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THE BIOSYNTHESIS OF THE ALKALOIDS OF *STEPHANIA GLABRA* (ROXB.) MIERS

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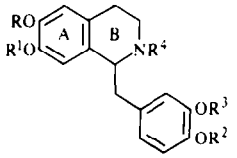
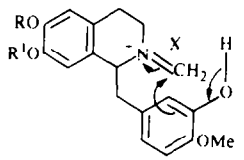
Abstract—Tracer experiments show that the tetrahydroprotoberberines stepholidine (**14**), corydalmine (**15**), capaurine (**19**) and corynoxidine (**20**) are stereospecifically biosynthesized from (*S*)-reticuline (**22**) whereas the bisbenzylisoquinoline alkaloids cycleanine (**52**), N-desmethylcycleanine (**51**) and the proaporphine alkaloids, pronuciferine (**27**) and stepharine (**26**) are derived from (*R*)-N-methylcoclaurine in young *Stephania glabra*. The quaternary protoberberine alkaloids (**40** to **44**) of the plant are formed by dehydrogenation of the corresponding tetrahydroprotoberberines.

Stephania glabra (Roxb.) Miers (Menispermaceae), a glabrous climber indigenous to the lower Himalayas of India, has long been used by the natives in the treatment of various ailments.[1] The plant has been extensively investigated for its alkaloidal constituents.[2] The alkaloids isolated from the rhizome, leaves and stems of the plant are tetrahydroprotoberberines, stepholidine (**14**), corydalmine (**15**), tetrahydropalmatine (**18**), capaurine (**19**) and corynoxidine (**20**); the quaternary protoberberines palmatine (**40**), dehydrocorydalmine (**41**), jatrorrhizine (**42**) and stepharanine (**43**); the bisbenzylisoquinolines, cycleanine (**52**) and N-desmethylcycleanine (**51**); and the spasmolytic proaporphines, pronuciferine (**27**) and stepharine (**26**).

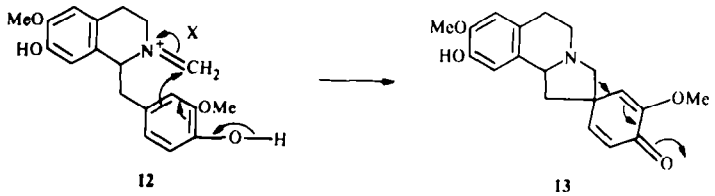
According to biogenetic theory[3] the tetrahydroprotoberberine and quaternary protoberberine bases of *S. glabra* could be derived in nature from norlaudanosoline (**1**) derivatives. Reticuline (**2**) could be



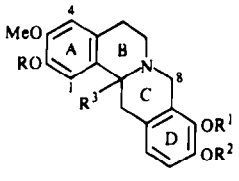
1: R = R¹ = R² = R³ = R⁴ = H
2: R¹ = R³ = H, R = R² = R⁴ = Me
3: R¹ = R² = H, R = R³ = R⁴ = Me
4: R = R³ = H, R¹ = R² = R⁴ = Me
5: R³ = H, R = R¹ = R² = R⁴ = Me
6: R¹ = R³ = R⁴ = H; R = R² = Me
7: R¹ = R² = R⁴ = H, R = R³ = Me
8: R³ = R⁴ = H, R = R¹ = R² = Me



9: R = Me, R¹ = H
10: R¹ = Me, R = H
11: R = R¹ = Me



12 **13**



14: R = R² = H; R¹ = Me; R³ = α H
15: R² = H; R = R¹ = Me; R³ = α H
16: R = R¹ = H; R² = Me, R³ = H
17: R¹ = H; R = R² = Me; R³ = H
18: R = R¹ = R² = Me, R³ = α H

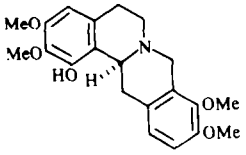
oxidised to give the iminium salt (**9**) which would then cyclise to yield scoulerine (**16**), the key intermediate. The bases **14**, **15** and **18** could then be formed from **16** by unexceptional steps. The tetrahydroprotoberberines (**14**–**18**) and the quaternary protoberberines (**40**–**44**), however, could also be formed in nature from orientaline (**3**), protosinomenine (**4**) and laudanidine (**5**) *via* the iminium intermediates **12**, **10** and **11** respectively. The dienone intermediate (**13**) derived from **12** could undergo dienone–phenol rearrangement as shown in **13** to give corydalmine (**15**). The tetrahydroprotoberberine alkaloid capaurine (**19**) could be formed in the plants from tetrahydropalmatine (**18**) by nuclear hydroxylation at position 1 in ring A and corynoxidine by N-oxidation of **18**.

The quaternary protoberberine alkaloids (**40**–**44**) of *S. glabra* could be formed in nature by dehydrogenation of the corresponding tetrahydroprotoberberines.

The proaporphine alkaloids pronuciferine (**27**) and stepharine (**26**) of *S. glabra* could be formed in the plant from coclaurine (**28**) *via* the dienone (**25**) intermediate.[4] The bisbenzylisoquinoline alkaloids cycleanine (**52**) and N-desmethylcycleanine (**51**) could be derived in nature from coclaurine derivatives.[4] Oxidative dimerization of (*R*)-coclaurine (**23**, R = H) could give the dimeric base (**49**). O-Methylation of the phenolic groups in **49** could give norcycleanine (**50**). Finally N-methylation could yield cycleanine (**52**). Selective N-methylation of the secondary amine functions in **50** could give N-desmethylcycleanine (**51**). Cycleanine (**52**) could also be formed by direct oxidative dimerization of (*R*)-N-methylcoclaurine (**23**, R = Me) *via* (**53**).

(*L*)-[U-^{14}C] Tyrosine (expt 1) was initially fed to young plants of *S. glabra* (Roxb.) Miers (Menispermaceae) and young cut branches of *Cocculus laurifolius* DC. (Menispermaceae) and it was found that both the plants were biosynthesising the proaporphine alkaloids stepharine (**26**) and pronuciferine (**27**) and the bisbenzylisoquinoline alkaloids cycleanine (**52**) and N-desmethylcycleanine (**51**). In subsequent experiments the labelled hypothetical precursors were fed to the plants. The results of several feedings are recorded in Table 1.

Feeding of tyrosine in parallel with labelled coclaurine



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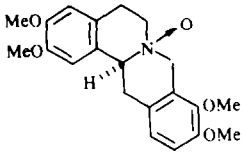

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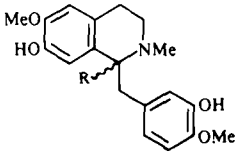
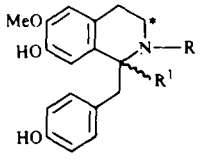


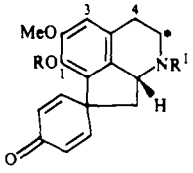
21: R = H (R)

22: R = H (S)



23: R^1 = H (R)

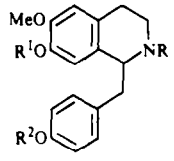
24: R^1 = H (S)



25: $R = R^1 = H$

26: R = Me, R^1 = H

27: $R = R^1$ = Me



28: $R = R^1 = R^2 = H$

29: R = Me; $R^1 = R^2$ = H

30: $R = R^1 = R^2$ = Me

31: $R = R^1$ = Me; R^2 = H

Table 1. Tracer experiments on *S. glabra*

Sl. No.	Precursor fed	% Incorporation into (27)	(26)	(52)	(51)
1.	(L)-[U-^{14}C]Tyrosine	0.04	0.06	0.05	0.04
		0.02†	0.03†	0.02†	0.01†
2.	(±)-[3′,5′,8-$^3H_3$]Coclaurine (**28**)	0.42	0.23	0.32	0.22
3.	(±)-[3′,5′,8-$^3H_3$]N-Methylcoclaurine (**29**)	0.68	0.02	0.74	0.60
4.	(±)-[3′,5′,8-$^3H_3$]N,O,O-Trimethylcoclaurine(**30**)	0.003	0.001	0.002	0.002
5.	(±)-[3-^{14}C]Coclaurine(**28**)	0.50	0.48	0.52	0.38
6.	(S)-[3′,5′,8-$^3H_3$]N-Methylcoclaurine(24, R = Ma)	0.017	0.01	0.014	0.012
7.	(R)-[3′,5′,8-$^3H_3$]N-Methylcoclaurine(**23**, R = Me)	0.70	0.02	0.62	0.58

†Feeding in *C. laurifolius*. Pronuciferine(**27**), Stepharine(**26**), Cycleanine(**52**), N-desmethylcycleanine(**51**).

(**28**) (expt 2) and N-methylcoclaurine (**29**) (expt 3) revealed that both were efficient precursors of the alkaloids **26**, **27**, **51** and **52**. As expected, labelled N,O,O-trimethylcoclaurine (**30**) (expt 4) was not incorporated into these alkaloids, thus supporting the phenol oxidative coupling theory[4] of proaporphine and bisbenzylisoquinoline alkaloids.

The regiospecificity of ^{14}C label in biosynthetic stepharine (**26**) derived from the feeding of (±)-[3-^{14}C] coclaurine (**28**; expt 5) was established as follows: Biosynthetic **26** was treated with $HCHO/HCO_2H$ to give radioactive pronucifierine (**27**). Borohydride reduction of radioactive **27** furnished the radioactive dienol (**32**) which on treatment with 3N HCl afforded labelled nuciferine (**33**) with essentially no loss of radioactivity. Labelled **33** was treated with methyl iodide to furnish nuciferine methiodide (**35**) with no loss of radioactivity. **35** Was converted into the corresponding methohydroxide (**36**) by IR-410 anion exchange resin. Hofmann degradation of **36** yielded the radioactive methine-I (**34**) with practically no loss of radioactivity. **34** Was treated with methyl iodide to give the corresponding methiodide (**37**) which was converted into its methohydroxide (**38**) and then subjected to a second Hofmann degradation to yield the radioactive methine-II (**39**) with essentially no loss of radioactivity. Ozonolysis of **39** furnished radioactive formaldehyde (dimedone derivative, 96% of original activity).

Biosynthetic pronuciferine (**27**) derived from [3-^{14}C] coclaurine (**28**; expt 5) was degraded as above to give the radioactive formaldehyde (dimedone derivative, 97% of original activity).

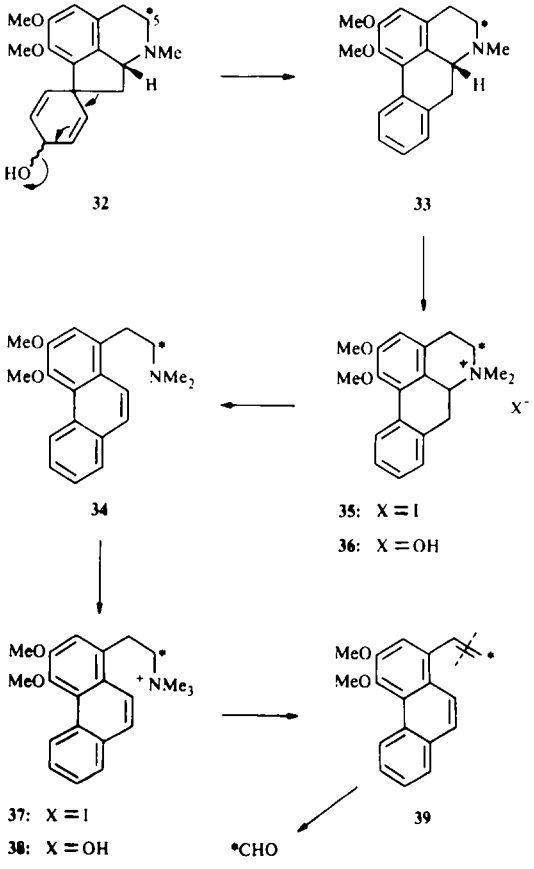
Na/NH_3 Reductive cleavage of the biosynthetic cycleanine (**52**) derived from the feeding of (±)-[3′, 5′, 8-$^3H_3$]N-methylcoclaurine (**29**) (expt 3) gave radioactive (*R*)-(-)-armepavine (**31**) having essentially the same molar activity as the parent base (95% of original activity), establishing thus that both the halves of **52** were derived from N-methylcoclaurine.

The foregoing experiments established that coclaurine (**28**) and N-methylcoclaurine (**29**) are specific precursors in *S. glabra* of proaporphine and bisbenzylisoquinoline alkalôids. The precursors used were, however, racemic. The enzyme system involved in the relevant biotransformation would be expected to be stereospecific. Parallel feedings with labelled (*S*)- and (*R*)-N-methylcoclaurines (expt 6 and 7 respectively) showed that the stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursor into proaporphine and bisbenzylisoquinoline alkaloids. (*R*)-N-Methylcoclaurine (**23**, R = Me) was incorporated more efficiently than the (*S*)- isomer (**24**, R = Me). The presence of N-methylcoclaurine in the plant was confirmed by trapping experiment with (*L*)-[U-^{14}C] tyrosine (incorporation 0.16%).

The foregoing tracer experiments strongly supported the following sequence for the biosynthesis of proaporphine and bisbenzylisoquinoline alkaloids in *S. glabra*.

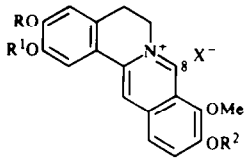
Tyrosine → (*R*)-coclaurine (**23**, R = H) → stepharine (**26**) → pronuciferine (**27**). (*R*)-Coclaurine (**23**, R = H) — (dimerisation) → cycleanine (**52**).

Tracer experiments that defined the biosynthetic pathways of tetrahydroprotoberberine and quaternary protoberberine alkaloids in *S. glabra* are as follows.



Initial feeding of (*L*)-[U-^{14}C]tyrosine (expt 1) demonstrated that the plants were biosynthesising the tetrahydroprotoberberine alkaloids stepholidine (**14**), corydalmine (**15**), scoulerine (**16**) and tetrahydropalmatine (**18**) and the quaternary protoberberine alkaloids palmatine (**40**), dehydrocorydalmine (**41**), jatrorrhizine (**42**) and stepharanine (**43**). In subsequent experiments the labelled hypothetical precursors were fed to the plants. The results of several feedings are recorded in Table 2.

Feeding of tyrosine in parallel with norlaudanosoline (**1**) (expt 2), norreticuline (**6**) (expt 3), reticuline (**2**) (expt 4), nororientaline (**7**) (expt 6), protosinomenine (**4**) (expt 7) and norlaudanidine (**8**; expt 8) and (±)-coclaurine (**28**) and (±)-N-methylcoclaurine (**29**) (experiment 9 and 10 respectively) demonstrated that **1**, **2**, and **6** were efficient precursors of stepholidine (**14**) and corydalmine (**15**) whereas **4**, **7**, **8**, **28** and **29** were not metabolised by the plants to form **14** and **15**.



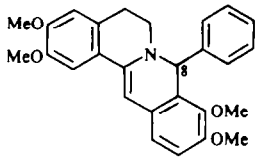
40: R = R^2 = Me, X = NO_3

41: R = R^1 = Me; R^2 = H; X = Cl

42: R^1 = R^2 = Me, R = H, X = Cl

43: R = Me; R^1 = R^2 = H; X = Cl

44: R = R^1 = $-CH_2-$, R^2 = Me; X = Cl



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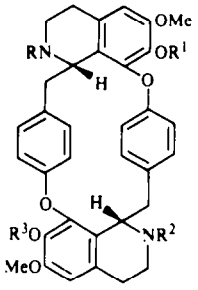


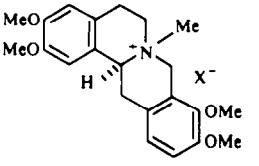
49: R = R^1 = R^2 = R^3 = H

50: R^1 = R^3 = Me; R = R^2 = H

51: R = H, R^1 = R^2 = R^3 = Me

52: R = R^1 = R^2 = R^3 = Me

53: R^1 = R^3 = H, R = R^2 = Me



46: X = I

47: X = OH

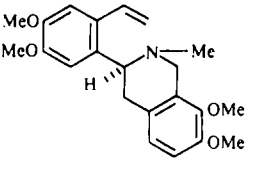


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Table 2. Tracer experiments on *S. glabra*

Sl. No.	Precursor fed	% Incorporation into (**14**)	% Incorporation into (**15**)
1.	(L)-[U-^{14}C]Tyrosine	0.06	0.04
		0.03†	0.02†
2.	(±)-[1-^{3}H]Norlaudanosoline(**1**)	0.22	0.18
		0.08†	0.06†
3.	(±)-[3-^{14}C]Norreticuline(**6**)	0.43	0.50
		0.12†	0.28†
4.	(±)-[2′,6′,8-$^{3}H_3$]Reticuline(**2**)	0.54	0.40
5.	(±)-[N-$^{14}CH_3$]Reticuline(**2**)	0.67	0.57
6.	(±)-[5′,8-$^{3}H_2$]Nororientaline(**7**)	0.001	0.002
7.	(±)-[Aryl-^{3}H]Protosinomenine(**4**)	0.003	0.003
8.	(±)-[2′,6′-$^{3}H_2$]Norlaudanidine(**8**)	0.005	0.002
9.	(±)-[3′,5′,8-$^{3}H_3$]Coclaurine(**28**)	0.001	0.003
10.	(±)-[3′,5′,8-$^{3}H_3$]N-Methylcoclaurine(**29**)	0.002	0.001
11.	(±)-[1-^{3}H,3-^{14}C]Norreticuline(**6**)	0.92	0.62
		0.40†	0.28†
12.	(S)-[2′,6′,8-$^{3}H_3$]Reticuline(**22**)	0.88	0.92
13.	(R)-[2′,6′,8-$^{3}H_3$]Reticuline(**21**)	0.022	0.03
14.	(±)-[1-^{3}H,4′-O-$^{14}CH_3$]Norreticuline(**6**)	0.72	0.52
15.	(±)-[Aryl-^{3}H]Scoulerine(**16**)	1.30	1.10
16.	(S)-[Aryl-^{3}H]Stepholidine(**14**)	—	0.03
17.	(±)-[Aryl-^{3}H]Tetrahydropalmatrubine(**17**)	—	1.50

†Feeding in *C. laurifolius*. Stepholidine (**14**), Corydalmine (**15**).

The regiospecificity of labelling in the biosynthetic stepholidine (**14**) derived from [3-^{14}C] norreticuline (**6**; expt 3) was shown as follows: Labelled **14** was treated with diazomethane to afford radioactive tetrahydropalmatine (**18**) which was refluxed with methyl iodide to give radioactive tetrahydropalmatine methiodide (**46**) with essentially no loss of radioactivity. **46** Was converted into the corresponding methohydroxide (**47**) by IR-410 anion exchange resin. Hofmann degradation of radioactive **47** afforded the radioactive methine (**48**) which had essentially the same radioactivity as the parent base. Ozonolysis of the radioactive **48** yielded radioactive formaldehyde trapped as its dimedone derivative (95% of original activity).

The position of label in biosynthetic corydalmine (**15**) derived from feeding of [3-^{14}C] norreticuline (**6**; expt 3) was established as follows: Labelled **15** was treated with diazomethane to furnish radioactive tetrahydropalmatine (**18**) which was degraded as above to the radioactive formaldehyde (dimedone derivative, 98% of original activity).

Carbon atoms **8** both in stepholidine (**14**) and corydalmine (**15**) were derived from N–Me group of reticuline was shown as follows: N-[$^{14}CH_3$] Reticuline (**2**; expt 5) was fed to young cut branches of *C. laurifolius* and the biosynthetic **14** and **15** were isolated. Labelled **14** was treated with diazomethane to give the radioactive tetrahydropalmatine (**18**) which was dehydrogenated with iodine to furnish radioactive palmatine (**40**) with essentially no loss of radioactivity. The radioactive **40** was treated with phenylmagnesium bromide to give the radioactive 8-phenyldihydropalmatine (**45**). Chromic acid oxidation (Kuhn–Roth) of **45** afforded radioactive benzoic acid (95% of original activity).

Biosynthetic corydalmine (**15**) derived from N-[$^{14}CH_3$] reticuline (**2**; expt 5) was treated with diazomethane to give radioactive tetrahydropalmatine (**18**) which was degraded as above to give radioactive benzoic acid (96% of original activity).

The foregoing experiments although established that reticuline (**2**) is specifically incorporated into stepholidine (**14**) and corydalmine (**15**), the orientation of OH and OMe groups in ring C in the precursor (**2**) and in the corresponding ring D in the biosynthetic bases (**14** and **15**) were different. The change in orientation of the functional groups during biotransformation could take place either by demethylation—remethylation process or by Me migration. To examine which of the two processes is in operation in the plant [1-^{3}H, 4′, O-$^{14}CH_3$] norreticuline (**6**; expt 14) was fed to young cut branches of *C. laurifolius* and biosynthetic stepholidine (**14**) and corydalmine (**15**) were isolated. ^{3}H and ^{14}C activities in biosynthetic **14** and **15** were determined and it was found that both the bases were essentially devoid of ^{14}C activity. The results thus demonstrated that change in orientation of OH and OMe group probably occurs by demethylation-remethylation process. Feeding of labelled scoulerine (**16**; expt 15) suggested that it takes place after the formation of **16**.

Whether reticuline (**2**) is dehydrogenated in the biosynthesis was then examined by feeding [1-^{3}H, 3-^{14}C] norreticuline (**6**; expt 11) to young cut branches of *C. laurifolius* and biosynthetic stepholidine (**14**) and corydalmine (**15**) were isolated. The ^{14}C and ^{3}H ratios in the precursor was 1:30 and in the biosynthetic **14** and **15** it was 1:28 and 1:31 respectively, demonstrating thus that the H atom at the asymmetric centre in **6** remains untouched during the biotransformation of 1-benzyltetrahydroisoquinoline precursor into the tetrahydroprotoberberines.

The experiments discussed, so far, established that reticuline is a specific precursor of tetrahydroprotoberberine alkaloids, the precursors used, however, were racemic. It would be expected that the enzyme involved in the biosynthesis would be stereospecific and that only one of the enantiomers of reticuline should normally act as a direct substrate. Parallel feedings with labelled (*S*)-(**22**) and (*R*)-(**21**), reticulines (expt 12 and 13 respectively) demonstrated that the stereospecificity is maintained in the biotransformation of reticuline into stepholidine (**14**) and corydalmine (**15**). The former was incorporated about 40 and 30 times more efficiently into **14** and **15** respectively than the latter. Reticuline[5] has been isolated from the plants and its presence in the plants was again confirmed by trapping experiment.[5] (*S*)-Reticuline (**22**), is thus, the biological precursor of tetrahydroprotoberberine alkaloids.

Quaternary protoberberine, palmatine (**40**) has been shown to be derived from tetrahydropalmatine[5] (**18**). [Aryl-^{3}H] Corydalmine (**15**) and [aryl-^{3}H] stepholidine (**14**) when fed to young *S. glabra*, were efficiently incorporated into dehydrocorydalmine (41) (incorporation 0.3%) and stepharanine (**43**) (incorporation 0.40%).

[Aryl-^{3}H] Tetrahydropalmatine (**18**) when fed to the plants, it was poorly metabolized to form capaurine (**19**) (incorporation 0.02%) and corynoxidine (**20**) (incorporation 0.03%). The results suggested that either **18** is not on the direct biosynthetic pathway or it is not reaching the site of biosynthesis.

Labelled coclaurine (**28**) and N-methylcoclaurine (**29**) were fed to young plants of *S. glabra*. Biosynthetic corydalmine (**15**), stepholidine (**14**), tetrahydropalmatine (**18**) and quaternary protoberberines, dehydrocorydalmine (**41**) and palmatine (**40**) were isolated. Very low incorporations of the precursors into the bases were observed. The results demonstrated that although alkaloids both derived from trisubstituted 1-benzyltetrahydroisoquinoline and tetrasubstituted 1-benzyltetrahydroisoquinoline precursors co-exist in *S. glabra*, the latter are not derived from the former precursors.

The feeding results demonstrate that protoberberinium salts in nature are formed by dehydrogenation of tetrahydroprotoberberine alkaloids, the rate of their conversion is, however, very different. In some plants both tetrahydroprotoberberine and their corresponding quaternary protoberberine alkaloids co-exist for example in *S. glabra* tetrahydropalmatine (**18** and palmatine (**40**); corydalmine (**15**) and dehydrocorydalmine (**41**); stepholidine (**14**) and stepharanine (**43**). The other quaternary protoberberines jatrorrhizine (**42**), palmatrubine, glabrine and glabrinine also exist in *S. glabra*, however, their corresponding tetrahydroprotoberberines have not yet been isolated. It appears that the rate of conversion of the tetrahydroprotoberberines into quaternary protoberberine alkaloids in the plant is very fast.

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see Ref. 6.

Synthesis of precursors. The racemates of coclaurine derivatives (**28–30**), norlaudanosoline derivatives[5] (**1** to **8**), (*S*)-(**22**), (*R*)-(**21**) reticulines[5]; (*S*)-(**24**, R = Me)-, (*R*)-(**23**, R = Me)-N-methylcoclaurines[7] were prepared as described earlier

Labelling of precursors. Specifically labelled 1-benzyltetrahydroisoquinoline precursors were prepared by base catalysed

tritiation. Non specifically labelled tetrahydroprotoberberines were prepared by acid catalysed tritiation. ^{14}C Labelled norreticuline, reticuline and coclaurine were prepared by the procedure described earlier.[5,7]

Feeding experiment. For feeding purposes labelled precursors were dissolved in H_2O (1 ml) containing tartaric acid (12 mg). Freshly cut young branches of *C. laurifolius* were dipped into the soln of the precursors to take up the precursor. When uptake was complete H_2O was added for washing. The twigs were then dipped in H_2O, left for 6–8 days and worked up for alkaloids of interest. The young *S. glabra* were fed by wick feeding technique.

Isolation of alkaloids

Tetrahydropalmatine (**18**), *capaurine* (**19**), *corydalmine* (**15**) *and stepholidine* (**14**). Young branches and leaves (typically 600 g wet wt.) of *C. laurifolius* were macerated in EtOH (250 ml) with radio inactive **18** (100 mg), **19** (60 mg), **15** (100 mg) and **14** (120 mg) and left overnight. The EtOH was then decanted and the plant material was percolated with EtOH (4 × 200 ml). The combined ethanolic extract was concentrated *in vacuo* to afford a greenish viscous mass which was extracted with 5% HCl (4 × 15 ml). The acidic extract was defatted with *n*-hexane (4 × 15 ml) and basified (pH 8–9) with Na_2CO_3. The liberated bases were extracted with $CHCl_3$ (4 × 20 ml), washed with water, dried and solvent removed. The crude bases (480 mg), so obtained, were purified by preparative TLC (*plates*: silica gel; *solvent*: $CHCl_3$:MeOH, 97:3). The desired bands were cut and eluted with $CHCl_3$:MeOH (80:20) to give **18** (65 mg) m.p. 141–142° (lit.[8] 141–142°), **19** (25 mg) m.p. 164–165° (lit.[9] 164°), **15** (60 mg) m.p. 174–175° (lit.[10] 174–175°) and **14** (70 mg) m.p. 156–157° (lit.[10] 158–160°). The radio chemical purity of each base was checked by dilution method.

Corynoxidine (**20**). Young cut branches (Typically 250 g wet wt.) of *C. laurifolius* were macerated in EtOH (250 ml) with **20** (45 mg). The plant material was worked up as above to give $CHCl_3$ soluble crude base (85 mg) which was subjected to preparative TLC (*plates*: Silica gel; *solvent*: $CHCl_3$: MeOH, 94:6) to give **20** (25 mg) m.p. 181° (lit.[2] 181–182°).

Pronuciferine (**27**) *and stepharine* (**26**). Young cut branches (Typically 300 g wet wt.) of *C. laurifolius* were macerated in EtOH (250 ml) with radio inactive **27** (100 mg) and **26** (70 mg) and worked up as above to give $CHCl_3$ soluble crude bases (250 mg) which were purified by preparative TLC (*plates*: Silica gel; *solvent*: $CHCl_3$:MeOH, 94:6) to give **27** (65 mg) m.p. 127–128° (lit.[11] 127–129°) and **26** (45 mg) m.p. 177–178° (lit.[11], 179–181°). The bases were crystallised from acetone to constant activity.

Cycleanine (**52**) *and N-Desmethylcycleanine* (**51**). Young branches and leaves (typically 450 g wet wt.) of *C. laurifolius* were macerated in EtOH (250 ml) with radio inactive **52** (100 mg) and **51** (35 mg). The plant material was extracted with EtOH and the extract worked up as above to give $CHCl_3$ soluble crude alkaloidal mixture (200 mg) which was subjected to preparative TLC (*plates*: Silica gel; *solvent*: $CHCl_3$:MeOH, 92:8) to give **52** (70 mg) m.p. 272–273° (lit.[12] 272–273°) and **51** (20 mg) m.p. 102–103° (lit.[2] 102–103°).

Palmatine (**40**), *dehydrocorydalmine* (**41**) *and stepharanine* (**43**. Young branches and leaves (typically 300 g wet wt.) of *C. laurifolius* were macerated in EtOH (300 ml) with radio inactive **40** (120 mg), **41** (100 mg) and **43** (100 mg) and left overnight. The EtOH was decanted and the plant material was percolated with fresh EtOH (6 × 200 ml). The extract was concentrated *in vacuo* to give a greenish viscous residue which was extracted with 5% HCl (5 × 15 ml). The aqueous acidic extract was defatted with n-hexane (4 × 20 ml), basified with Na_2CO_3(pH 8) and extracted with n-BuOH (5 × 20 ml). The n-BuOH extract was washed with H_2O (3 × 15 ml), dried (Na_2SO_4) and the solvent removed. The crude bases (420 mg), so obtained, were purified by preparative TLC (plates: Silica gel; *solvent*; $CHCl_3$:MeOH 76:24). The bands containing the desired alkaloids were cut and eluted with MeOH. The solvent from the eluates was removed and the residues crystallised from MeOH to afford **40** (60 mg) m.p. 239–241° (lit.[8], 239°), **41** (70 mg) m.p. 252–253° (lit.[13], 253°) and **43** (55 mg) m.p. 274–275° (lit.[5], 274–275°).

Berberine (**44**) and *jatrorrhizine* (**42**). Young cut branches (Typically 300 g wet wt.) of *C. laurifolius* were macerated in EtOH (300 ml) with radio inactive **44** (120 mg) and **42** (60 mg). The plant material was extracted with EtOH and the extract worked up as above to give n-BuOH soluble crude alkaloidal mixture (220 mg) which was subjected to preparative TLC (*plates*: Silica gel; *solvent*: $CHCl_3$:MeOH 80:20) to furnish **44** (75 mg) m.p. 145° (lit.[14], 145°) and **42** (30 mg) m.p. 204–205° (lit.[2] 205–206°).

Degradation of the biosynthetic alkaloids

[5-^{14}C] *Stepharine* (26) *derived from* (±)-[3-^{14}C] *coclaurine.* Labelled **26** (60 mg; molar activity 4.9×10^4 dis.min^{-1} m mol^{-1}) was treated with HCHO/HCO_2H to give radioactive *pronuciferine* (**27**) m.p. 128–129° (lit.[11], 127–129°) (56.8 mg) (molar activity 4.8×10^4 dis.min^{-1} mmol^{-1}). Radioactive **27** was reduced with $NaBH_4$ to **32** which was treated with 3N HCl to yield radioactive *nuciferine* (**33**) m.p. 165° (lit.[15], 165–166°) (50 mg) (molar activity 4.78×10^4 dis.min^{-1} mmol^{-1}). Radioactive **33** (240 mg) in MeOH (4 ml) was treated with MeI (1 ml) to afford radioactive *nuciferine methiodide* (**35**) m.p. 178–179° (lit.[15], 177–178°) (molar activity 9.48×10^3 dis.min^{-1} m). Radioactive **35** in MeOH (100 ml) was passed through a column of amberlite IR-410 anion exchange resin (OH^- form) (10 g). The eluate recycled 5 times and finally washed with MeOH (100 ml). The solvent from the eluates was removed to give the radioactive *methohydroxide* (**36**). **36** Was refluxed with KOH (3.5 g, in 10 ml MeOH and 10 ml H_2O) for 2 hr. to give the radioactive *methine-I* (**34**; 180 mg) as an oil (molar activity 9.38×10^3 dis.min^{-1} m mol^{-1}).

Radioactive **34** (170 mg) in MeOH (4 ml) was treated with MeI to give the corresponding *methine-I methiodide* (**37**; 175 mg) (molar activity 9.40×10^3 dis.min^{-1} m mol^{-1}) which was converted into its hydroxide form (**38**) with IR-410 anion exchange resin. The radioactive methohydroxide (**38**) was heated with KOH (1.4 g in 5 ml MeOH and 5 ml H_2O) at 100° for 2 hr to afford radioactive 3,4-*dimethoxy*-1-*vinylphenanthrene* (**39**; 90 mg) (molar activity 9.30×10^3 dis.min^{-1} m mol^{-1}).

Ozonised O_2 was passed through a soln of the radioactive **39** (85 mg) in EtOAc (6 ml) at −78° for 30 min. The solvent from the resulting mixture was removed. To the residue, H_2O (18 ml) Zn dust (200 mg) and $AgNO_3$ (9 mg) were added. The mixture was refluxed for 20 min. The liberated radioactive HCHO was distilled and the distillate was collected in a soln of dimedone (250 mg) in aqueous EtOH (80 ml). After 1 hr the soln was concentrated to 10 ml and then left at ambient temp for 12 hr. The ppt thus obtained was taken in $CHCl_3$ and chromatographed over SiO_2 column. Elution with $CHCl_3$ (TLC control) gave the radioactive *formaldehyde-dimethone* (12 mg) m.p. 186–188° (lit.[16], 188°) (molar activity 9.22×10^3 dis.min^{-1}.mmol^{-1}) (96% of original activity).

[5-^{14}C] *Pronuciferine* (**27**) *derived from* [3-^{14}C] *coclaurine.* Labelled **27** (molar activity 9.86×10^4 dis.min^{-1} mmol^{-1}) was reduced with $NaBH_4$ to give the dienol which was treated with 3N HCl to yield finally radioactive *nuciferine* (**33**) (58 mg). Radioactive **33** (55 mg) was diluted with radio inactive nuciferine (200 mg) and crystallised to give radioactive **33** (240 mg) (molar activity 2.10×10^4 dis. min.$^{-1}$ mmol^{-1}). Labelled **33** was then degraded as above to give radioactive 3,4-*dimethoxy*-1-*vinylphenanthrene* (**39**). Ozonolysis of radioactive **39** afforded the radioactive formaldehyde Radioactivity of the various degradation products is given in Table 3.

Cycleanine (**52**) *derived from* (±)-[3′, 5′, 8-$^3H_3$]*N-methylcoclaurine.* Radioactive **52** (80 mg; molar activity 8.086×10^4 dis.min.$^{-1}$ mmol^{-1}) was diluted with radio inactive **52** (200 mg) and crystallised to give radioactive **52** (275 mg; molar activity 2.30×10^4 dis.min.$^{-1}$ mmol^{-1}). The radioactive **52** in dry toluene (10 ml) was added dropwise to liquid ammonia (150 ml) pretreated with NaH (1g) and Na (500 mg). The mixture was stirred at −68° and again Na (200 mg) was added till a blue colour persisted. The mixture was stirred for 3 hr at −68° and allowed to stand overnight at ambient temperature. Water was added to the residue and the product extracted with ether (5 × 20 ml). To the aqueous layer NH_4Cl was added (pH7) and the liberated bases extracted with $CHCl_3$ (5 × 15 ml), dried (anhyd. Na_2SO_4) and the solvent removed *in vacuo* to afford (*R*)-armepavine (**31**) (100 mg; molar activity 2.18×10^4 dis. min^{-1} mmol^{-1}), m.p. 144 – 145° (lit.[17] 145 – 146°).

Table 3.

Compound	Molar activity ($dis.min^{-1}$ $mmol^{-1}$)
Pronuciferine	2.10×10^4
Nuciferine methiodide	1.88×10^4
Nuciferine methine – I	1.78×10^4
Nuciferine methine – I methiodide	1.82×10^4
3,4-Dimethoxy-1-vinylphenanthrene	1.86×10^4
Formaldehyde-dimethone	2.03×10^4
	(97% of original)

[6-^{14}C] *Stepholidine* (**14**) *derived from* (±)-[3-^{14}C] *norreticuline.* A soln of labelled **14** (80 mg; molar activity 1.046×10^4 $dis.min^{-1}$ $mmol^{-1}$) in MeOH (4 ml) and THF (6 ml) was treated with an excess of ethereal soln of CH_2N_2 to give the radioactive *tetrahydropalmatine* (**18**; 70 mg) m.p. 141–142° (lit.[8] 141–142°). Radioactive **18** (68 mg) was diluted with radio inactive **18** (95 mg) and crystallised to give radioactive **18** (molar activity 3.905×10^3 $dis.min^{-1}$ $mmol^{-1}$). Radioactive **18** in MeOH (5 ml) was treated with MeI (1 ml) to afford the radioactive *tetrahydropalmatine methiodide* (**46**; 175 mg) m.p. 249–251° (lit.[18] 248–251°) (molar activity 3.92×10^3 $dis.min^{-1}$ $mmol^{-1}$). **46** Was converted into the corresponding *methohydroxide* (**47**) with IR-410 anion exchange resin. Radioactive **47** in MeOH (10 ml) was refluxed with KOH (3.5 g in 1 ml H_2O) for 2 hr to give the radioactive *tetrahydropalmatine methylmethine* (**48**; 98.5 mg) m.p. 114–115° (lit.[8] 115–116°) (molar activity 3.85×10^3 $dis.min^{-1}$ $mmol^{-1}$). Ozonolysis of **48** as described above afforded the radioactive HCHO (dimedone derivative) m.p. 187–188° (lit.[16] 188°) (molar activity 3.72×10^3 $dis.min^{-1}$ $mmol^{-1}$) (95% of original activity).

[6-^{14}C] *Corydalmine* (**15**) *derived from* (±)-[3-^{14}C] *norreticuline.* A soln of labelled **15** (85 mg; molar activity 1.09×10^4 $dis.min^{-1}$ $mmol^{-1}$) in MeOH (4 ml) and THF (5 ml) was treated with an excess of ethereal CH_2N_2 to give radioactive **18** (81 mg; molar activity 1.02×10^4 $dis.min^{-1}$ $mmol^{-1}$). Radioactive **18** (80 mg) was diluted with radioinactive **18** (90 mg) and crystallized to yield radioactive **18** (molar activity 4.796×10^3 $dis.min^{-1}$ $mmol^{-1}$). Labelled **18** was degraded as described above to furnish radioactive formaldehyde dimethone. The radioactivities of the degradation products are recorded in Table 4.

[6-^{14}C] *Stepholidine* (**14**) *derived from* (±)-N-[$^{14}CH_3$] *reticuline.* A soln of labelled **14** (62 mg; molar activity 9.50×10^5 $dis.min^{-1}$ $mmol^{-1}$) in MeOH (4 ml) and THF (3 ml) was treated with an excess of ethereal CH_2N_2 to give radioactive **18** (60 mg; molar activity 9.00×10^5 $dis\ min^{-1}$ $mmol^{-1}$). Radioactive **18** (58 mg) was diluted with radioinactive **18** (100 mg) and crystallised to give radioactive **18** (152 mg; molar activity 3.307×10^3 $dis.min^{-1}$ $mmol^{-1}$). The radioactive **18** (150 mg) in EtOH (4 ml) was refluxed with I_2 (148 mg) to afford radioactive palmatine (**40**) (140 mg; molar activity 3.00×10^3 $dis.min^{-1}$ $mmol^{-1}$). Radioactive **40** was suspended in dry ether (10 ml) and an ethereal soln of PhMgBr was added to it at 0° and the mixture was then refluxed for 3 hr. The resulting mixture was worked up in the usual manner to give the radioactive 8-*phenyldihydropalmatine* (**45**) m.p. 157–59° (lit.[19] 158–160°) (82 mg; molar activity 2.5×10^3 $dis.min^{-1}$ $mmol^{-1}$). Radioactive **48** (80 mg) was oxidised with CrO_3 (3.8 g) in 10% H_2SO_4 (12 ml) in the usual way to afford radioactive *benzoic acid* (molar activity 3.14×10^3 $dis.min^{-1}$ $mmol^{-1}$) (95% of original activity).

[8-^{14}C] *Corydalmine* (**15**) *derived from* (±)-N[$^{14}CH_3$]*reticuline.* Radioactive **15** (64 mg; molar activity 2.47×10^5 $dis.min^{-1}$ $mmol^{-1}$) was treated with an excess of ethereal CH_2N_2 to furnish radioactive **18** (58 mg) which was diluted with radioinactive **18** (100 mg) and converted into radioactive *palmatine* (**40**) as described earlier. Radioactive **40** was treated with PhMgBr to give radioactive 8-*phenyldihydro palmatine* (**45**). Kuhn–Roth oxidation of radioactive **45** gave radioactive *benzoic acid.* The radioactivities of the various degradation products are recorded in Table 5.

Trapping experiment. (*L*)-[U-^{14}C]Tyrosine (activity 0.1 mCi) was fed to young cut branches of *S. glabra.* After 7 days the plants were harvested. The plant material (160 g, wet wt.) was macerated in EtOH (200 ml) with inactive (±)-reticuline (**2**; 110 mg) and (±)-N-methylcoclaurine (**29**; 95 mg). After 10 hr EtOH was decanted and the plant material percolated with fresh EtOH (6 × 150 ml) containing 2% AcOH. The solvent from the ethanolic extract was distilled under reduced pressure. The alcoholic extractive thus obtained was worked up in the usual manner to give a mixture of bases which was subjected to preparative TLC of SiO_2 plates to give reticuline (80 mg) (incorporation 0.31%) and N-methylcoclaurine (70 mg) (incorporation 0.16%).

Table 5.

Compound	Molar activity ($dis.min^{-1}$ $mmol^{-1}$)
Corydalmine (**15**)	2.474×10^5
Tetrahydropalmatine (**18**)	8.87×10^4
Palmatine (**40**)	8.76×10^4
8-Phenyldihydropalmatine (**45**)	7.97×10^4
Benzoic acid	8.51×10^4
	(96% of original)

Table 4.

Compound	Molar activity ($dis.min^{-1}$ $mmol^{-1}$)
Corydalmine (**15**)	1.09×10^4
Tetrahydropalmatine (**18**)	4.796×10^3
Tetrahydropalmatine methiodide (**46**)	4.77×10^3
Tetrahydropalmatine methylmethine (**48**)	4.68×10^3
Formaldehyde-dimethone	4.70×10^3 (98% or original)

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