The Biosynthesis of Glaucine in *Litsea glutinosa*

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Incorporation of ^{14}C labelled (±)-reticuline and tritiated racemic reticuline, protosinomenine, orientaline, laudanosine, isoboldine, boldine, thaliporphine, *N*-methyl-laurotetanine, and dicentrine into glaucine in young *Litsea glutinosa* (Lour) C. B. Rob. var. *glabraria* Hook f. (Lauraceae) has been studied. The results provide evidence that (*S*)-reticuline (**5**) is converted by oxidative coupling into (*S*)-isoboldine (**6**) and methylation *via* (*S*)-thaliporphine (**7**) to form (*S*)-glaucine (**8**).

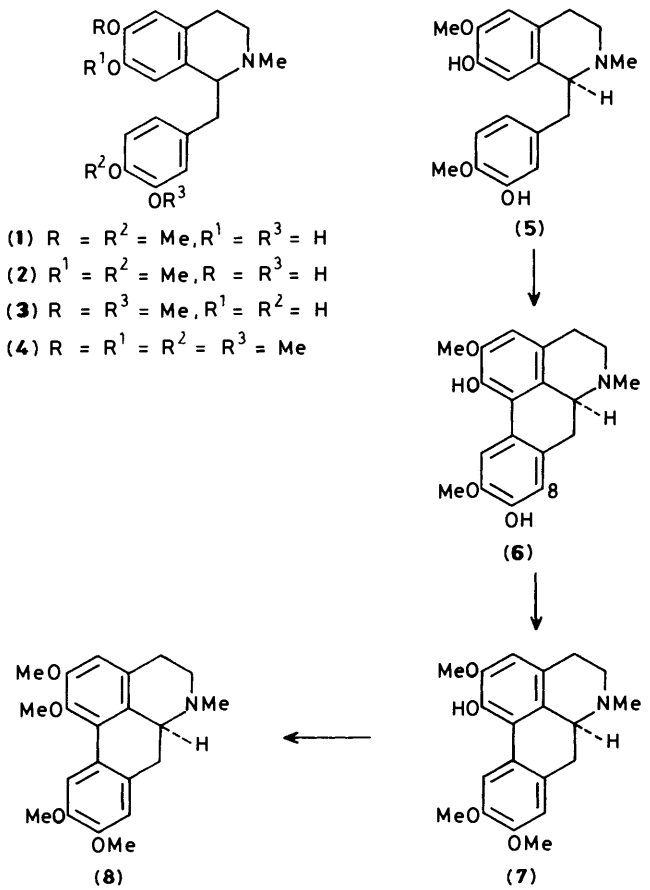
Glaucine (**8**),[1] the antithrombotic, anti-inflammatory, and analgesic principle of several plant species[2] has been isolated both in (+)- and (−)-forms. The structure and stereochemistry of (+)-glaucine (**8**)[3] is well established.[4] (−)-Glaucine (**17**) has been isolated from Korean *Corydalis* tubers.[5]

Glaucine (**8**) could be formed in Nature from reticuline (**1**), protosinomenine (**2**), and orientaline (**3**) by alternative biosynthetic routes.[6] However, it has been reported that glaucine (**8**) in *Dicentra eximia* (Ker). Toer (Fumariaceae) is biosynthesized from norprotosinomenine.[7] We have studied the biosynthesis of several aporphine alkaloids[8–10] and we now find that (**8**) is biosynthesized from (*S*)-reticuline (**5**) in *Litsea glutinosa* (Lour) C. B. Rob. var. *glabraria* Hook f. (Lauraceae).

Initially reticuline (**1**) (experiment 2), protosinomenine (**2**) (experiment 3), and orientaline (**3**) (experiment 4) were fed in parallel with tyrosine (experiment 1) to young *Litsea glutinosa* var. *glabraria* plants and it was found that reticuline (**1**) was incorporated with higher efficiency into glaucine (**8**) than protosinomenine (**2**), and orientaline (**3**). The incorporation of (**2**) and (**3**) was low but not negligible. As expected, laudanosine (**4**) (experiment 5) was not incorporated into (**8**). The incorporation of various labelled precursors into glaucine is recorded in the Table.

Table. Tracer experiments on *Litsea glutinosa*

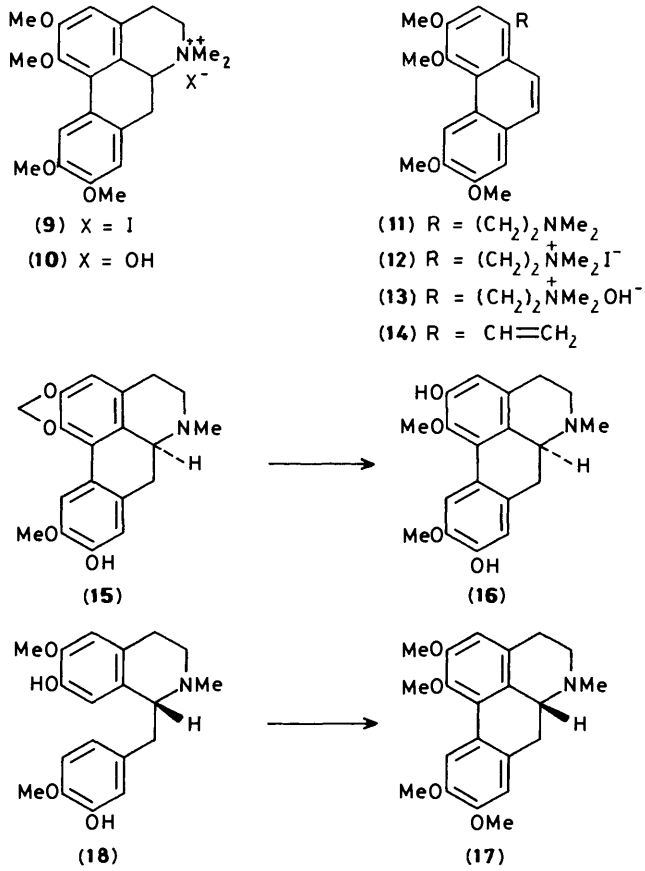
Expt.	Precursor fed	% Incorporation into Glaucine (**8**)	
1	(L)-[U-^{14}C]Tyrosine	0.02	0.02
2	(±)-[2′,6′,8-$^{3}H_3$]Reticuline (**1**)		0.14
3	(±)-[*Aryl*-^{3}H]Protosinomenine (**2**)		0.04
4	(±)-[*Aryl*-^{3}H]Orientaline (**3**)		0.02
5	(±)-[*Aryl*-^{3}H]Laudanosine (**4**)		0.002
6	(±)-[3-^{14}C]Reticuline (**1**)		0.16
7	(±)-[3,8-$^{3}H_2$]Boldine (**16**)		0.06
8	(±)-[*Aryl*-^{3}H]Isoboldine (**6**)		0.21
9	(+)-[*Aryl*-^{3}H]Thaliporphine (**7**)		0.27
10	(±)-[*Aryl*-^{3}H]-*N*-Methyl-laurotetanine		0.06
11	(+)-[*Aryl*-^{3}H]Dicentrine (**15**)		0.01
12	(*S*)-(+)-[2′,6′,8-$^{3}H_3$]Reticuline (**5**)		0.18
13	(*R*)-(−)-[2′,6′,8-$^{3}H_3$]Reticuline (**18**)		0.012



The regiospecificity of the label in the biosynthetic glaucine (**8**) derived from (±)-[3-^{14}C]reticuline (**1**) (experiment 6) was determined as follows. Biosynthetic glaucine (**8**) was treated with methyl iodide to afford glaucine methiodide (**9**) having essentially the same molar radioactivity as the parent base. Radioactive (**9**) was converted into the corresponding methohydroxide (**10**) by IR-410 anion exchange resin. Hofmann degradation of compound (**10**) gave the methine-I (**11**) with essentially no loss of radioactivity. Treatment of (**11**) with methyl iodide afforded glaucine methyl methine methiodide (**12**) which was converted into its methohydroxide (**13**). Second Hofmann degradation of the labelled compound (**13**) yielded radioactive 3,4,6,7-tetramethoxy-1-vinylphenanthrene (**14**). Ozonolysis of compound (**14**) gave a radioactive formaldehyde dimethone derivative (85% of original activity).

Although the foregoing experiments established that reticuline (**1**) was specifically incorporated into glaucine (**8**) in *L. glutinosa* var. *glabraria*, the precursors used were racemic. Parallel feedings of labelled (+)-reticuline (**5**) (experiment 12) and (−)-reticuline (**18**) (experiment 13) demonstrated that stereospecificity is maintained in the bioconversion of (**5**) into (**8**) in *L. glutinosa* var. *glabraria* plants. The former was incorporated about 10 times more efficiently than the latter. The incorporation of (−)-reticuline into glaucine (**8**) is due to the optical impurity of (+)-reticuline (**5**).

Specific incorporation of (*S*)-reticuline (**5**) into glaucine (**8**) implied isoboldine (**6**) as an intermediate. Labelled (**6**)



(experiment 8) when fed to young *L. glutinosa* var. *glabraria* plants was efficiently incorporated into (**8**). Labelled boldine (**16**) (experiment 7) when fed to young *L. glutinosa* var. *glabraria* plants gave rise to radioactive biosynthetic (**8**), suggesting thereby that the enzymes present in the plant can metabolize boldine (**16**) to give (**8**).

In the bioconversion of isoboldine (**6**) into glaucine (**8**), selective *O*-methylation at position 1 in isoboldine (**6**) could give *N*-methyl-laurotetanine. Alternatively selective *O*-methylation at position 9 in isoboldine (**6**) could give thaliporphine (**7**). Glaucine (**8**) could then be formed finally both from *N*-methyl-laurotetanine and from (**7**) by *O*-methylation at positions 9 and 1, respectively. Labelled thaliporphine (**7**) (experiment 9), when fed in parallel with labelled *N*-methyl-laurotetanine (experiment 10) to young *L. glutinosa* var. *glabraria* plants, was incorporated into (**8**) about five times more efficiently than *N*-methyl-laurotetanine demonstrating thereby that selective *O*-methylation at position 9 in isoboldine (**6**) occurs to give thaliporphine (**7**) which is then converted into (**8**). Labelled dicentrine (**15**) (experiment 11) when fed to young *L. glutinosa* var. *glabraria* plants was poorly metabolized to give (**8**).

(*S*)-Reticuline (**5**) and (*S*)-isoboldine (**6**) have been isolated from *L. glutinosa* var. *glabraria*.[11] The presence of these bases in young *L. glutinosa* var. *glabraria* plants was confirmed by a trapping experiment with (L)-[U-^{14}C]tyrosine (incorporation 0.3 and 0.2% respectively). (*S*)-Reticuline (**5**) and isoboldine (**6**) are, thus, the biological precursors of glaucine (**8**) in young *L. glutinosa* var. *glabraria* plants. The foregoing tracer experiments support the following sequence for the biosynthesis of glaucine (**8**) in young *L. glutinosa* (Lour.) C. B. Rob. var. *glabraria* Hook f. (Lauraceae): Tyrosine ⟶ (*S*)-(+)-reticuline (**5**) ⟶ (*S*)-isoboldine (**6**) ⟶ (*S*)-thaliporphine (**7**) ⟶ (*S*)-glaucine (**8**).

Several aporphine,[8-10,12] protoberberine, and tetrahydroprotoberberine[13-18] alkaloids, in recent years have shown to be derived in Nature from reticuline. Although in the majority of the cases the orientation of hydroxy and methoxy functions in the isoquinoline moiety of the precursor and the corresponding position in the biosynthetic bases is the same, in a few cases the orientation of these groups is different.[10] It has been shown that the methylenedioxy function in berberine is formed from *ortho*-hydroxy methoxy function.[19,20] It is possible that dicentrine (**15**) could be an intermediate in the biosynthesis of boldine (**16**) from isoboldine (**6**).

Glaucine has been isolated both in (+)- and (−)-forms from plants. We have demonstrated with the help of tracer experiments that (*S*)-glaucine (**8**) is stereospecifically derived in *L. glutinosa* var. *glabraria* plants from (*S*)-reticuline (**5**). It is logical that (*R*)-glaucine (**17**) is also stereospecifically derived in plants from (*R*)-reticuline (**18**).

Experimental

For general directions (spectroscopy details and counting method) see ref. 21.

Synthesis of Precursors.—The racemates of reticuline (**1**),[22] protosinomenine (**2**),[23] orientaline (**3**),[24] and laudanosine (**4**)[10] were prepared by standard procedures. (+)-Isoboldine (**6**), (+)-*N*-methyl-laurotetanine, (+)-dicentrine (**15**), and (±)-thaliporphine (**7**) used, were natural products.[25]

Resolution.—(±)-Di-*O*-benzylreticuline was resolved[26] by treatment with (+)- and (−)-*O*,*O*-dibenzoyltartaric acids. Hydrogenolysis of the benzyl ethers with HCl furnished (*S*)-(+)- and (*R*)-(−)-reticulines, (**5**) and (**18**) respectively, of known absolute configuration.

Labelling of Precursors: Tritiation.—Both acid- and base-catalysed exchange reactions[27,28] were employed.

(±)-[2′,6′,8-^{3}H$_3$]*Reticuline.* (±)-Reticuline (100 mg) in tritiated water (0.2 ml, 200 mCi) containing Bu^{t}OK (220 mg) was heated under N_2 (sealed tube) for 110 h at 100 °C. Work-up afforded (±)-[2′,6′,8-^{3}H$_3$]reticuline. (*S*)-(+)- and (*R*)-(−)-[2′,6′,8-^{3}H$_3$] Reticulines, (**5**) and (**18**) respectively, and (+)-[3,8-^{3}H$_2$]boldine (**16**) were prepared in the same way.

(±)-[Aryl-^{3}H]*Protosinomenine* (**2**). Compound (**2**) (110 mg) was added to a mixture of tritiated water (0.2 ml, 200 mCi) and $SOCl_2$ (0.1 ml), and the mixture under N_2 (sealed tube) was heated for 110 h at 100 °C. The resulting mixture was worked up to give (±)-[*aryl*-^{3}H]protosinomenine (**2**). (±)-Orientaline (**3**), (±)-laudanosine (**4**), isoboldine (**6**), thaliporphine (**7**), *N*-methyl-laurotetanine, and dicentrine (**15**) were tritiated in the same way.

(±)-[3-^{14}C]Reticuline (**1**) was prepared by total synthesis.

Feeding Experiments.—For feeding purposes, the labelled precursors were either dissolved in water (1 ml) containing tartaric acid (10 mg) or in aqueous dimethyl sulphoxide (0.2 ml). Twigs of *Litsea glutinosa* (Lour.) C. B. Rob. var. *glabraria* Hook f. (Lauraceae) were dipped into the solution of the precursor. When uptake was complete, the twigs were washed with water and worked up to provide biosynthetic glaucine (**8**).

Isolation of Glaucine (**8**).—The precursor-fed twigs (typically 40 g, wet wt.) of *L. glutinosa* var. *glabraria* plants were macerated in EtOH (250 ml) with radioinactive glaucine (**8**) (100 mg) and left for 10 h. The EtOH was decanted and the plant material was percolated with fresh EtOH (6 × 250 ml). The combined washings were concentrated under reduced pressure to afford a greenish viscous mass which was extracted with 5% hydrochloric acid (5 × 20 ml). The acidic extract was defatted

with light petroleum (3 × 20 ml), basified with Na_2CO_3 (pH 8), and extracted with $CHCl_3$ (6 × 30 ml). The combined $CHCl_3$ extracts were washed with water, dried (Na_2SO_4), and evaporated. The crude base was subjected to preparative t.l.c. on silica gel GF_{254} (solvent: $CHCl_3$–MeOH, 97:3). The region containing glaucine (**8**) was removed and eluted with $CHCl_3$–MeOH (80:20). The solvent from the eluate was removed under reduced pressure to give radioactive glaucine (**8**) (78 mg), m.p. 120—121 °C (from MeOH–H_2O) (lit.,[29] 120 °C).

Degradation of the Biosynthetic Glaucine (**8**) *Derived from* (±)-[3-^{14}C]*Reticuline.*—Labelled glaucine (**8**) (250 mg) (molar activity 6.60 × 10^4 disint. min^{-1} $mmol^{-1}$) in MeOH (10 ml) was heated with MeI (5 ml) for 2 h to give radioactive glaucine methiodide (**9**) (275 mg), m.p. 222—223 °C (lit.,[30] 221 °C) (molar activity 6.62 × 10^4 disint. min^{-1} $mmol^{-1}$). A solution of (**9**) in MeOH (60 ml) was passed through a column of freshly generated Amberlite IR-410 anion exchange resin (OH^- form) to afford the corresponding radioactive methohydroxide (**10**). Labelled (**10**) in MeOH (15 ml) was refluxed with KOH (4 g in 4 ml water) for 2.5 h. The solvent from the resulting mixture was removed under reduced pressure, and the residue diluted with water (10 ml) and extracted with Et_2O–$CHCl_3$ (3:1; 5 × 30 ml). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to give radioactive glaucine methylmethine (**11**) (190 mg) as an oil.

Labelled (**11**) (188 mg) in MeOH (5 ml) was refluxed with MeI for 2 h to give radioactive glaucine methylmethine methiodide (**12**) (184 mg), m.p. 278—280 °C (lit.,[30] 276—280 °C) (molar activity 6.48 × 10^4 disint. min^{-1} $mmol^{-1}$). Labelled (**12**) was converted into the corresponding methohydroxide (**13**) by passage through Amberlite IR-410 anion exchange resin (OH^- form). Labelled (**13**) in MeOH (10 ml) was refluxed with KOH (3.5 g) in water (3 ml) for 2 h. The solvent from the resulting mixture was removed under reduced pressure and the concentrate was diluted with water and extracted with Et_2O–$CHCl_3$ (3:1; 4 × 30 ml). The combined extracts were washed with water, dried (Na_2SO_4), and concentrated under reduced pressure to afford radioactive 3,4,6,7-tetramethoxy-1-vinylphenanthrene (**14**) (102 mg), m.p. 142—143 °C (lit.,[30] 143 °C) (molar activity 6.00 × 10^4 disint. min^{-1} $mmol^{-1}$).

Ozonised O_2 was passed through a solution of radioactive (**14**) (100 mg) in EtOAc (8 ml) at −78 °C for 20 min. The solvent from the resulting mixture was removed under reduced pressure and water, Zn dust (320 mg), and $AgNO_3$ (15 mg) were added to the residue. The mixture was refluxed for 20 min and distilled. The distillate was collected in a solution of dimedone (310 mg) in aqueous EtOH (80 ml) which after being kept for 1 h, was concentrated to 10 ml and left overnight. The precipitate was chromatographed over silica gel column. Elution with C_6H_6–$CHCl_3$ and $CHCl_3$ (t.l.c. control) furnished labelled formaldehyde dimethone, m.p. 187—188 °C (lit.,[31] m.p. 188 °C) (molar activity 5.1 × 10^4 disint. min^{-1} $mmol^{-1}$; 85% of original activity).

Trapping Experiment.—(L)-[U-^{14}C]Tyrosine (activity 0.5 mCi) was fed to the twigs of *L. glutinosa* var. *glabraria* plant and after 7 days the twigs were harvested. The plant material (90 g, wet wt.) was macerated in EtOH (250 ml) with inactive reticuline (**5**) (100 mg) and isoboldine (**6**) (48 mg) separately each being left for 12 h. The EtOH was decanted and the plant material percolated with fresh EtOH (5 × 200 ml). The combined washings were concentrated under reduced pressure to afford a greenish viscous mass which was extracted with 5% HCl (4 × 15 ml). The acidic extract was defatted with light petroleum (3 × 10 ml), basified with Na_2CO_3 (pH 8), and extracted with $CHCl_3$ (5 × 15 ml). The combined extracts were washed with water, dried (Na_2SO_4), and concentrated under reduced pressure to furnish the crude alkaloidal mixture which was subjected to preparative t.l.c. on silica gel GF_{254} (solvent: $CHCl_3$–MeOH, 90:10) to afford radioactive (**5**), base picrate, m.p. 189—190 °C (lit.,[32] 190—192 °C, incorporation 0.3%) and (**6**), m.p. 121—122 °C (lit.,[33] 122—123 °C, incorporation 0.2%).

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