more efficiently than into **4**. The results thus demonstrated specificity of enzyme reactions in the biosynthesis of (*R*)- and (*S*)-tetrahydropalmatines.

That reticuline (**3**) is not oxidised to didehydroreticuline (**7**) in the plants and racemisation of enantiomers of tetrahydropalmatines does not take place via the dehydrotetrahydropalmatine (**9**) was demonstrated as follows: [1-^{3}H, 4′-methoxy-^{14}C] reticuline (experiment 6) was fed to young cut branches of *C. laurifolius* and biosynthetic tetrahydropalmatines **4**, **5** and **6** were isolated. The ratios of ^{14}C: ^{3}H in the precursor was 1:38 and in the biosynthetic bases **4**, **5** and **6** 1:37, 1:40 and 1:36 respectively.

EXPERIMENTAL

For general directions (spectroscopy details, counting method, synthesis and labelling of precursors) see earlier paper in the series.[11,12]

Resolution

(+)-*Tetrahydropalmatine* (**5**). (±)-Tetrahydropalmatine (**6**) (2.5 g) in MeOH (30 ml) was treated with di-*p*-toluoyl-*d*-tartaric acid (2.0 g) in MeOH (30 ml). The resulting salt was fractionally crystallised from C_6H_6 (10 times) and then from MeOH (10 times) to give the crystalline salt (1.0 g), m.p. 148°; $[\alpha]_D$ +68.2° (c, 2.0 in EtOH). The salt in H_2O (5 ml) was treated with 4N NaOH (20 ml). The liberated base was extracted with $CHCl_3$ (4 × 25 ml), washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed *in vacuo* to afford (+)-tetrahydropalmatine (**5**) (400 mg), m.p. 140–41°, $[\alpha]_D$ +290° (c, 1.6 in EtOH) (lit[8] m.p. 142°, $[\alpha]_D$ +292.5° in EtOH).

(−)-*Tetrahydropalmatine* (**4**). Partially resolved tetrahydropalmatine (1.0 g) in MeOH, enriched with (−)-enantiomer was treated with di-p-toluoyl-*l*-tartaric acid (600 mg) in MeOH (15 ml). The solvent from the resulting

salt was removed and the residue fractionally crystallized first with C_6H_6 and then with MeOH as above to give the crystalline salt, m.p. 154°, $[\alpha]_D$ −77.8° (c, 1.2 in EtOH). The salt was treated with 4N NaOH (10 ml) and the liberated base extracted with $CHCl_3$ and crystallised from MeOH to yield (−)-tetrahydropalmatine (**4**), m.p. 140°, $[\alpha]_D$ −289.2° (c, 0.8 in EtOH) (lit[9] m.p. 141–42°, $[\alpha]_D$ −290.8° in EtOH).

Feeding experiments. Labelled reticulines were fed as their tartrates and didehydroreticuline (**7**) in H_2O (1 ml) containing DMSO (0.2 ml) was fed to young cut branches of *C. laurifolius* DC.

Isolation of tetrahydropalmatines. Young branches with leaves (typically 220 g wet wt) of *C. laurifolius* were macerated in EtOH (300 ml) with radioinactive 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 from which tetrahydropalmatine was isolated by the procedure described earlier.[2] In each case isolated tetrahydropalmatine was crystallised from MeOH to constant activity. The radiochemical purity of the sample was established by dilution technique.

Feeding of (±)-[1-^{3}H, 4′-methoxy-^{14}C] reticuline. Young cut branches of *C. laurifolius* 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. (±)-, (+)- And (−)-tetrahydropalmatines (80 mg) was added in separate experiments and reisolated in each case in the usual way. The biosynthetic bases were crystallised from MeOH to constant activity and counted for ^{3}H and ^{14}C activities. The ratios of ^{3}H and ^{14}C in biosynthetic (−)-, (+)- and (±)-tetrahydropalmatines were found to be 37:1, 39:1 and 36:1 respectively.

Degradation of (±)-[8-^{14}C] tetrahydropalmatine (**6**). Labelled tetrahydropalmatine (300 mg; molar activity 2.98×10^5 disint min^{-1} mmol^{-1}) in EtOH (4 ml) was refluxed with I_2 (220 mg) to give radioactive palmatine (178 mg), m.p. 238–40° (lit[13] m.p. 241°) (molar activity 2.97×10^5 disint min^{-1} mmol^{-1}). Radioactive palmatine (125 mg) was treated with PhMgBr to give **11** (63 mg; molar activity 2.80×10^5 disint min^{-1} mmol^{-1}), m.p. 158–59° (lit[14] 158–60°). Kuhn–Roth oxidation of **11** (60 mg) gave radioactive benzoic acid (molar activity 2.80×10^5 disint min^{-1} mmol^{-1}; 94% of original).

Degradation of (+)-tetrahydropalmatine derived from didehydro-N-[^{14}CH$_3$] reticuline. Labelled (+)-**5** (70 mg) in EtOH (2.5 ml) was refluxed with I_2 to afford palmatine (**10**) (34 mg) which was then diluted with inactive palmatine (160 mg) and crystallized from MeOH to constant activity (molar activity 2.72×10^4 disint min^{-1} mmol^{-1}). Radioactive **10** was then degraded to labelled benzoic acid as above.

Compound	Molar activity (disint min^{-1} mmol^{-1})
(+)-Tetrahydropalmatine (**5**)	1.36×10^5
Palmatine (**10**)	2.72×10^4
8-Phenyldihydropalmatine (**11**)	2.68×10^4
Benzoic acid	2.53×10^4 (93% of original)

Degradation of (−)-[8-^{14}C] tetrahydropalmatine derived from didehydro-N-[^{14}CH$_3$] reticuline. Labelled (−)-tetrahydropalmatine (**4**) (72.4 mg) was diluted with inactive (−)-**4** (130.2 mg) and crystallized to constant activity (molar activity 3.20×10^5 disint min^{-1} mmol^{-1}). The preceding radioactive **4** was degraded as described earlier to furnish labelled benzoic acid.

Compound	Molar activity (disint min^{-1} mmol^{-1})
(−)Tetrahydropalmatine (**4**)	3.23×10^5
Palmatine (**10**)	3.20×10^5
8-Phenyldihydropalmatine (**11**)	3.18×10^5
Benzoic acid	3.26×10^5 (101% of original)

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