Aberrant Biosynthesis of 12-Aminoprotoberberines

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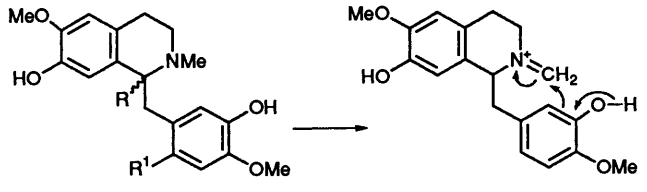
The incorporation of labelled racemic 2'-aminoreticuline **2** into racemic 12-aminotetrahydropalmatine **6**, 12-aminocanadine **8**, 12-aminopalmatine **10** and 12-aminoberberine **11** has been studied in *Cocculus laurifolius* DC (Menispermaceae) and specific incorporation of **2** into **6**, **8**, **10** and **11** demonstrated. Further, it has been shown that **2** is not metabolized by the plants to form tetrahydropalmatine **5**, palmatine **9** and berberine **12**.

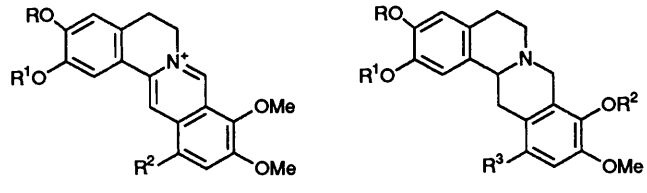
Protoberberines constitute a large group of 1-benzyltetrahydroisoquinoline derived alkaloids, several of which exhibit biological activities. The protoberberine nucleus has been transformed chemically into several biogenetically related skeletons.[1]

Tracer experiments have demonstrated that 1,2,9,10- and 1,2,10,11-tetrasubstituted protoberberines and tetrahydroprotoberberines in plants are derived from 1-benzyltetrahydroisoquinoline precursors such as reticuline.[2,3] It has been demonstrated that in protoberberine and berberine alkaloids C-8, known as the 'berberine bridge', is formed in Nature by oxidative cyclization of the *N*-methyl group of reticuline[4–7] **1**, possibly through the iminium intermediate **3**. Specific incorporation of (*S*)-reticuline **1** into tetrahydropalmatine[8] **5**, palmatine[9] **9**, berberine[7] **12** and canadine **7** *via* scoulerine **4** has been



1 R = ---H, R^1 = H
2 R = ~~H, R^1 = NH_2

3



9 R = R^1 = Me, R^2 = H
10 R = R^1 = Me, R^2 = NH_2
11 R, R^1 = $-CH_2-$, R^2 = NH_2
12 R, R^1 = $-CH_2-$, R^2 = H

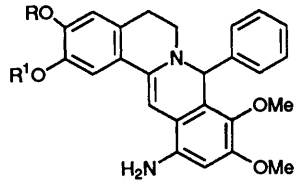
4 R = Me, R^1 = R^2 = R^3 = H
5 R = R^1 = R^2 = Me, R^3 = H
6 R = R^1 = R^2 = Me, R^3 = NH_2
7 R, R^1 = $-CH_2-$, R^2 = Me, R^3 = H
8 R, R^1 = $-CH_2-$, R^2 = Me, R^3 = NH_2

shown. Further, the enzymes involved in the biotransformations of reticuline into berberine have been studied.[10–13] We have demonstrated the conversion of 2'-bromonorreticuline and 2'-nitronorreticuline into 2'-bromopapaverine and 2'-nitropapaverine, respectively, in *Papaver somniferum* (Papaveraceae)[14] and of 5-bromoreticuline and 2',5-dibromoreticuline into 3-bromoglaucine and 3,8-dibromoglaucine respectively in *Litsea glutinosa* (Lour) C.B. Roxb. Var *glabraria* Hook[15] while exploring the potential of higher plants for carrying out transformations of organic molecules that they normally do not produce.

In a study we have now shown the specific incorporation of (±)-2'-aminoreticuline **2** into 12-aminotetrahydropalmatine **6**, 12-aminocanadine **8**, 12-aminopalmatine **10** and 12-aminoberberine **11** in *Cocculus laurifolius* DC (Menispermaceae) (Table 1).

(±)-2'-Amino[*aryl*-^{3}H]reticuline **2** (expt. 1) (Table 1) was initially fed to twigs of *C. laurifolius* DC and the biosynthetic 12-aminotetrahydropalmatine **6**, 12-aminocanadine **8**, 12-aminopalmatine **10** and 12-aminoberberine **11** were found to be radioactive. Tetrahydropalmatine **5**, palmatine **9** and berberine **12** were also isolated from the plants fed with the labelled precursor **2**. However, these bases were found to be radioinactive. The twigs were then fed with (±)-2'-amino[N-$^{14}CH_3$]reticuline **2** (expt. 2). The biosynthetic bases **6**, **8**, **10** and **11** were again found to be radioactive and the bases **5**, **9** and **12** radioinactive.

The regiospecificity of label in the biosynthetic 12-aminotetrahydropalmatine **6** derived from the feeding of (±)-2'-amino[N-$^{14}CH_3$]reticuline **2** (expt. 2) was shown as follows. Labelled compound **6** in ethanol was refluxed with I_2 to give 12-aminopalmatine **10** having essentially the same molar radioactivity as the parent base. Radioactive **10** was treated with phenylmagnesium bromide to afford the radioactive 12-amino-8-phenyldihydropalmatine **13** essentially with no loss of radioactivity. Kuhn–Roth oxidation of **13** afforded radioactive benzoic acid (95% of original activity).



13 R = R^1 = Me
14 R, R^1 = $-CH_2-$

The regiospecificity of label in biosynthetic 12-aminopalmatine **10**, 12-aminocanadine **8** and 12-aminoberberine **11** derived from feeding of (±)-2'-amino[N-$^{14}CH_3$]reticuline **2** (expt. 2) was determined as follows. Treatment of labelled 12-aminopalmatine **10** with phenylmagnesium bromide gave radioactive 12-amino-8-phenyldihydropalmatine **13**. Kuhn–Roth oxidation of **13** afforded radioactive benzoic acid (95% of original activity). Dehydrogenation of the biosynthetic 12-

Table 1 Tracer experiments on *Cocculus laurifolius* DC

Expt.	Precursor fed	% Incorporation into 6	8	10	11
1	(±)-2′-Amino[*aryl*-^{3}H]reticuline **2**	0.45	0.32	0.30	0.28
2	(±)-2′-Amino[N-$^{14}CH_3$]reticuline **2**	0.67	0.30	0.47	0.27

aminocanadine **8** with I_2 yielded 12-aminoberberine **11** which on reaction with phenylmagnesium bromide, afforded 12-amino-8-phenyldihydroberberine **14**. Kuhn–Roth oxidation of **14** finally gave radioactive benzoic acid (94% of original activity). Reaction of the biosynthetic 12-aminoberberine **11** with phenylmagnesium bromide gave 12-amino-8-phenyldihydroberberine **14**. Kuhn–Roth oxidation of **14** afforded radioactive benzoic acid (92% of original activity).

Experimental

For general directions (spectroscopy details and counting method) see ref. 16.

Synthesis of Precursors.—The racemate of 2′-aminoreticuline **2** was prepared by a standard procedure.[17]

Labelling of Precursors: Tritiation.—(±)-2′-Aminoreticuline **2** hydrochloride (110 mg) in tritiated water (0.2 cm^3, 150 mCi) was heated under N_2 (sealed tube) for 110 h at 100 °C. Work-up afforded (±)-2′-amino[aryl-^{3}H]reticuline **2** hydrochloride (80 mg) (specific activity 0.12 mCi mg^{-1}).

(±)-2′-*Amino*[N-$^{14}CH_3$]*reticuline* **2**. Treatment of the corresponding dihydroisoquinoline with [^{14}C]methyl iodide followed by reduction of the methiodide with $NaBH_4$ and subsequent hydrogenolysis of the benzyl ether afforded (±)-2′-amino[N-$^{14}CH_3$]reticuline (specific activity 0.048 mCi mg^{-1}).

12-*Aminopalmatine* **10**.—A mixture of 12-aminotetrahydropalmatine[17] **6** (250 mg), iodine (100 mg) and EtOH (10 cm^3) was refluxed for 2 h after which the resulting mixture was filtered and excess of iodine decomposed by sulfurous acid. The mixture was then extracted with $CHCl_3$ (4 × 10 cm^3) and the combined extracts washed with water, dried and evaporated. The product crystallized from EtOH to give 12-*aminopalmatine* **10** iodide (155 mg) (yield 63%), m.p. 271–272 °C; λ_{max}(MeOH)/nm 226, 293, 320 and 400, *m/z* 367 (M^+) and 251 (M^+–NH_2) (Found: C, 68.9; H, 6.2; N, 7.7. $C_{21}H_{23}N_2O_4$ requires C, 68.7; H, 6.23; N, 7.6%).

(±)-12-*Aminocanadine* **8**.—A mixture of 12-nitrocanadine[17] (1.1 g), 10% Pd/C (150 mg) and MeOH (150 cm^3) was shaken for 5 h (under hydrogen). The resulting mixture was filtered and evaporated. The residue was treated with ethereal HCl to give 12-*aminocanadine* **8** hydrochloride (850 mg, 69.5%), m.p. 235–236 °C (MeOH); λ_{max}(MeOH)/nm 210 and 292; ν_{max}(KBr)/cm^{-1} 3310 (NH_2); δ_H(90 MHz; $CDCl_3$–[$^2H_6$]-DMSO) 3.64 (s, 3 H, OCH_3), 3.7 (s, 3 H, OCH_3), 5.82 (s, 2 H, OCH_2O), 6.16 (s, 1 H), 6.5 and 6.76 (s, 1 H); *m/z* 254 (M^+), 179, 164 and 149 (Found: C, 67.7; H, 6.15; N, 7.85. $C_{20}H_{24}N_2O_4$ requires C, 67.79; H, 6.21; N, 7.9%).

12-*Aminoberberine* **11**.—A mixture of 12-aminocanadine **8** (300 mg), iodine (200 mg) and EtOH (15 cm^3) was refluxed for 2 h after which it was filtered and the excess of iodine decomposed by sulfurous acid. Work-up gave a crystalline product, which was recrystallized from MeOH to give 12-*aminoberberine* **11** iodide (160 mg, 53.8%), m.p. 300 °C; λ_{max}(MeOH)/nm 225, 287, 318 and 296; *m/z* 351 (M^+) 335 (Found: C, 68.5; H, 5.2; N, 7.92. $C_{20}H_{19}N_2O_4$ requires C, 68.37; H, 5.41; N, 7.97%).

Feeding Experiments.—Labelled 2′-amino[*aryl*-^{3}H]reticuline (1 mg; specific activity 0.12 mCi mg^{-1}) and 2′-amino[N-$^{14}CH_3$]reticuline (10.2 mg; specific activity 0.048 mCi mg^{-1}) were separately dissolved in water (1 cm^3) containing tartaric acid (10 mg). The twigs of *C. laurifolius* DC were dipped into the solution of the precursors. When uptake was complete water was added to wash off the remaining precursors. The plants were left for 6 to 8 days and then worked up for alkaloids of interest.

Isolation of Racemic 12-*Aminotetrahydropalmatine* **6**.—The twigs (typically 130 g, wet wt.) of *C. laurifolius* DC were macerated in EtOH (250 cm^3) with radioinactive racemic 12-aminotetrahydropalmatine[17] **6** (130 mg) and left for 10 h. The EtOH was decanted and the plant material was percolated with fresh EtOH (6 × 200 cm^3). The combined ethanolic extracts were concentrated under reduced pressure to give a greenish viscous mass which was extracted with 10% hydrochloric acid (5 × 15 cm^3). The aqueous acidic extract was defatted with light petroleum (5 × 15 cm^3) and basified with Na_2CO_3. The liberated bases were extracted with $CHCl_3$ (5 × 30 cm^3) and the combined extracts were washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The crude bases thus obtained were subjected to preparative thin layer chromatography (PTLC) on silica gel GF_{254}, (solvent $CHCl_3$–MeOH, 98:2). The region containing the desired alkaloid was excised and eluted with $CHCl_3$–MeOH (80:20). Evaporation of the eluate gave the base which was crystallized from MeOH to afford radioactive 12-*aminotetrahydropalmatine* **6** (80 mg), m.p. 134–135 °C (decomp.) [lit.,[17] 133–134 °C (decomp.)].

Isolation of Racemic 12-*Aminocanadine* **8**.—The twigs (120 g, wet wt.) of *C. laurifolius* DC were macerated in EtOH (250 cm^3) with radioinactive 12-aminocanadine **8** (100 mg). The plant material was extracted with EtOH and the extract worked up as above to give radioactive, 12-*aminocanadine* **8** (58 mg); hydrochloride, m.p. 235–236 °C.

Isolation of 12-*Aminopalmatine* **10**.—The twigs (110 g, wet wt.) of *C. laurifolius* DC were macerated in EtOH (250 cm^3) with radioinactive 12-aminopalmatine **10** iodide (120 mg). The plant material was extracted with EtOH and the extract worked up as above to give radioactive 12-*aminopalmatine* (85 mg) iodide, m.p. 271–272 °C.

Isolation of 12-*Aminoberberine* **11**.—The twigs (115 g, wet wt.) of *C. laurifolius* DC were macerated in EtOH (250 cm^3) with radioinactive 12-aminoberberine **11** iodide (110 mg). The plant material was extracted with EtOH and the extract worked up as above to give radioactive 12-*aminoberberine* **11** iodide (80 mg), m.p. 300 °C.

Isolation of Racemic Tetrahydropalmatine **5**.—The twigs (140 g, wet wt.) of *C. laurifolius* DC fed with labelled 2′-aminoreticuline **2** were macerated in EtOH (250 cm^3) with radioinactive tetrahydropalmatine **5** (100 mg). The plant material was extracted with EtOH and worked up to give radioactive *tetrahydropalmatine* **5** (180 mg), m.p. 140–141 °C (lit.,[18] 141–142 °C).

Table 2 Radioactivity of 12-aminotetrahydropalmatine products

Compd.	Molar activity (disint. min^{-1} $mmol^{-1}$)
6	1.36×10^4
10	1.37×10^4
13	1.29×10^4
Benzoic acid	1.29×10^4

Table 3 Radioactivity of 12-aminopalmatine products

Compd.	Molar activity (disint. min^{-1} $mmol^{-1}$)
10	1.12×10^4
13	1.10×10^4
Benzoic acid	1.07×10^4

Table 4 Radioactivity of 12-aminocanadine products

Compd.	Molar activity (disint. min^{-1} $mmol^{-1}$)
8	3.89×10^3
11	3.75×10^3
14	3.60×10^3
Benzoic acid	3.65×10^3

Table 5 Radioactivity of 12-aminoberberine products

Compd.	Molar activity (disint. min^{-1} $mmol^{-1}$)
11	1.58×10^4
14	1.50×10^4
Benzoic acid	1.45×10^4

Isolation of Racemic Canadine **7**.—The twigs (100 g, wet wt.) of *C. laurifolius* fed with labelled 2'-aminoreticuline **2** (expt. 1) were macerated in EtOH (250 cm^3) with radioinactive canadine **7** (80 mg). The plant material was extracted with EtOH and the extract worked up to give radioinactive (±)-*canadine* **7** (60 mg), m.p. 167–168 °C (Et_2O–hexane) (lit.,[19] 165 °C).

Degradation of the Biosynthetic 12-*Aminotetrahydropalmatine* **6** *derived from* 2'-*Amino*[N-$^{14}CH_3$]*reticuline* **2**.—A mixture of labelled **6** (300 mg), EtOH (4 cm^3) and I_2 (350 mg) was refluxed for 2 h and then worked up to give radioactive 12-*aminopalmatine* **10** iodide (180 mg), m.p. 272–273 °C. To a suspension of radioactive **10** (170 mg) in dry ether (10 cm^3) at 0 °C was added an ethereal solution of PhMgBr and the mixture was refluxed for 2 h. It was then worked up to give radioactive 12-*amino*-8-*phenyldihydropalmatine* **13** (80 mg), m.p. 148–150 °C; *m/z* 444. Labelled **13** (75 mg) was treated with Cr_2O_3 (4 g) in 10% H_2SO_4 (14 cm^3) (Kuhn–Roth method) to give radioactive benzoic acid. The radioactivity of the products is given in Table 2.

Degradation of the Biosynthetic 12-*Aminopalmatine* **10** *derived from* 2'-*Amino*[N-$^{14}CH_3$]*reticuline*.—Labelled compound **10** (250 mg) in ether was treated with PhMgBr as above to give radioactive 12-amino-8-phenyldihydropalmatine **13** (110 mg). Oxidation of **13** with Cr_2O_3–10% H_2SO_4 gave radioactive benzoic acid. The radioactivity of the products is recorded in Table 3.

Degradation of the Biosynthetic 12-*Aminocanadine* **8** *derived from* 2'-*Amino*[N-$^{14}CH_3$]*reticuline*.—A mixture of labelled **8** (250 mg), iodine (150 mg) and EtOH (15 cm^3) was refluxed for 2 h as above to give radioactive 12-aminoberberine **11** (130 mg). A suspension of radioactive **11** (120 mg) in ether was treated with PhMgBr to give radioactive 12-amino-8-phenyldihydroberberine **14** which was oxidised with Cr_2O_3–10% H_2SO_4 to give radioactive benzoic acid. The radioactivity of the products is given in Table 4.

Degradation of the Biosynthetic 12-*Aminoberberine* **11** *derived from* 2'-*Amino*[N-$^{14}CH_3$]*reticuline*.—Labelled **11** (200 mg) was treated with PhMgBr to give radioactive 12-amino-8-phenyldihydroberberine **14**, which was oxidised with Cr_2O_3–10% H_2SO_4 to give radioactive benzoic acid. The radioactivity of the products is given in Table 5.

Acknowledgements

We thank the Council of Scientific & Industrial Research, New Delhi for financial assistance.

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Paper 3/04282K
Received 21*st July* 1993
Accepted 21*st September* 1993