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Stereospecificity in the Biosynthesis of Papaverine

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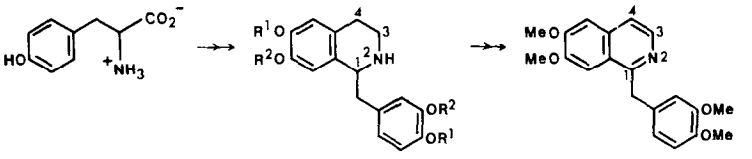
The stereochemistry of hydrogen removal from C-3 and C-4 in the aromatization of the heterocyclic ring of papaverine has been determined by incorporation experiments with stereospecifically tritiated norreticulines.

INTRODUCTION

Early work on the biosynthesis of papaverine in the opium poppy, *Papaver somniferum*, confirmed the proposal of Winterstein and Trier (*1*) that the benzylisoquinoline skeleton is derived from two units of tyrosine (*2*). Subsequently it has been shown that norlaudanosoline (2) (*3*), norreticuline (3) (*4*), and tetrahydropapaverine (4) (*5*) are successive biosynthetic intermediates.

The exceptional feature of this biosynthesis lies in the final steps which bring about aromatization of the heterocyclic ring. Formally, the process can be considered to involve the generation of two double bonds, presumably in a stepwise manner. One of these is an imino linkage between C-1 and N-2; this could be formed by a redox reaction common in alkaloid biosynthesis which has already been shown to occur in the opium poppy (*6*). Two possible mechanisms are given as Routes 1 and 2. The first involves transfer of a hydride from C-1 to a suitable acceptor such as nicotinamide coenzyme, assisted by the electron-donating power of the adjacent nitrogen. The alternative involves hydroxylation at carbon (*7*) followed by a breakdown of the intermediate carbinolamine to an imine. The generation of the C–C double bond between C-3 and C-4 is, however, a less common biosynthetic process. Possible mech-



(1) (2) $R^1 = R^2 = H$; (3) $R^1 = Me$; $R^2 = H$; (4) $R^1 = R^1 = Me$ (5)

anisms are shown as Routes 3, 4, and 5. First there is the possibility, shown in Route 3, of a direct and possibly concerted removal of two hydrogens from these carbons in a

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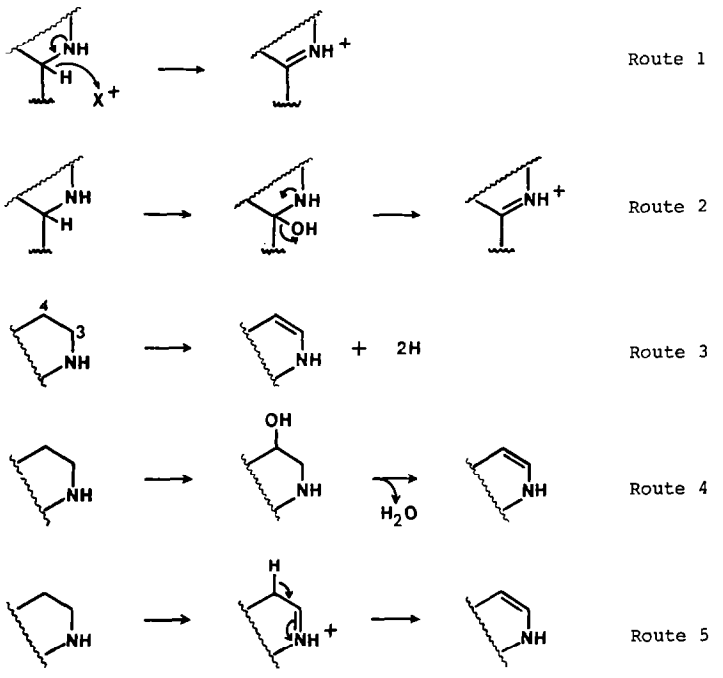

process of the type which has been shown to occur in the biosynthesis of testosterone (*8*) and the gibberellins (*9*). An alternative indirect process is shown as Route 4 where C-4 is first hydroxylated, and then the double bond is generated by dehydration; a precedent for such benzylic hydroxylation can be found in the biosynthesis of narcotine

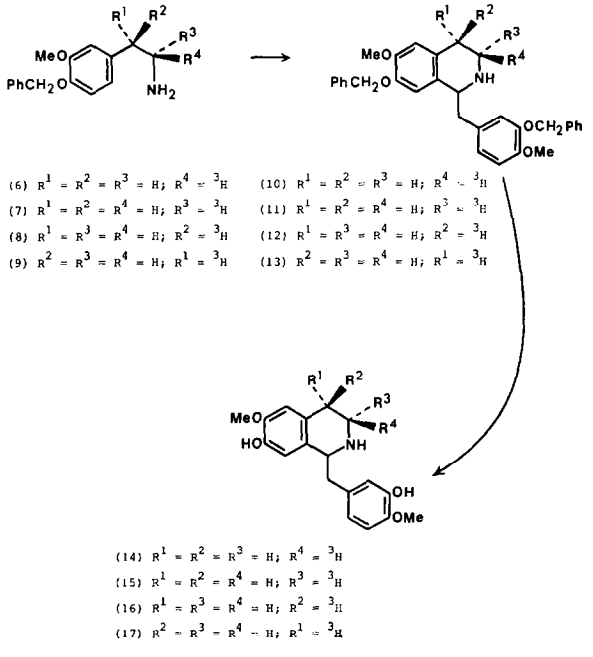

TABLE 1

INCORPORATION EXPERIMENTS WITH [$^3H_1$] NORRETICULINES

Experiment	Precursor	$^3H/^{14}C$ Ratio[a]	Papaverine[b]		Morphine[c]	
			$^3H/^{14}C$ Ratio	^3H Retention (%)	$^3H/^{14}C$ Ratio	^3H Retention (%)
1	(*3R*)-[3-$^3H_1$]Isomer (14)	9.4	9.7	103 ± 3	—	—
2	(*3RS*)-[3-$^3H_1$]Isomer (14 + 15)	10.1	4.9	49 ± 2	—	—
3	(*3S*)-[3-$^3H_1$]Isomer (15)	10.6	0.22	2 ± 0.5	—	—
4	(*4R*)-[4-$^3H_1$]Isomer (16)	10.0	6.2	62 ± 2	9.4	94 ± 3
5	(*4RS*)-[4-$^3H_1$]Isomer (16 + 17)	8.7	6.5	75 ± 2	8.4	97 ± 3
6	(*4S*)-[4-$^3H_1$]Isomer (17)	9.8	8.6	88 ± 3	10.0	102 ± 3

[a] Determined on the *N*-benzoyl derivative of the corresponding *O,O*-dibenzylnorreticuline.
[b] Incorporation ca. 1%.
[c] Incorporation ca. 0.3%.

in the opium poppy (*10*). Route 5 shows an alternative indirect process in which an imine double bond is generated between C-3 and N-2 (by one of the mechanisms given in Routes 1 and 2), followed by deprotonation at C-4 to give an enamine.

RESULTS AND DISCUSSION

It should be possible, in principle, to distinguish between the mechanisms in Routes 3, 4, and 5 by determining the stereochemistry of hydrogen removal from C-3 and C-4. To this end, we have prepared (*11*, *12*) the four chirally labeled phenethylamines (6), (7), (8), and (9) of high enantiomeric purity (>98%) and have converted them into the corresponding *O*,*O*-dibenzylnorreticuline isomers, (10), (11), (12), and (13), by established procedures (*3*) which do not affect the chiral integrity of the labeled center. The corresponding nonstereospecifically labeled compounds, (10) + (11) and (12) + (13), were similarly prepared from the equivalent nonstereospecifically tritiated phenethylamines, (6) + (7) and (8) + (9), respectively. Each of the tritiated compounds was mixed with a suitable amount of *O*,*O*-[3-^{14}C]-dibenzylnorreticuline as internal standard, and the benzyl groups were then removed by hydrogenolysis.

The six samples of doubly labeled norreticuline were dissolved in aqueous hydrochloric acid, and the solution was neutralized and injected into the capsules of *P. somniferum* plants (variety Noordster) at the time of petal fall. Two weeks later, the capsules were harvested and worked up for papaverine and morphine.

The results of the incorporation experiments are shown in Table 1. Experiments 1, 2, and 3 rigorously establish that in the conversion of norreticuline (3) to papaverine (5) a stereospecific reaction takes place at C-3 resulting in the exclusive loss of the *pro-S* hydrogen atom. In contrast, experiments 4, 5, and 6 show that hydrogen-removal from C-4 is essentially nonstereospecific. The fact that the morphine formed concurrently in the experiments retains virtually all the tritium at C-4 rules out a trivial loss of isotope from a common early intermediate. A nonstereospecific reaction at C-4 makes Routes 3 and 4 very unlikely since both would be expected to involve a stereospecific reaction at that center. However, the results can be accommodated by the process shown in Route 5 if it is assumed that proton loss from C-4 takes place independently of an enzyme. The requisite isomerization of an imine to an enamine is a facile chemical reaction so this is plausible; the small degree of stereoselectivity in the process of hydrogen removal may be induced by the chirality at C-1.

Thus it seems very probable that the generation of the C-3–C-4 double bond is an indirect reaction which hinges on the presence of the nitrogen atom at C-3.

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