Biosynthesis of Isocorydine (1,2,10-Trimethoxy-6aα-aporphin-11-ol)

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The incorporation of (±)-nororientaline, (±)-norprotosinomenine, (±)-reticuline, and (±)-norlaudanidine into isocorydine in *Annona squamosa* has been studied, and the specific utilization of (±)-reticuline demonstrated. The evidence supports the direct *ortho–ortho* oxidative coupling of (+)-nor-reticuline to give the isocorydine nucleus.

Isocorydine (1), a 1,2,10,11-tetrasubstituted aporphine alkaloid, could be formed in nature from suitable 1-benzyltetrahydroisoquinoline precursors by three possible alternative biogenetic pathways. Direct *ortho–ortho* oxidative coupling of reticuline [1] (9) could provide corytuberine (2), from which isocorydine could be formed by methylation at C-1. In alternative proposals, isocorydine could be biosynthesised from orientaline [2] (5) or protosinomenine [3] (7) *via* the dienone (12) or (13), respectively.

Feeding of (±)-nororientaline (4) (Table) (experiment 1) in parallel with (±)-norprotosinomenine (6) (experiment 2) and (±)-reticuline (9) (experiment 3) established that only reticuline was efficiently metabolised by *Annona squamosa* Linn. (Annonaceae) plants to form isocorydine. As expected the partially methylated 1-benzyltetrahydroisoquinoline, (±)-norlaudanidine (10) (experiment 4) was not incorporated. The labelled isocorydine derived from (±)-reticuline feeding was brominated to afford 8-bromoisocorydine (3), which was virtually radioinactive.

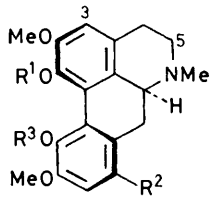
Tracer experiments on *A. squamosa*

Expt.	Precursor fed	% Incorporation into isocorydine (1)
1	(±)-[5′,8-$^3H_2$]Nororientaline (4)	0.006
2	(±)-[2′,6′,5-$^3H_3$]Norprotosinomenine (6)	<0.001
3	(±)-[2′,6′,8-$^3H_3$]Reticuline (9)	0.21
4	(±)-[2′,6′-$^3H_2$]Norlaudanidine (10)	<0.001
5	(+)-[2′,6′,8-$^3H_3$]Nor-reticuline (11)	0.18
6	(−)-[2′,6′,8-$^3H_3$]Nor-reticuline	0.002

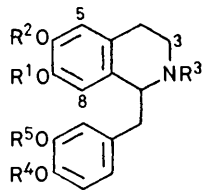
The foregoing experiments thus established that (±)-

[1] D. H. R. Barton and T. Cohen, *Festschr. A. Stoll*, 1957, 117.
[2] A. R. Battersby in ' Oxidative Coupling of Phenols,' eds. A. R. Battersby and W. I. Taylor, Dekker, New York, 1967, p. 119.
[3] A. R. Battersby, J. L. McHugh, J. Staunton, and M. Todd, *Chem. Comm.*, 1971, 985.

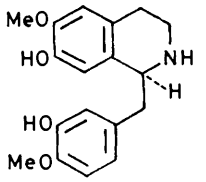
reticuline is a specific precursor of isocorydine in *A. squamosa*. The precursor used, however, was racemic. Parallel feeding with (+)-nor-reticuline (experiment 5) and (−)-nor-reticuline (experiment 6) demonstrated that stereospecificity was maintained in the bioconversion



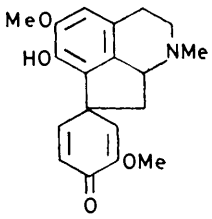
(1) R^1 = Me, R^2 = R^3 = H
(2) R^1 = R^2 = R^3 = H
(3) R^1 = Me, R^2 = Br, R^3 = H



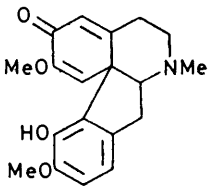
(4) R^1 = R^3 = R^4 = H, R^2 = R^5 = Me
(5) R^1 = R^4 = H, R^2 = R^3 = R^5 = Me
(6) R^1 = R^4 = Me, R^2 = R^3 = R^5 = H
(7) R^1 = R^3 = R^4 = Me, R^2 = R^5 = H
(8) R^1 = R^3 = R^5 = H, R^2 = R^4 = Me
(9) R^1 = R^5 = H, R^2 = R^3 = R^4 = Me
(10) R^1 = R^2 = R^4 = Me, R^3 = R^5 = H



(11)



(12)



(13)

of 1-benzyltetrahydroisoquinoline precursors into isocorydine. The former was incorporated 90 times more efficiently than the latter. The specificity of the label in biosynthetic isocorydine derived from (+)-nor-reticuline (11) feeding was established by the bromination procedure as well as by refluxing radioactive isocorydine with aqueous sodium hydroxide to give essentially inactive (1).

The presence of reticuline in *A. squamosa* was confirmed by a trapping experiment with (−)-[U-^{14}C]-tyrosine (incorporation 0.28%). Reticuline thus fulfils both requirements of a true precursor. The foregoing experiments strongly support the following sequence for the biosynthesis of isocorydine in *A. squamosa*: (+)-(11) ⟶ (9) ⟶ (1).

EXPERIMENTAL

For general directions (spectroscopy details, counting method, synthesis, and labelling of precursors) see refs. 4 and 5.

Feeding Experiments.—Labelled reticuline and nor-protosinomenine were fed as their hydrochlorides, and nor-orientaline, nor-reticuline, and norlaudanidine were fed as their tartrates, by the stem cut method, to *A. squamosa* shoots. The plants were left for 5—6 days for metabolisation and then worked up for isocorydine.

Isolation of Isocorydine.—The stems and leaves (typically 75 g wet wt.) of the plants were macerated in ethanol (500 ml) with inactive isocorydine (70 mg) and left overnight. The ethanolic extract was decanted and the residue was percolated with fresh ethanol (4 × 200 ml). The combined ethanolic extract was concentrated under reduced pressure. The green viscous mass so obtained was extracted with N-hydrochloric acid (2 × 5 ml). The acidic solution was defatted with light petroleum (6 × 50 ml) and then basified with aqueous sodium hydrogen carbonate. The liberated bases were extracted with chloroform (6 × 50 ml); the extracts were washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to afford crude isocorydine (50 mg), which was purified by chromatography over a column of neutral alumina (2.0 g) [elution with chloroform and chloroform–methanol (99 : 1)]. The fractions containing pure isocorydine (t.l.c.) were mixed and evaporated, and the residue was crystallized from chloroform–petroleum to afford isocorydine (35 mg), m.p. 185° (lit.,[6] 185°). The radiopurity of the biosynthetic isocorydine was checked by the reverse dilution technique and further conversion into its hydrochloride.

Degradation of Isocorydine.—(±)-*Reticuline feeding.* The [8-^{3}H]isocorydine (50 mg; specific activity 2.66 × 10^4 disint. min^{-1} mg^{-1}; molar activity 8.93 × 10^6 disint. min^{-1} mmol^{-1}) was treated with a 1% solution of bromine in carbon tetrachloride until the mixture furnished no more precipitate. It was then kept overnight at 5 °C. The precipitate was filtered off, washed with carbon tetrachloride (3 × 5 ml), and dried. The residue was dissolved in water (3 ml) and basified with aqueous sodium hydrogen carbonate, and the liberated base was extracted with chloroform (5 × 20 ml). The combined organic layer was washed with water, dried (Na_2SO_4), and concentrated and the residue was chromatographed on a column of neutral alumina (6.0 g) [elution with chloroform and chloroform–methanol (99 : 1)]. Appropriate fractions (t.l.c.) were mixed to afford 8-*bromoisocorydine* (20.0 mg) as an oil (Found: C, 56.95; H, 5.3; N, 3.2. $C_{20}H_{22}BrNO_4$ requires C, 57.15; H, 5.3; N, 3.35%) (specific activity 12 disint. min^{-1} mg^{-1}; molar activity 5.04 × 10^3 disint. min^{-1} mmol^{-1}).

(±)-*Nor-reticuline feeding.* (*A*) To a solution of [8-^{3}H]-isocorydine (30 mg; specific activity 1.89 × 10^4 disint. min^{-1} mg^{-1}; molar activity 7.02 × 10^6 disint. min^{-1} mmol^{-1}) in carbon tetrachloride (10 ml) was added dropwise a 1% solution of bromine in carbon tetrachloride until the mixture furnished no more precipitate. After 12 h at 5 °C the mixture was worked up to furnish 8-bromoisocorydine (15 mg) (specific activity 10 disint. min^{-1} mg^{-1}; molar activity 4.20 × 10^3 disint. min^{-1} mmol^{-1}).

(*B*) A solution of [8-^{3}H]isocorydine (10 mg; specific activity 1.89 × 10^4 disint. min^{-1} mg^{-1}; molar activity 7.02 × 10^6 disint. min^{-1} mmol^{-1}) in aqueous 0.5N-sodium hydroxide (5 ml) was heated under nitrogen at 98 °C for 20 h.

[4] D. S. Bhakuni, S. Tewari, and R. S. Kapil, *J.C.S. Perkin I*, 1977, 706.

[5] D. S. Bhakuni, A. N. Singh, S. Tewari, and R. S. Kapil, *J.C.S. Perkin I*, 1977, 1662.

[6] T. Kametani, T. Sugahara, and K. Fukumoto, *Tetrahedron*, 1971, **27**, 5367.

The resulting mixture was worked up in the usual manner to afford radioinactive isocorydine.

Feeding of (−)-[U-^{14}C]*tyrosine*. A solution of (−)-[U-^{14}C]tyrosine (total activity 10.21×10^7 disint. min^{-1}) in water (0.5 ml) was fed to young *A. squamosa* plants (8 nos.). The plants were kept alive for 4 days, harvested, macerated in ethanol (500 ml) with inactive reticuline (118.8 mg), and left overnight. The ethanolic extract was decanted and the residue percolated with fresh ethanol (5×400 ml). The combined ethanolic extract was concentrated under reduced pressure and the residue was extracted with N-hydrochloric acid (2×5 ml). The acidic solution was defatted with light petroleum (6×50 ml) and basified with aqueous sodium hydrogen carbonate. The liberated bases were extracted with chloroform (6×50 ml). The combined chloroform layer was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to afford crude reticuline (100 mg), which was chromatographed over a column of neutral alumina (6.0 g) [elution with chloroform and chloroform–methanol (99 : 1)]. Fractions containing pure reticuline (t.l.c.) were mixed and concentrated to afford radioactive reticuline (75.5 mg), which was further purified through its perchlorate, m.p. 144—145° (lit.,[7] 144—145°) and crystallized to constant activity from absolute ethanol (specific activity 2.32×10^3 disint. min^{-1} mg^{-1}; molar activity 1.04×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.28%).

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[7] K. W. Gopinath, T. R. Govindachari, B. R. Pai, and N. Viswanathan, *Chem. Ber.*, 1959, **92**, 1657.