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BIOSYNTHESIS OF PAPAVERINE*

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Key Word Index—*Papaver somniferum*; Papaveraceae; papaverine; alkaloid biosynthesis.

Abstract—Tracer experiments have shown that in *Papaver somniferum* papaverine arises from (−)-norreticuline via norlaudanidine and norlaudanosine.

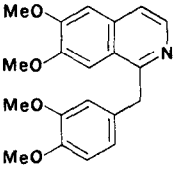
INTRODUCTION

Papaverine (**1**), one of the major 1-benzylisoquinoline alkaloids of *Papaver somniferum* L. (Papaveraceae), was first isolated from opium by Merck [1] in 1848, and its structure has been confirmed by several syntheses [2–4]. Winterstein and Trier [5] suggested that benzylisoquinoline alkaloids might be derived in *Nature* from two molecules of 3,4-dihydroxyphenylalanine (DOPA) *via* norlaudanosoline [6] (**2**). The bioconversion of norlaudanosoline to papaverine then requires methylation and dehydrogenation. Since biological reactions proceed in a definite sequence and generally show a high order of stereoselectivity it was considered of interest to study these latter aspects of the biosynthesis of papaverine.

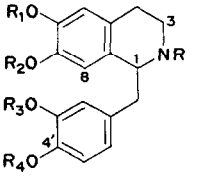
METHODS AND RESULTS

The racemates of norreticuline [7] (**3**), reticuline [8] (**4**), norcodamine [9] (**5**) and norlaudanidine [10] (**6**) were prepared by standard procedures. The resolution of (±)-di-*O*-benzylnorreticuline was carried out with (+) and (−)-di-*p*-toluoyltartaric acids, the chilarity of the enantiomers being established by conversion to the corresponding *N*-methyl derivatives of known absolute configurations [11]. The acid catalysed debenzylation furnished (−) and (+)-norreticulines (**9**, **10**). The tritium in the phenolic precursors (Table 1) was introduced by base catalysed exchange [12] in tritiated water at 100° and [2′,6′,8-$^3H_3$]-norlaudanosine (**7**) was obtained by *O*-methylation of labelled norreticuline with diazomethane.

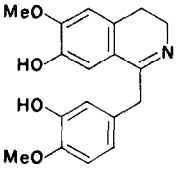
The mature capsules of *P. somniferum* were then injected with tritiated precursors as their tartarates. The plants were left for 6 days to metabolize



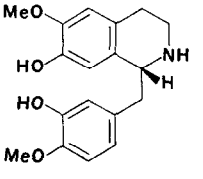
(**1**)



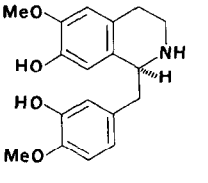
(**2**) R = R_1 = R_2 = R_3 = R_4 = H
(**3**) R = R_2 = R_3 = H, R_1 = R_4 = Me
(**4**) R = R_1 = R_4 = Me, R_2 = R_3 = H
(**5**) R = R_2 = H, R_1 = R_3 = R_4 = Me
(**6**) R = R_3 = H, R_1 = R_2 = R_4 = Me
(**7**) R = H, R_1 = R_2 = R_3 = R_4 = Me



(**8**)



(**9**)



(**10**)

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Table 1. Incorporation of labelled precursors into papaverine in *P. somniferum*

Precursor fed	Incorporation papaverine (%)
(±)-[2′,6′,8-$^3H_3$]reticuline (**4**)	<0·001
(±)-[2′,6′,8-$^3H_3$]norreticuline (**3**)	0·42
(−)-[2′,6′,8-$^3H_3$]norreticuline (**9**)	0·81
(+)-[2′,6′,8-$^3H_3$]norreticuline (**10**)	<0·001
(±)-[8-^{3}H]norcodamine (**5**)	0·14
(±)-[2′,6′-$^3H_2$]norlaudanidine (**6**)	1·48
(±)-[2′,6′,8-$^3H_3$]norlaudanosine (**7**)	1·21

the precursors and then harvested and the alkaloids isolated. The incorporations obtained in each case are recorded in Table 1.

DISCUSSION

Papaverine has been shown to be biosynthesized in *P. somniferum* from two units of tyrosine [13] *via* norlaudanosoline [14] and norreticuline [15]. The dehydrogenation of the benzyltetrahydroisoquinoline precursor is an important step in the biosynthesis of (**1**). The mechanism of this reaction could be stepwise or it could proceed in a concerted manner. Further, this process could occur in a partially methylated 1-benzyltetrahydroisoquinoline precursor, such as, norreticuline (**3**) or it could take place in a completely methylated derivative, i.e. norlaudanosine (**7**). The efficient incorporation of (±) norlaudanosine (**7**) (Table 1) into papaverine when coupled with the earlier data [15] that 1,2-dehydronorreticuline (**8**) did not participate in the biosynthesis of (**1**) clearly demonstrates that the dehydrogenation step occurs after complete methylation, presumably at the norlaudanosine level and probably in a concerted manner.

Since biological methylation normally occurs in a definite sequence, partial *O*-methylation of norreticuline at C-7 or C-3′ could give rise to norlaudanidine (**6**) or norcodamine (**5**) respectively. Norlaudanosine then of course be reached from both of these isomers by further *O*-methylation. However when norcodamine and norlaudanidine were fed in parallel experiments to *P. somniferum*, the former was incorporated 10 times less efficiently than the latter (Table 1) strongly suggesting that norlaudanidine is an intermediate between norreticuline and norlaudanosine.

Normally in Nature, *N*-norbases are the precursors of *N*-methyl alkaloids. However, in some cases it has been reported that reverse is also true [16]. The parallel feeding experiments with reticuline and norreticuline in *P. somniferum* (Table 1) clearly domonstrate that *N*-demethylation of reticuline is not a favoured process, in the biosynthesis of papaverine.

Although papaverine does not possess an asymmetric centre, yet enzymatic reactions are generally stereospecific and one can expect that either of the enantiomers of norreticuline would be the true biological precursor of papaverine. Indeed when (−) and (+)-norreticulines were fed to *P. somniferum* plants, papaverine was exclusively biosynthesized from (−)-norreticuline.

The incorporation of a hypothetical precursor into an alkaloid does not prove it to be a true precursor. The true precursor must also exist in the plant. The isolation of reticuline [17], codamine [18], laudanidine [18] and laudanosine [18] from *P. somniferum* indirectly lend support to the possible existence of the corresponding *N*-norbases in this plant.

The tracer experiments carried out so far on the biosynthesis of papaverine in *P. somniferum* supports the following sequence: tyrosine → norlaudanosoline (**2**) → (−)-norreticuline (**9**) → norlaudanidine (**6**) → norlaudanosine (**7**) → papaverine (**1**).

EXPERIMENTAL

(−)-Di-O-benzylnorreticuline. The salt from (±)-OO-dibenzylnorreticuline (1·20 g) and (+)-di-*p*-toluoyl-*d*-tartaric acid (0·59 g) was fractionally crystallized successively from MeOH–Et_2O and MeOH to give colourless needles, mp 183–84°, $(\alpha)_D^{21}$ −17° (*c* 1·0, $CHCl_3$). It was decomposed with aq. 4 N NaOH to yield (−)-OO-dibenzylnorreticuline as an oil (300 mg), $(\alpha)_D^{21}$ −24° (*c* 1·0, $CHCl_3$).

(−)-Norreticuline. A solution of (−)-OO-dibenzylnorreticuline (200 mg) in MeOH (8 ml) and 12 N HCl (7 ml) was refluxed for 2 hr and the solvent removed. Residue was diluted with H_2O and extracted with Et_2O (3 × 10 ml). The aq. acidic layer was basified with aq. Na_2CO_3 solution and the liberated base was extracted with $CHCl_3$ (4 × 20 ml). The combined $CHCl_3$ layer was washed with H_2O, dried (anhyd. Na_2SO_4) and the solvent removed to furnish (−)-norreticuline (140 mg) as amorphous powder, $(\alpha)_D^{21}$ −36·6° (*c* 0·5, MeOH).

(+)-OO-Dibenzylnorreticuline. The salt from (±)-OO-dibenzylnorreticuline (0·86 g) and (−)-di-*p*-toluoyl-*l*-tartaric acid (0·43 g) was fractionally crystallized successively from MeOH–Et_2O and MeOH to afford colourless needles, mp 182–83°, $(\alpha)_D^{21}$ +15° (*c* 1·0, $CHCl_3$). It was decomposed with aq. 4 N NaOH to give (+)-OO-dibenzylnorreticuline as an oil (200 mg), $(\alpha)_D^{21}$ +23° (*c* 1·0, $CHCl_3$).

(+)-*Norreticuline*. A mixture of (+)-OO-dibenzylnorreticuline (200 mg) in MeOH (8 ml) and 12 N HCl (7 ml) was refluxed for 2 hr. The resulting mixture when worked up in the usual manner furnished (+)-norreticuline (150 mg), $(\alpha)_D^{21°}$ +34·5° (*c* 0·5, MeOH).

(+)-OO-Dibenzylreticuline. A soln of (+)-OO-dibenzylnorreticuline (50 mg) in MeOH (5 ml) and HCHO (1 ml) was treated with powdered $NaBH_4$ (40 mg) during 30 min at 5–10°. The reaction mixture was further stirred at room temp. for 1 hr, concentrated, the residue was neutralised with dil. HCl and extracted with Et_2O (3 × 10 ml). The aq. acidic layer was basified with aq. Na_2CO_3 soln. to pH 8·0–9·0 and extracted with $CHCl_3$ (3 × 20 ml). The combined $CHCl_3$ layer was washed with H_2O, dried (anhyd. Na_2SO_4) and solvent removed to yield (+)-OO-dibenzylreticuline (45 mg), $(\alpha)_D^{21°}$ + 46° (*c* 1·0, $CHCl_3$) (Lit. [11] $(\alpha)_D$ + 44° (*c* 1·0, $CHCl_3$)).

(−)-*Di-O-benzylreticuline*. Similar *N*-methylation of (−)-OO-dibenzylnorreticuline (50 mg) as described above furnished (−)-OO-dibenzylreticuline (46 mg), $(\alpha)_D^{21°}$ −43° (*c* 1·0, $CHCl_3$) (Lit. [11] $(\alpha)_D$ −42° (*c* 1·0, $CHCl_3$)).

Feeding experiments. The labelled precursors were dissolved in dil. tartaric acid and injected into the mature capsules of *P. somniferum*. The plants were kept alive for 6 days, then harvested and worked up for papaverine.

Isolation of papaverine. Plant material (typically 300–400 g, wet wt) and inactive papaverine (150 mg) were macerated with EtOH (500 ml) and kept overnight. EtOH was decanted and residual plant material percolated with additional EtOH (4 × 500 ml). Combined ethanolic extract was concentrated under red pres to 100 ml and dild with 0·5 N HCl (50 ml). The soln was then extracted with EtOAc (5 × 50 ml) and aq. acidic layer was extracted successively with Et_2O (5 × 50 ml) and $CHCl_3$ (5 × 50 ml). The $CHCl_3$ layer was washed with aq. $NaHCO_3$ soln, H_2O, dried (anhyd. Na_2SO_4) and evaporated to dryness to afford crude papaverine (140 mg), which was chromatographed on a column of neutral Al_2O_3. The elution was effected with C_6H_6 to furnish papaverine (110 mg), which was converted to the hydrochloride, mp 222–24° and crystallized to constant activity from MeOH–Et_2O. The radiochemical purity of the labelled papaverine in each expt was checked by reverse diln technique and further through its methiodide and conversion to laudanosine [19].

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