Norcoclaurine as Biosynthetic Precursor of Thebaine and Morphine

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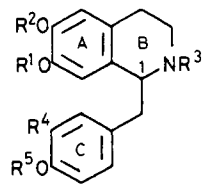
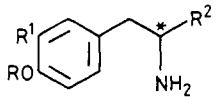
(*R,S*)-[1-^{13}C]Norcoclaurine and (*R,S*)-[1-^{13}C]coclaurine fed to *Papaver somniferum* plants afford highly enriched thebaine and morphine which are specifically labelled at position C-9; the results are interpreted as showing that the morphine skeleton is built up from the condensation product of dopamine with 4-hydroxyphenylacetaldehyde.

Numerous tracer experiments have established that the morphine skeleton is derived from two molecules of L-tyrosine (**7**).[1] Two C_6–C_2 units derived from this amino acid combine to form the first alkaloid precursor. (*S*)-Norlaudanosoline (**2**), resulting from the condensation of dopamine (**10**) with 3,4-dihydroxyphenylacetaldehyde (**12**) both derived from a dihydroxylated tyrosine derivative, was recognised as this first intermediate.[2] This suggestion was in full agreement with earlier predictions.[3,4] However, the unequal distribution of radioactivity between the two labelled centres in tyrosine-derived thebaine (**13**)[1] and the non-random incorporation of $^{14}CO_2$ into morphine[5] shows that the C_6–C_2 units must differ from one another in their biogenetic origin. This discrepancy prompted us to reinvestigate the early precursors of the morphine skeleton.

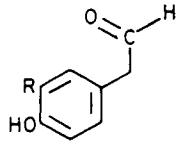
In order to prove unequivocally the specific incorporation of potential precursors, ^{13}C-labelled molecules were utilized. To achieve sufficiently high incorporation, 5-day-old seedlings of *Papaver somniferum*, which are known to accumulate appreciable amounts of thebaine (**13**) at this stage,[6,7] were used. Between 30 and 100 μg of the labelled material was administered to each seedling through the root system. Typically, 200 seedlings were extracted after a metabolic period of 48 h at 2000 lux (neon), 20 °C and 83% humidity. Thebaine (**13**) was isolated by standard procedures[8] supplemented by t.l.c., yielding spectroscopically (u.v., m.s.)



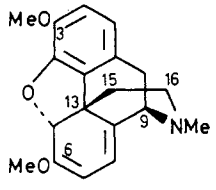
(1) $R^1 = R^2 = R^3 = R^4 = R^5 = H$
(2) $R^1 = R^2 = R^3 = R^5 = H$; $R^4 = OH$
(3) $R^1 = R^3 = R^4 = R^5 = H$; $R^2 = Me$
(4) $R^1 = R^3 = R^5 = H$; $R^2 = Me$; $R^4 = OH$
(5) $R^1 = R^3 = H$; $R^2 = R^5 = Me$; $R^4 = OH$
(6) $R^1 = H$; $R^2 = R^3 = R^5 = Me$; $R^4 = OH$
1-H = α = (S); 1-H = β = (R)



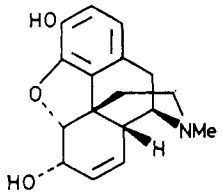
(7) $R^1 = H$; $R^2 = CO_2H$
(8) $R^1 = OH$; $R^2 = CO_2H$
(9) $R^1 = R^2 = H$
(10) $R^1 = OH$; R^2 = H



(11) R = H
(12) R = OH



(13)



(14)

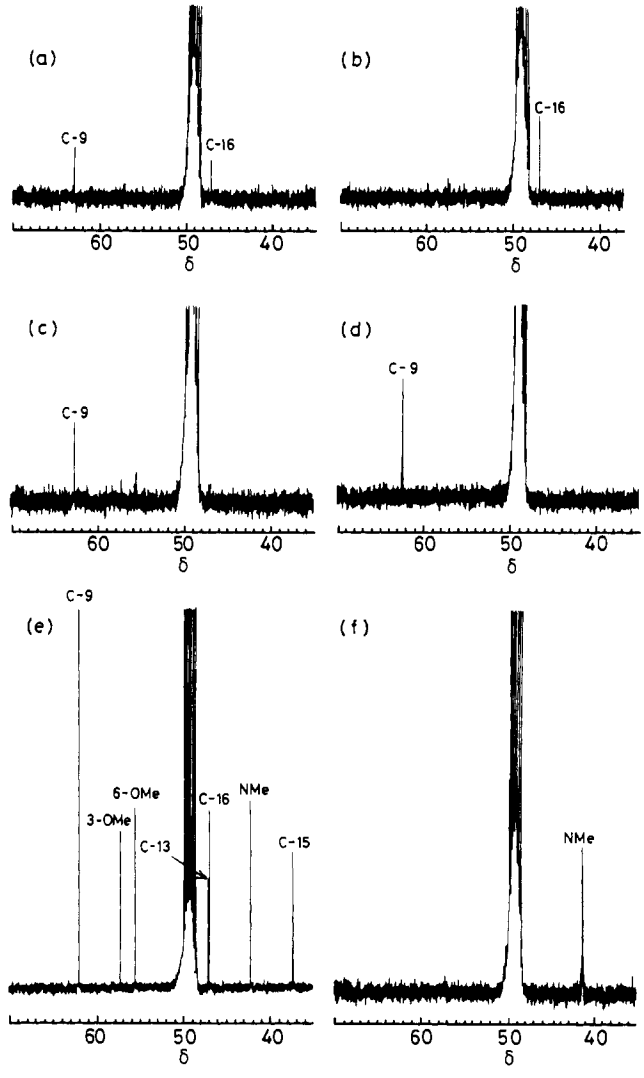


Figure 1. ^{13}C N.m.r. partial spectra of thebaine (**13**). (a) Biosynthesized from L-[8-^{13}C]tyrosine (**7**); (b) biosynthesized from [2-^{13}C]tyramine (**9**); (c) biosynthesized from (*R,S*)-[1-^{13}C]norcoclaurine (**1**); (d) biosynthesized from (*R,S*)-[1-^{13}C]coclaurine (**3**); (e) unlabelled thebaine added to biosynthesized thebaine from experiment (d); (f) biosynthesized from (*S*)-[*N*-$^{13}CH_3$]reticuline (**6**).

pure (**13**) at an average of 150 μg, sufficient for ^{13}C n.m.r. analysis (90 MHz, Bruker Aspect 3000) in CD_3OD. ^{13}C-Precursors were synthesized by standard techniques and the correct position of the label verified by n.m.r. analyses. L[2-^{13}C]Tyrosine (**7**) yielded (**13**) as shown in Figure 1(a) which was labelled as expected[8—10] at C-9 and C-16 (enrichment factor of 41 ^{13}C atom % excess, calculated from mass spectral data).

[8-^{13}C]Tyramine (**9**) labelled solely C-16 [Figure 1(b), enrichment factor 42%] which clearly demonstrates that in this tissue tyramine (**9**) and dopamine (**10**)[11] are incorporated into the isoquinoline portion of the morphinandienones only. Both (*R,S*)-[1-^{13}C]norcoclaurine (**1**) and (*R,S*)-[1-^{13}C]coclaurine (**3**) label C-9 of thebaine (**13**), as shown in Figures 1(c) and (d) respectively. Application of both of the ring-C monohydroxylated derivatives (**1**,**3**) resulted in considerable browning of the seedling root system. This explains the lower enrichment factor (20%), since a portion of the precursors was obviously converted into polyphenolic material. Addition of unlabelled alkaloid (**13**) (3 mg) to the labelled thebaine (**13**) sample [biosynthesized from coclaurine (**3**)] and subsequent n.m.r. measurement unequivocally demonstrated the incorporation of (**3**) into the specific site (C-9) of the morphinandienone (**13**) without scrambling of the label to other carbons [Figure 1(e)].

(*S*)-[*N*-$^{13}CH_3$]Reticuline (**6**) was incorporated [55% enrichment, Figure 1(f)] with specific labelling of the *N*-CH_3 of (**13**), once again confirming the well established role of (**6**) as precursor for the hydrophenanthrene molecules.[1] Supplying 5-week-old plants [containing about 20 μg morphine (**14**) per plant] with (*R,S*)-[1-^{13}C]coclaurine (**3**) resulted in the incorporation of label into C-9 of (**14**) (enrichment factor of 3%). Administration of the (*R*)-[6-O$^{14}CH_3$] enantiomer of coclaurine (**3**) resulted in no incorporation into (**13**), while (*S*)-(**3**) was selectively incorporated (5.7%).[12] Doubly labelled (*R,S*)-[6-O$^{3}CH_3$,1-^{14}C]coclaurine (**3**) (^3H: ^{14}C ratio 32) was incorporated (0.5%) into thebaine (**13**) (^3H: ^{14}C ratio 33.7) intact without noticeable O-de- and/or re-methylation from the C-1 pool.

Based on the results presented here and previously recorded data[12] on the incorporation of (*S*)-coclaurine (**3**) into protoberberine and benzophenanthridine alkaloids, we now firmly believe that the key entry reaction into the benzylisoquinoline alkaloids and derivatives thereof is the enzymatic

and stereospecific condensation[13] of dopamine (**10**) with 4-hydroxyphenylacetaldehyde (**11**) to yield (*S*)-norcoclaurine (**1**). Subsequent 6-O-methylation (**3**) and 3′-hydroxylation (**4**), followed by 4′-O-(**5**), and *N*-methylation, yields (*S*)-reticuline (**6**), the established branch point intermediate for a multitude of benzylisoquinoline derived alkaloids. In *P. somniferum* tissue, tyramine (**9**) is not transformed [Figure 1(b)] by action of amine oxidase into 4-hydroxyphenylacetaldehyde (**11**), contrary to findings in *Berberis*[14] and *Eschscholtzia*[15] cell cultures. This aldehyde (**11**) is most likely generated in *Papaver via* decarboxylation of 4-hydroxyphenylpyruvic acid [derived from tyrosine (**7**) by transamination] by an enzyme recently observed in isoquinoline alkaloid containing plants.[14] The role of norcoclaurine (**1**), established here as a precursor for the morphinandienone alkaloids, gives a clue as to the hitherto unexplainable fact[1] that tyrosine (**7**) labels both halves of the alkaloids of the morphine family (C-9 and C-16), while DOPA (**8**) or its amine (**10**) labels only the isoquinoline portion (C-16) of these alkaloids.

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