

REVISION OF THE EARLY STEPS OF RETICULINE BIOSYNTHESIS

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Abstract: Precursor feeding experiments to Annona reticulata leaves demonstrated that coclaurine and reticuline are both derived from the common intermediate norcoclaurine; furthermore (S)-coclaurine was found to be the specific precursor to the major classes of isoquinoline alkaloids: protoberberines, benzophenanthridines and morphinandienones.

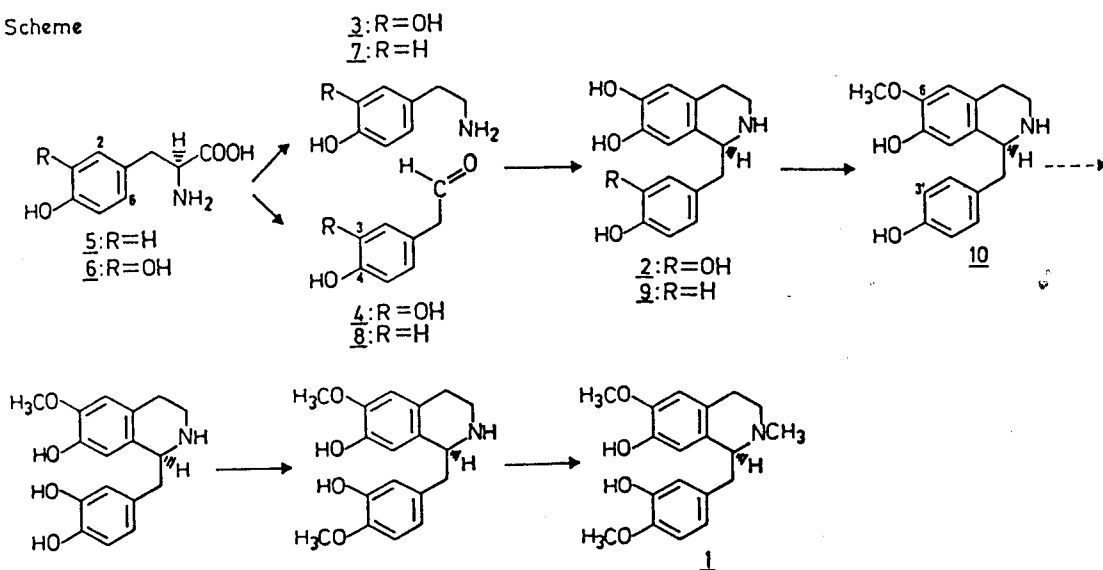
Reticuline (1) is firmly established as the biosynthetic precursor of a vast number of isoquinoline alkaloids [1,2]. Norlaudanosoline (2) has up till now been assumed the universal precursor for reticuline [3,4]. This compound was proposed purely on chemical reasoning as precursor for isoquinoline alkaloids already in 1910 by Winterstein and Trier [5] and later by R. Robinson [6,7], representing the first major contribution to biogenetic hypothesis [8]. This tetrahydroxybenzylisoquinoline derivative (2) is supposed to be formed by the enzyme catalyzed stereospecific condensation of dopamine (3) with 3,4-dihydroxyphenylacetaldehyde (4). For both building blocks tyrosine (5) is an amply demonstrated precursor, however, 3,4-dihydroxyphenylalanine (6) or its amine (3) which already possess the required 3,4-dihydroxy-configuration in the aromatic rings label only the isoquinoline and not the benzylic portion of the alkaloids [8,9]. These findings prove that the two C₆-C₂ units derived from tyrosine (5) differ from one another. Recently it was demonstrated that not only (5), but in addition tyramine (7), can serve as precursor of the benzylic portion of these alkaloids [10,11]. Since monohydroxylated (7) but not dopamine (3) enters this part of the alkaloids [8], it could not be excluded that 4-hydroxyphenylacetaldehyde (8) and therefore norcoclaurine (9) and coclaurine (10) are the true precursors of reticuline (1) and not norlaudanosoline (2) as hitherto assumed.

In order to test this possibility, mature leaves of greenhouse grown Annona reticulata L., which contain both (S)-reticuline (1, 0.09% dwt) [12] and (S)-coclaurine (10, 0.06% dwt) [13], were fed with a mixture of L-[2,6-³H]-tyrosine and L-[ring-U-¹⁴C]-tyrosine in a ratio of 7.4 : 1. Both (10) and (1) were isolated, purified to constant specific activity and methylated (diazomethane) to laudanosine (11) (ratio found 6.3 : 1) and 4'-O-methylnorarmepavine (12) (ratio 5.5 : 1), respectively. A theoretical ratio of 5.5 : 1, calculated on the basis that one tritium atom of dopamine (3) is removed during the condensation of the amine (3) with a phenylacetaldehyde [14,15] was expected. Within experimental limits, these results demonstrate that both benzylisoquinoline derivatives (10,1) are formed from tyrosine as predicted and no degradation of tyrosine [16] occurs in this

plant. Since both (R,S)-[3-¹⁴C]-norlaudanosoline and (R,S)-[3-¹⁴C]-norcoclaurine were not incorporated into the alkaloidal fraction but instead led to browning of the leaf base indicative of oxidation of these compounds, (S)-[6-0-¹⁴CH₃]-coclaurine (10) was fed to the plant. Good incorporation (2.6%) into (S)-reticuline (1) was observed. Methylation of this compound yielded laudanosine (11) (2716 dpm/μmole) which was subjected to ceric ammonium nitrate oxidation [17]. The resulting veratraldehyde (13) corresponding to the benzylic portion of (11) was devoid of any radioactivity, while the 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (14) was labelled (2784 dpm/μmole), demonstrating that (S)-coclaurine (10) is specifically incorporated into reticuline (1). (R)-[6-0-¹⁴CH₃]-Coclaurine was not incorporated into (1) showing that no racemisation occurs during this transformation and that the (S)-derivative is the true precursor. The incorporation of labelled (S)-(10) into (S)-reticuline (1) was confirmed using calli of *Fumaria capreolata* (0.87% incorporation). In addition, (S)-(10) labelled the entire set of benzyliisoquinoline alkaloids present in this culture [18].

If it is assumed that (S)-coclaurine (10) is the biosynthetic precursor of (S)-reticuline (1), identical ratios of C₆-C₂ units derived from tyrosine should be found in the two halves of both the coclaurine (10) and reticuline (1) molecules. L-[Ring-U-¹⁴C]-tyrosine was fed to *Annona* leaves, (10) and (1) were purified to constant specific activity, methylated and subjected to Ce⁴⁺ oxidation as described above. 4'-O-Methylnorarmepavine (12) showed a ratio of isoquinoline: benzyl portion of 1 : 4.90 while laudanosine (11) yielded a ratio of 1 : 4.71. Within experimental error, these results demonstrate that both compounds must have one and the same biogenetic origin. Two C₆-C₂ units, namely dopamine (3) and 4-hydroxyphenylacetaldehyde (8) condense in a stereospecific manner to (9). 6-O-Methylation yields (S)-coclaurine (10) which is transformed by 3'-hydroxylation and subsequent 4'-O- as well as N-methylation to reticuline (1) as shown in the Scheme.

Scheme



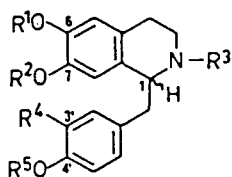
It is of interest to note that clearly the majority of label from tyrosine was found in the lower (benzylic) half of (10) and (1) which has not been observed previously [8,9]. Chemical examination of the leaf tissue revealed the presence of an exceedingly large pool of dopamine (3) (ca.35 mM, 3.03% dwt) while only trace quantities of tyramine were observed. This fact indicates that unlike 4-hydroxyphenylacetaldehyde (8), labelled dopamine (3) derived from (6) will be highly diluted prior to incorporation into norcoclaurine (9). This most plausibly explains the higher levels of radioactivity found in the lower halves of (10) and (1).

Reticuline (1) is the building block for protoberberines, benzophenanthridines and morphinandienones [2] as well as many other alkaloid species. If coclaurine (10) is truly the biosynthetic precursor for reticuline (1), then (S)-(10) should also be specifically incorporated into these classes of reticuline derived alkaloids. (S)-Coclaurine (10) is indeed incorporated into the target molecules. In *Berberis stolonifera* calli [19], (S)-[6-0-¹⁴CH₃]- (10) is not only incorporated into the bisbenzylisoquinoline alkaloid berbaminine (15, 1.73%), but also into the protoberberines columbamine (16, 2.18%) and jatrorrhizine (17, 5.94%). Demethylation (HBr) of the acetylated tetrahydroprotoberberine showed that only 3.5% of the original radioactivity remained in the tetrahydroxyprotoberberine skeleton. Good incorporation (4.6%) of (S)-(10) into the benzophenanthridine alkaloid macarpine (18) was also observed in cell cultures of *Eschscholzia californica*. Finally, (S)-[6-0-¹⁴CH₃]- (10) was an excellent precursor (5.7% incorporation) in seedlings of *Papaver somniferum* of the morphinandienone alkaloid, thebaine (19), a known precursor of codeine and morphine. Label from the precursor was exclusively located in the 6-0-methyl position of thebaine (19) as shown by mild and selective demethylation [20]. The resultant codeinone was unlabelled. Repetition of the feeding experiments with the (R)-enantiomer of [6-0-¹⁴CH₃]-cocclaurine (10) showed absolutely no incorporation into protoberberines, benzophenanthridines or morphinandienone alkaloids with the exception of berbaminine (15) where (R)-(10) was incorporated (1.92%) predominantly (82.1%) into the (R)-half of this molecule.

On the basis of these experiments we conclude that coclaurine and reticuline derived alkaloids have not, as previously assumed, two different precursors, norcoclaurine (9) and norlaudanosoline (2), but instead have a common origin, namely norcoclaurine (9). The revision of the biosynthetic pathway leading to the central intermediate, reticuline (1), is shown in the Scheme. All of the enzymes involved in the (S)-reticuline pathway have been discovered [10] except for the hydroxylase which introduces the 4'-hydroxyl group into the coclaurine (10) skeleton. This proposed pathway is consistent with experimental data in the literature which until now have been difficult to interpret.

Acknowledgement:

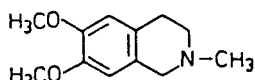
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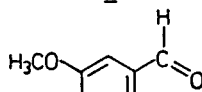
11: $R^1=R^2=R^3=R^5=CH_3, R^4=OCH_3$

12: $R^1=R^2=R^5=CH_3, R^3=R^4=H$

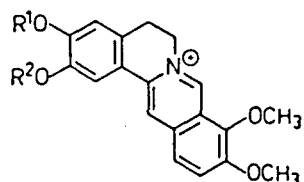
$1H=\alpha=(S), 1H=\beta=(R)$



14

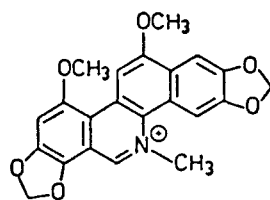


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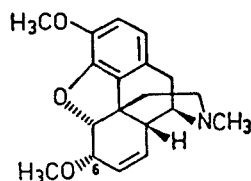


16: $R^1=CH_3, R^2=H$

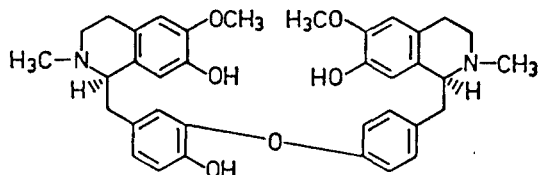
17: $R^1=H, R^2=CH_3$



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