The Biosynthesis of Reticuline

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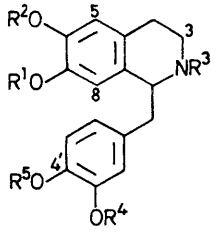
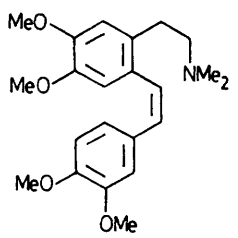
Summary Tracer experiments prove that dopa contributes only to the formation of the phenethylamine portion of reticuline in *Litsea glutinosa* and that the benzylic portion is biosynthesised from 3,4-dihydroxyphenylpyruvic acid not derived from dopa.

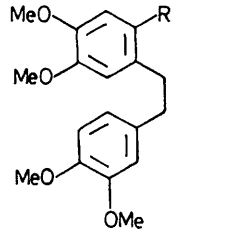
RETICULINE (**1**), the putative precursor for the biosynthesis of a large number of 1-benzyltetrahydroisoquinoline derived alkaloids[1] could itself be formed in Nature from norlaudanosoline (**3**), which is considered to be derived from two fragments (**16**) [or (**17**)] and (**19**) each derivable from



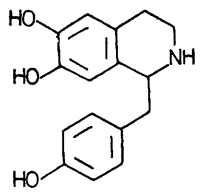
(1) $R^1 = R^4 = H$, $R^2 = R^3 = R^5 = Me$
(2) $R^1 = R^3 = R^4 = H$, $R^2 = R^5 = Me$
(3) $R^1 = R^2 = R^3 = R^4 = R^5 = H$
(4) $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Me$
(5) $R^1 = R^3 = R^4 = R^5 = H$, $R^2 = Me$
(6) $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = Me$
(7) $R^1 = R^2 = R^3 = R^4 = R^5 = Me$



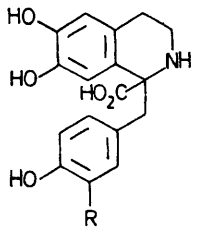
(8)



(9) R = $(CH_2)_2NMe_2$
(10) R = vinyl



(11)



(12) R = H
(13) R = OH

dopa[2] (**18**). Since tyrosine (**14**) is an established precursor of (**18**) in micro-organisms,[3] an identical mode of biosynthesis for dopa was considered to follow in higher plants. This assumption was apparently supported by feeding radioactive tyrosine.[4]

Recently, a considerable body of evidence has emerged concerning the duality of the role of tyrosine in 1-benzylisoquinoline biosynthesis. Tyrosine has been suggested to give rise to two different constituent units by independent pathways.[5] Furthermore, dopa has been shown to be a precursor of only a portion of the molecules of glaucine[6] and morphine.[7]

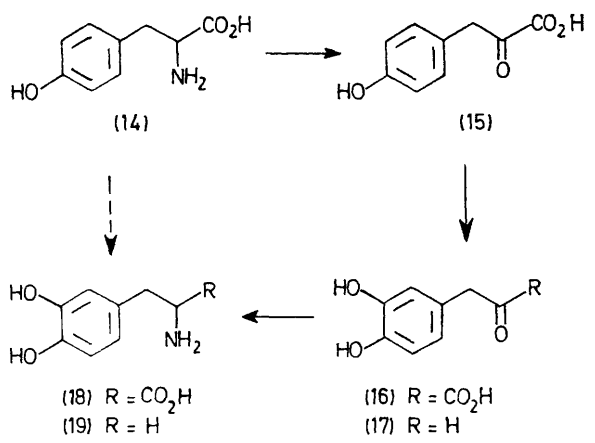
TABLE. Tracer experiments on *L. glutinosa*

Expt.	Precursor fed	% Incorporation into reticuline (**1**)
1	(±)-[2-^{14}C]Dopa (**18**)	0·35
2	(±)-[2-^{14}C]Tyrosine (**14**) and (±)-[3-^{14}C]Dopa (**18**) (specific activity ratio 50 : 61)	0·34
3	(±)-[aryl-^{3}H]Norcoclaurine (**11**) ..	0·002
4	(±)-[3-^{14}C]6,7-Dihydroxy-1-(4-hydroxybenzyl)-1,2,3,4-tetrahydroisoquinaldic acid (**12**) ..	<0·001
5	(±)-[1-^{3}H]6-*O*-Methylnorlaudanosoline (**5**)	0·12
6	(±)-[1-^{3}H]4′-*O*-Methylnorlaudanosoline (**6**)	0·18
7	(±)-[aryl-^{3}H]Laudanosoline (**4**) ..	0·002
8	(±)-[2′,6′, 8-$^3H_3$]Norreticuline (**2**) ..	0·45

Feeding of (±)-[2-^{14}C]dopa (expt. 1) and a mixture of (±)-[2-^{14}C]tyrosine and (±)-[3-^{14}C]dopa (specific activity ratio 50 : 61) (expt. 2) (Table) to *Litsea glutinosa* (Lauraceae) plants showed that both these compounds were efficient precursors of (**1**). The labelled reticuline derived from the first experiment was converted into laudanosine (**7**) with no loss of activity. Hofmann elimination[4] gave (**8**) (98% of original activity). Catalytic hydrogenation of (**8**) gave (**9**) with no loss of activity. A second Hofmann degradation yielded (**10**), which on ozonolysis gave formaldehyde, trapped as its dimedone derivative (97% of original activity). The labelled reticuline derived from the second experiment (calc. value for tyrosine: 45% of the mixture) was similarly degraded to give formaldehyde dimedone (24% of original activity). Controlled potassium permanganate oxidation of (**8**) gave veratric acid (radioinactive). These results suggest that while tyrosine gives rise to both the portions, dopa contributes only in the formation of the phenethylamine portion of (**1**).

Feeding of (±)-[aryl-^{3}H]norcoclaurine (**11**) (expt. 3) and (±)-[3-^{14}C]6,7-dihydroxy-1-(4-hydroxybenzyl)-1,2,3,4-tetrahydroisoquinaldic acid (**12**) (expt. 4) showed that a 4′,6,7-trihydroxy-1-benzyltetrahydroisoquinoline† precursor was not involved. Parallel feedings of (±)-[1-^{3}H]-6-*O*-methylnorlaudanosoline (**5**) (expt. 5), (±)-[1-^{3}H]4′-*O*-methylnorlaudanosoline (**6**) (expt. 6), (±)-[aryl-^{3}H]laudanosoline (**4**) (expt. 7) and (±)-[2′,6′,8-$^3H_3$]nor-reticuline (**2**) (expt. 8) suggest that *O*-methylation precedes *N*-methylation in the biosynthesis of reticuline.

† Independently of us Prof. A. R. Battersby has carried out the feeding of (**12**) and (**13**) to *Papaer somniferum* plants. While the former was not incorporated, (**13**) was shown to be the specific precursor of morphine.



SCHEME

Direct hydroxylation of (**14**) could afford (**18**); alternatively deamination of (**14**) to *p*-hydroxyphenylpyruvic acid (**15**), followed by hydroxylation to 3,4-dihydroxyphenylpyruvic acid (**16**) and finally amination of (**16**) could yield (**18**). From the present experiments it is not possible to decide whether in these plants dopa is biosynthesised by both these pathways or if the latter route is preferred (Scheme). However, they do strongly support the fact that the enzyme system present is not capable of deaminating (**18**) to (**16**) and thus, dopa does not give rise to the two constituent units of the 1-benzylisoquinoline system as exemplified by reticuline.

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