Biosynthesis of the Alkaloid Boldine

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Summary Tracer experiments prove that boldine is biosynthesised in *Litsea glutinosa* from (+)-reticuline *via* (+)-isoboldine.

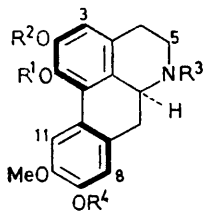
BOLDINE, the choleretic principle of *Peumus boldus*[1] Molina (Monimiaceae) was first isolated in 1872. Its structure[2] has been assigned to be (**1**) (undefined stereochemistry), and its stereochemistry and absolute configuration has recently been determined.[3]

Boldine could be biosynthesised from suitable 1-benzylisoquinoline precursors. Direct oxidative coupling of reticuline[4] (**8**) could give rise to the boldine nucleus, whereas oxidative coupling of orientaline[5] (**10**) and protosinomenine[6] (**12**), involving the dienones (**15**) and (**16**) respectively as intermediates, would furnish the required aporphine system by dienone–phenol rearrangement.

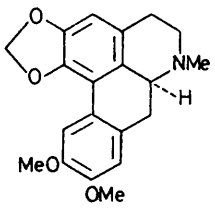
Feeding of (±)-tyrosine (expt. 1) in parallel with (±)-nororientaline (**9**) (expt. 3) and (±)-norprotosinomenine (**11**) (expt. 4) (Table) established that *Litsea glutinosa* (Lour.) C. B. Rob. var. *glabraria* Hook. f. (Lauraceae) plants were actively biosynthesising boldine. Both (**9**) and (**11**) were less efficiently incorporated. Feeding with (±)-nor-reticuline (**7**) (expt. 5) and (±)-reticuline (**8**) (expt.

TABLE Tracer experiments on *L. glutinosa*

Expt.	Precursor fed	Incorporation (%) into boldine (**1**)
1	(±)-[2-^{14}C]Tyrosine	0·16
2	(±)-[1-^{3}H]4-*O*-Methylnorlaudanosoline (**6**)	0·13
3	(±)-[5′,8-$^{3}H_2$]Nororientaline (**9**)	0·01
4	(±)-[3-^{14}C]Norprotosinomenine (**11**)	0·06
5	(±)-[3-^{14}C]Nor-reticuline (**7**)	0·62
6	(±)-[2′,6′,8-$^{3}H_3$]Reticuline (**8**)	0·44
7	(+)-[2′6′,8-$^{3}H_3$]Reticuline (**14**)	0·43
8	(−)-[2′,6′,8-$^{3}H_3$]Reticuline	0·01
9	(±)-[1-^{3}H, 6-*O*-$^{14}CH_3$]Reticuline (**8**)	0·90
10	(±)-[2′,6′,8-$^{3}H_3$]Laudanosine (**13**)	0·006
11	(+)-[8-^{3}H]Isoboldine (**2**)	2·00
12	(+)-[aryl-^{3}H]Norboldine (**3**)	1·86



(1) $R^1=R^3=Me$, $R^2=R^4=H$
(2) $R^1=R^4=H$, $R^2=R^3=Me$
(3) $R^1=Me$, $R^2=R^3=R^4=H$
(4) $R^1=R^2=R^3=R^4=Me$



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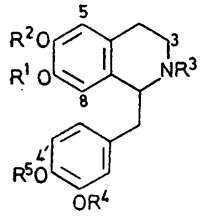
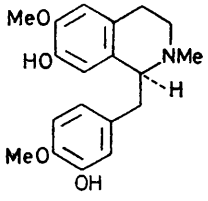


(6) $R^1=R^2=R^3=R^4=H$, $R^5=Me$
(7) $R^1=R^3=R^4=H$, $R^2=R^5=Me$
(8) $R^1=R^4=H$, $R^2=R^3=R^5=Me$
(9) $R^1=R^3=R^5=H$, $R^2=R^4=Me$
(10) $R^1=R^5=H$, $R^2=R^3=R^4=Me$
(11) $R^1=R^5=Me$, $R^2=R^3=R^4=H$
(12) $R^1=R^3=R^5=Me$, $R^2=R^4=H$
(13) $R^1=R^2=R^3=R^4=R^5=Me$



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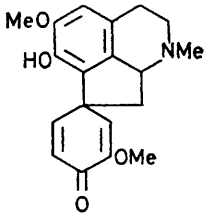

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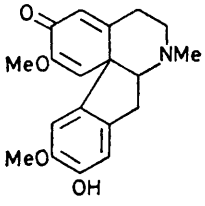

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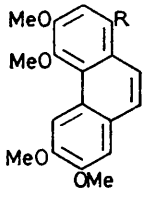

(17) R=vinyl
(18) R=CHO

6) showed that both these compounds were efficient precursors of boldine. As expected the completely methylated 1-benzylisoquinoline (±)-laudanosine (**13**) (expt. 10) was not incorporated.

The radioactive boldine derived from (±)-reticuline feeding was brominated to afford a mixture of 3-bromo- and 3,8-dibromo-boldines. The former was found to possess all the activity (97·22% of original), while the latter was virtually inactive (<1·00%). Labelled boldine derived from (±)-nor-reticuline was converted into glaucine (**4**) (no loss of activity). Degradation of (**4**) by double Hofmann elimination[2] gave (**17**)(no loss of activity), which was cleaved by OsO_4–$NaIO_4$ to (**18**) (radioinactive). Ozonolysis of (**17**) furnished formaldehyde, trapped as its dimedone derivative (98% of original activity).

Both (+)-isoboldine (**2**) (expt. 11) and (+)-norboldine (**3**) (expt. 12) were efficiently incorporated into boldine. The specificity of the label in the biosynthetic boldine (expt. 11) was again shown by bromination, the label being found at the expected position. Feeding with (+)-reticuline (**14**) (expt. 7) and (−)-reticuline (expt. 8) showed that stereospecificity is maintained in the bioconversion of 1-benzylisoquinoline into boldine. The loss of activity in the methoxy-group (64% of original) in the biosynthesis of boldine from doubly labelled (±)-reticuline (**8**) (expt. 9) provided evidence against methyl migration. Similar results have been obtained by Barton and his co-workers[7] in the biosynthesis of crotonosine.

Reticuline and isoboldine have previously been isolated from *L. glutinosa*[8] and we confirmed the presence of the former by feeding (−)-[*U*-^{14}C]tyrosine (incorporation, 0·16%); reticuline and isoboldine are thus true intermediates. The young shoots of *L.glutinosa* were also found to metabolise (±)-4′-*O*-methylnorlaudanosoline (**6**) (expt. 2) and its presence in the plant was showed by a trapping experiment [(−)-(*U*-^{14}C)tyrosine; incorporation, 0·17%]. The foregoing results thus strongly support the following sequence for the biosynthesis of boldine in *L. glutinosa*:

(**6**) → (**7**) → (**14**) → (**2**) → (**1**).

It is interesting that the aporphine alkaloids, glaucine (**4**) and dicentrine (**5**), are biosynthesised in *Dicentra eximia*[6] (Ker) Torr. (Fumariaceae) from norprotosinomenine.

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