THE ABERRANT BIOSYNTHESIS OF BROMOGLAUCINES[+]

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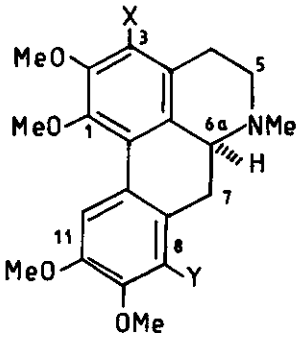
Abstract - The incorporation of racemic 5-bromoreticuline (8) and 2',5-dibromoreticuline (9) into 3-bromoglaucine (2) and 3,8-dibromoglaucine (3) respectively in Litsea glutinosa (Lour.) C.B. Roxb. var. glabraria Hook. (Menispermaceae) has been studied and specific incorporation of 8 and 9 into 2 and 3 was respectively demonstrated. Further, it was demonstrated that the plants do not metabolize 8 and 9 to form glaucine (1).

The biotransformation of unnatural substrates into unnatural products in higher plants is well documented[1-5]. There are also reports where an unnatural substrate has been metabolized by the plants into normal products[6,7]. The biotransformation of 5-bromoreticuline (8) and 2',5-dibromoreticuline (9) into 3-bromoglaucine (2) and 3,8-dibromoglaucine (3) respectively in Litsea glutinosa var. glabraria has been demonstrated by us. Further, it was found that 8 and 9 are not metabolized by the plants to form glaucine (1). Glaucine (1) is biosynthesized from (S)-reticuline (4) in L. glutinosa[8]. (±)-[2',6',8-$^{3}H_{3}$]-Reticuline (7) was initially fed to young L. glutinosa var. glabraria and it was found that the plants were actively biosynthesizing glaucine (1) (1.1% incorporation). Subsequently, suitably bromosubstituted reticulines were fed to the plants. The results of the feeding are recorded in Table 1.

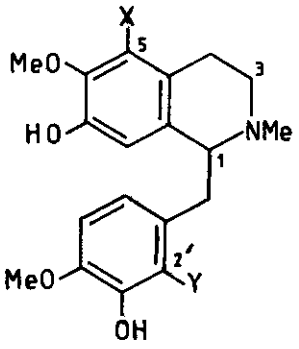
Table 1. Tracer experiments on Litsea glutinosa (Lour.) C.B. Roxb. var. glabraria Hook.

Expt.	Precursor fed	% Incorporation into	
		3-Bromoglaucine (2)	3,8-Dibromoglaucine (3)
1	(±)-5-Bromo[aryl-^{3}H]reticuline (8)	0.11	-
2	(±)-5-Bromo[N-$^{14}CH_{3}$]reticuline (8)	0.42	-
3	(±)-2',5-Dibromo[aryl-^{3}H]reticuline (9)	-	0.21
4	(±)-2',5-Dibromo[N-$^{14}CH_{3}$]reticuline (9)	-	1.03

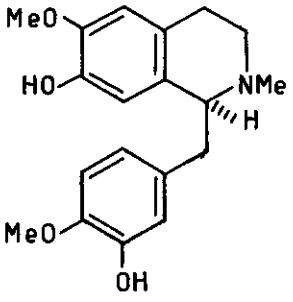
[+]Dedicated to Sir Derek H.R. Barton on the occasion of his 70th birthday.



1 X = Y = H
2 X = Br , Y = H
3 X = Y = Br



7 X = Y = H
8 X = Br , Y = H
9 X = Y = Br



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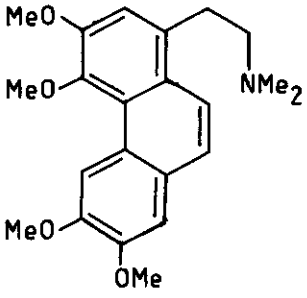

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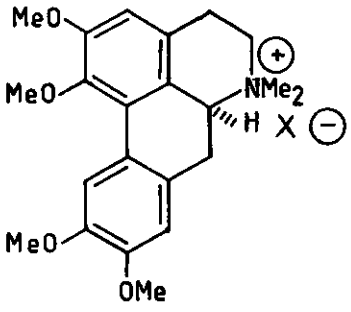
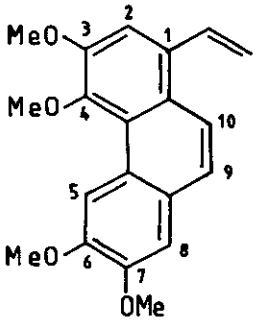


5 X = I
6 X = OH



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Racemic 5-bromo[aryl-^{3}H]reticuline (8) (experiment 1) was fed to young L. glutinosa var. glabraria plants and efficient incorporation of (8) into 3-bromoglaucine (2) was demonstrated. The precursor (8) used, however, was labeled with tritium in the aromatic ring only, which are vulnerable to exchange. Racemic 5-bromo[N-$^{14}CH_3$]reticuline (8) (experiment 2) was then fed to young plants. The precursor (8) was again efficiently incorporated into 3-bromoglaucine (2). The regiospecificity of the label in the biosynthetic 2 was established as follows. Treatment of labeled 2 with Zn-NaOH furnished glaucine (1) having essentially the same molar radioactivity as the parent base. Reaction of 1 with methyl iodide yielded glaucine methiodide (5) which was converted into the corresponding methohydroxide (6) by IR-410 anion exchange resin. Hofmann degradation of (6) yielded glaucine methyl methine (10) with essentially no loss of radioactivity. Treatment of (10) with dimethyl sulphate-potassium hydroxide afforded 3,4,6,7-tetramethoxy-1-vinylphenanthrene (11) (radioinactive) and trimethylamine (trapped as hydrochloride, 94% of original activity).

Feeding of racemic 2',5-dibromo[aryl-^{3}H]reticuline (9) (experiment 3) gave radioactive 3,8-dibromoglaucine (3). The experiment was repeated with racemic 2',5-dibromo[N-$^{14}CH_3$]reticuline (9) (experiment 4) and again (9) was efficiently incorporated into (3). The regiospecificity of the label in the biosynthetic 3,8-dibromoglaucine (3) was determined as follows. Biosynthetic (3) was debrominated with Zn-NaOH to give glaucine (1) having essentially the same molar radioactivity as the parent base. Labeled (1) was degraded to glaucine methyl methine (10) as described above with essentially no loss of radioactivity. Treatment of (10) with dimethyl sulphate-potassium hydroxide furnished 3,4,6,7-tetramethoxy-1-vinylphenanthrene (11) (radioinactive) and trimethylamine (trapped as hydrochloride, 92% of original activity). Glaucine (1) isolated from the feeding of 5-bromo[N-$^{14}CH_3$]reticuline (8) (experiment 2) and 2',5-dibromo-[N-$^{14}CH_3$]reticuline (9) (experiment 4) was found essentially radioinactive.

The foregoing tracer experiments thus demonstrated that the suitably bromosubstituted reticulines are specifically transformed in L. glutinosa var. glabraria into the corresponding bromoglaucines. Further the bromoreticulines are not metabolized by the plants to glaucine.

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see ref. 9.

SYNTHESIS OF PRECURSORS - The racemates of reticuline[9] (7), 5-bromoreticuline[10] (8) and 2',5-dibromoreticuline[10] (9) were prepared by standard method.

SYNTHESIS OF INACTIVE BROMOGLAUCINES - To a stirred solution of glaucine (1) (500 mg) in 10% AcOH (14 ml) was added 10% solution of bromine in glacial AcOH (0.3 ml) at 2-5°C. After stirring at the same temperature for 0.5 h, the reaction mixture was stirred at room temperature for 2 h, basified with Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried (anhyd. Na_2SO_4) and concentrated in vacuo to afford a residue (550 mg). The mixture was purified by preparative

tlc (plates: silica gel GF_{254}, solvent, $CHCl_3$: MeOH; 97:3). The two bands were cut and eluted with $CHCl_3$-MeOH (3:1) to afford 3-bromoglaucine (2) (260 mg); mp 121°C($CHCl_3$-MeOH); uv (MeOH) : 308, 285 and 222 nm; ir (KBr): 2870, 1580, 1510, 1440, 1390, 1250, 1215, 1090, 1000, 875 and 760 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ : 7.83 (s, 1H, H-11), 6.69 (s, 1H, H-8), 3.84 (s, 9H, 3 x OCH_3); 3.63 (s, 3H, OCH_3) and 2.46 (s, 3H, N-CH_3); mass m/z : 435 and 433 (M^+, ^{81}Br and ^{79}Br), 420, 418, 404, 402, 392, 390 and 354; Anal. Calcd for $C_{21}H_{24}NO_4Br$: C, 58.06; H, 5.53; N, 3.23. Found : C, 58.00; H, 5.40; N, 3.18 and 3,8-dibroglaucine (3) (250 mg); mp 114°C ($CHCl_3$-MeOH); uv (MeOH) : 310, 284 and 225 nm; ir (KBr) : 2860, 1570, 1415, 1380, 1220, 1090, 1010, 870 and 765 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ : 7.92 (s, 1H, H-11), 3.94 (s, 9H, 3 x OCH_3), 3.86 (s, 3H, OCH_3) and 2.98 (s, 3H, N-CH_3); mass m/z : 515, 513 and 511 (M^+, ^{81}Br and ^{79}Br). Anal. Calcd for $C_{21}H_{23}NO_4Br_2$: C, 49.12; H, 4.48; N, 2.73. Found : C, 49.04; H, 4.32; N, 2.66.

LABELING OF PRECURSORS : Tritiation - A mixture of (±)-reticuline (7) (100 mg) and tritiated H_2O (0.2 ml, 200 mCi, pretreated with $SOCl_2$, 0.02 ml) in a sealed tube (N_2 atmosphere) was heated at 100°C for 110 h. Work up in the usual manner yielded (±)-[aryl-^3H]reticuline (7).

(±)-5-Bromo[aryl-^3H]reticuline (8) and (±)-2',5-dibromo[aryl-^3H]reticuline (9) were similarly prepared.

(±)-5-Bromo[N-$^{14}CH_3$]reticuline (9) - The corresponding dihydroisoquinoline[10] (100 mg) in C_6H_6 (2 ml) was added to [^{14}C]MeI (frozen) and the mixture kept at 0°C for 2 h. Radioinactive MeI (1 ml) was added to complete the reaction. The radioactive methiodide in MeOH (5 ml) was reduced with $NaBH_4$ and the tetrahydroisoquinoline derivative was heated with methanolic hydrochloric acid to give (±)-5-bromo[N-$^{14}CH_3$]reticuline (8).

(±)-2',5-Dibromo[N-$^{14}CH_3$]reticuline (9) was similarly prepared from the corresponding dihydroisoquinoline.

FEEDING EXPERIMENTS - (±)-[Aryl-^3H]reticuline (7), 5-bromo[aryl-^3H]reticuline (8) and 2',5-dibromo-[aryl-^3H]reticuline (9) were separately dissolved in H_2O (1 ml) containing tartaric acid (5 mg). The young L. glutinosa var. glabraria plants (8 Nos.) were separately dipped into the solution of the precursor. When uptake was complete H_2O added for washing the precursor. The plants were then dipped into H_2O, left for 7 to 8 days to metabolize the precursor and harvested.

ISOLATION OF BIOSYNTHETIC 3-BROMOGLAUCINE (2) - Precursor fed young L. glutinosa var. glabraria plants (110 g, wet wt) were macerated in EtOH (200 ml) with radioinactive 3-bromoglaucine (2, 92 mg) and left at room temperature for 20 h. EtOH was then decanted and the plant material was percolated with fresh EtOH (4 x 200 ml). The solvent from the combined percolate was removed under reduced pressure and the greenish viscous mass was extracted with 10% hydrochloric acid (4 x 30 ml). The acidic extract was defatted with pet. ether (4 x 15 ml), basified with Na_2CO_3, the liberated bases were extracted with $CHCl_3$-MeOH (8:2, 6 x 25 ml), washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent was removed under reduced pressure. The crude base was subjected to preparative chromatography

on silica gel GF_{254} plates (solvent : $CHCl_3$-MeOH, 9:1). The region containing the desired base was cut and eluted with $CHCl_3$-MeOH (3:1) to give labeled 3-bromoglaucine (2, 80 mg), mp 121°C.

ISOLATION OF BIOSYNTHETIC 3,8-DIBROMOGLAUCINE (3) - The young L. glutinosa var. glabraria plants (90 g, wet wt) were macerated in EtOH (200 ml) with radioinactive 3,8-dibromoglaucine (3, 92 mg) and left at room temperature for 18 h. EtOH was then decanted and the plant material was extracted with fresh EtOH (4 x 150 ml). The solvent from the combined percolate was removed under reduced pressure. The greenish viscous mass was extracted with 5% HCl and worked up as above to give the crude base which was subjected to preparative chromatography on silica gel GF_{254} plates (solvent: $CHCl_3$-MeOH, 95:5) to furnish radioactive 3,8-dibromoglaucine (3, 72 mg), mp 114°C ($CHCl_3$-MeOH).

ISOLATION ON GLAUCINE (1) - The young L. glutinosa var. glabraria plants (80 g, wet wt) fed with the labeled bromoreticuline precursors (experiment 2 and 4) were macerated in EtOH (200 ml) with radioinactive glaucine (1, 80 mg) and left for 15 h. The plant material was percolated with EtOH (4 x 200 ml). The combined percolate was worked up as above to give the crude base which was subjected to preparative chromatography on silica gel GF_{254} plates (solvent : $CHCl_3$-MeOH, 9:1) to give glaucine (1, 60 mg), mp 119°C (lit.[11] 120°C).

DEGRADATION OF THE BIOSYNTHETIC 3-BROMOGLAUCINE (2) DERIVED FROM (±)-5-BROMO-[N-$^{14}CH_3$]-RETICULINE (8) - Labeled 2 (180 mg) in EtOH (12 ml) was refluxed with 40% aqueous NaOH (1.2 ml) and Zn powder (1.2 g) for 2 h. The resulting mixture was cooled, filtered and the solvent was removed under reduced pressure. The residue was extracted with $CHCl_3$ (4 x 10 ml), washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent was removed under reduced pressure to give labeled glaucine (1, 150 mg), mp 119°C (lit.[11] 120°C). Labeled 1 (140 mg) in MeOH (10 ml) was treated with MeI to furnish the methiodide (5) (145 mg), mp 222-223°C (lit.[12] 221°C) which was converted into methohydroxide (6) and subjected to Hofmann degradation to furnish radioactive glaucine methyl methine (10). A mixture of methine (10, 100 mg) and H_2O (6 ml) adjusted to pH 10 with KOH, was stirred with Me_2SO_4 (1 ml) and 10 N KOH (0.5 ml) for 2 h at room temperature. A mixture of Me_2SO_4 (0.5 ml) and 10 N KOH (0.25 ml) was added 3 times at an interval of 1 h. The mixture was finally refluxed with KOH (6 g) for 2 h and then subjected to distillation. The distillate was received in 0.2 N HCl and the radioactive trimethylamine hydrochloride was crystallized from MeOH to constant activity. 3,4,6,7-Tetramethoxy-1-vinylphenanthrene (11) (radioinactive) was isolated from the alkaline solution. The radioactivity of the products is given in Table 2.

DEGRADATION OF THE BIOSYNTHETIC 3,8-DIBROMOGLAUCINE (3) DERIVED FROM 2',5-DIBROMO-[N-$^{14}CH_3$]RETICULINE (9) - Labeled 3 (190 mg) was treated with Zn/NaOH as above to afford labeled glaucine (1, 138 mg). Radioactive 1 in MeOH was converted into its methiodide (5, 140 mg), mp 222-23°C

(lit.[12] 221°C), then into methohydroxide (6) and subjected to Hofmann degradation to furnish labeled glaucine methyl methine (10). Labeled 10 was then treated with Me_2SO_4-KOH as above to give radioactive trimethylamine hydrochloride and radioinactive 3,4,6,7-tetramethoxy-1-vinylphenanthrene (11). The radioactivity of the products is given in Table 3.

TABLE 2

Compound	Molar activity (disint. $min^{-1}m\ mol^{-1}$)
3-Bromoglaucine (2)	1.4×10^5
Glaucine (1)	1.39×10^5
Glaucine methiodide (5)	1.42×10^5
Glaucine methyl methine (10)	1.35×10^5
Trimethylamine hydrochloride	1.30×10^5
3,4,6,7-Tetramethoxy-1-vinylphenanthrene (11)	Inactive

TABLE 3

Compound	Molar activity (disint. $min^{-1}m\ mol^{-1}$)
3,8-Dibromoglaucine (3)	1.13×10^5
Glaucine (1)	1.12×10^5
Glaucine methiodide (5)	1.18×10^5
Glaucine methyl methine (10)	1.12×10^5
Trimethylamine hydrochloride	1.05×10^5
3,4,6,7-Tetramethoxy-1-vinylphenanthrene (11)	Inactive

REFERENCES

1. E. Leete and M. R. Chedekel, Phytochemistry, 1972, 11, 2751.
2. E. Leete, J. Org. Chem., 1979, 44, 165.
3. M. L. Rueppel and H. Rapoport, J. Am. Chem. Soc., 1970, 92, 5528.
4. M. L. Rueppel and H. Rapoport, J. Am. Chem. Soc., 1971, 93, 7021.
5. G. W. Kirby, S. R. Massey, and P. Steinreich, J. Chem. Soc., Perkin Trans 1, 1972, 1642.
6. G. Blaschke, H. I. Parker, and H. Rapoport, J. Am. Chem. Soc., 1967, 89, 1540.
7. T. J. Gilbertson and E. Leete, J. Am. Chem. Soc., 1967, 89, 7085.
8. D. S. Bhakuni and S. Jain, J. Chem. Soc., Perkin Trans 1, 1988, 1447.
9. D. S. Bhakuni, S. Tewari, and R. S. Kapil, J. Chem. Soc., Perkin Trans 1, 1977, 706.
10. D. S. Bhakuni and P. Kumar, Indian J. Chem., 1983, 22B, 5.
11. M. Tomita and H. Furukawa, J. Pharm. Soc. Japan, 1962, 82, 1199.
12. T. Kametani, K. Fukumoto, S. Shibuya, H. Nemoto, T. Nakano, T. Sugahara, T. Takahashi, Y. Aizawa, and M. Toriyama, J. Chem. Soc. (C), 1972, 1435.

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