THE EARLY STAGES OF PAPAVER ALKALOID BIOSYNTHESIS. CELL-FREE SYNTHESIS OF BENZYLISOQUINOLINES

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A cell-free system of Papaver somniferum has been isolated, capable of synthesizing norlaudanosoline-1-carboxylic acid, dehydronorlaudanosoline, and norlaudanosoline from 3,4-dihydroxyphenethylamine (dopamine) and 3,4-dihydroxyphenylpyruvic acid. The enzyme activity lies in a Triton X-100 solubilized protein fraction.

The biogenesis of the carbon framework of many alkaloids is believed to operate through the biochemical equivalent of the Pictet-Spengler condensation. For example, the key step in the biosynthesis of the indole alkaloids of Catharanthus roseus, uncovered by the evolution of cell-free methodology,[1] involves condensation of the aldehyde group of secologanin with tryptamine to form strictosidine, recently shown to be the pivotal precursor of the Corynanthé, Iboga and Aspidosperma by three independent investigations.[2,3,4] In a similar vein it has been suggested that dopamine (1), which constitutes the "upper" building block of the Papaver alkaloids,[5,6] condenses with the putative intermediate 3,4-dihydroxyphenylpyruvate (2), (itself derived from tyrosine) to form norlaudanosoline carboxylic acid (3). This condensation which can proceed non-enzymically has been used to produce (3) which is

transformed in intact P. somniferum plants into morphine.[6] The utilization of both R and S forms of norlaudanosoline (4) for morphine alkaloid biosynthesis[7] has implicated the imine 5 as a dynamic metabolite[5] and/or precursor which may be in redox equilibrium with (4). The similarity of the problems involving chirality at position 3 of the indole alkaloids[2-4] and the corresponding center (C-1) in (3) and (4) together with the necessary identification of the C_6-C_3 segment (e.g. 2) in the pathway suggested to us that, as before,[1] cell-free methodology could be applied to this problem.

Cell-free systems were prepared by the following procedure. Fifteen grams of capsules and stems of P. somniferum, harvested three to ten days after petal fall, were homogenized in 10 ml of phosphate buffer solution (pH 6.4) containing 0.1 M diethyldithiocarbamate (or Polyclar AT at 0.5 g/g tissue) at 0-4°C. The suspension was filtered through fine nylon cloth, and then the homogenate centrifuged at 37,000 g for 20 min. The sediment was washed with 5 ml of phosphate buffer (pH 6.4) and resuspended in 3 ml of phosphate buffer (pH 7.0) containing 1% Triton X-100. Similar procedures were used in extraction of enzymes from the callus and seedlings. Both suspension and supernatant were used for incubations described in Table 1.

It was found that the alkaloids, 3, 4 and 5,[8] were synthesized from dopamine and 3,4-dihydroxyphenylpyruvic acid by the cell-free system. However, the formation of reticuline, thebaine, codeine and morphine was not detected under these conditions.[14] The enzyme systems from capsules and stems were more active than those from seedlings or callus, while the pellet was more active than the supernatant. Triton X-100 increased the activity threefold by solubilization of the pellet. The following conclusions can be drawn from the data of Table 1. (a) A complete enzyme system is present in the pellet, which catalyzes the formation of norlaudanosoline-1-carboxylic acid (3), as the first tetrahydroisoquinoline alkaloid of the morphine alkaloid series. (b) The role of

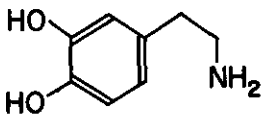
TABLE 1. Incorporation (%) of [1-^{14}C]dopamine into 3, 4 and 5

Exp. No.	Origin	Condition[b]	3	4	5	Dopamine (Unchanged)
1	Capsules and Stems[a]	Supernatant	0.4	<0.1	0.1	64.7
2	Capsules and Stems[a]	Pellet with Triton X-100	3.0	0.3	0.9	5.8
3	Capsules and Stems[a]	Pellet	0.9	<0.1	0.3	19.2
4	Seedlings (4 weeks old)	Supernatant	<0.1	-	-	57.3
5	Seedlings (4 weeks old)	Pellet	<0.1	-	-	61.2
6	Callus (1 month old)	Supernatant	<0.1	<0.1	<0.1	78.3
7	Callus (1 month old)	Pellet with Triton X-100	<0.1	<0.1	<0.1	75.2
8	Control	Boiled system of Exp. 2	<0.1	<0.1	<0.1	89.7

a) These were harvested at 3-10 days after petal fall.

b) Incubations of 1 μCi [1-^{14}C]dopamine were performed at 37°C for 3 hr (under nitrogen atmosphere in a Thunberg tube) in the above systems containing 1 μmole each of dopamine and 3,4-dihydroxyphenylpyruvic acid as substrates. After incubation, the reaction mixture was immediately treated with 1 N HCℓ, freeze-dried, and chromatographed (Silica gel plate) with three different solvent systems: (i) $CHCℓ_3$/MeOH = 85:15, (ii) n-BuOH/Acetic acid/H_2O = 25:4:10, and (iii) $CHCℓ_3$/acetone/dimethylamine = 5:4:1. Paper chromatography with CM-cellulose using acetate buffer at pH 4.0 as eluent was useful and separated norlaudanosoline-1-carboxylic acid (3) from the other alkaloids. Norlaudanosoline-1-carboxylic acid (3), norlaudanosoline (4), and 1,2-dehydronorlaudanosoline (5) have Rf values 0.32, 0.05 and 0.03, respectively. After addition of carrier compounds, the labeled 3 and 4 were further confirmed by recrystallization to constant specific activity. The alkaloids were further identified by methylation with diazomethane to the corresponding methylated products, 6, 7 and 8, which were separated at Rf 0.9, 0.67 and 0.77, respectively, by TLC plate (Silica gel) with the solvent of $CHCℓ_3$/MeOH (4:1).

dopamine (1) and 3,4-dihydroxyphenylpyruvic acid (2) as true precursors has been established at the cell-free level. (c) The putative intermediacy of 1,2-dehydronorlaudanosoline (5) is supported.



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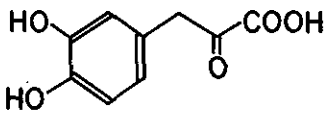

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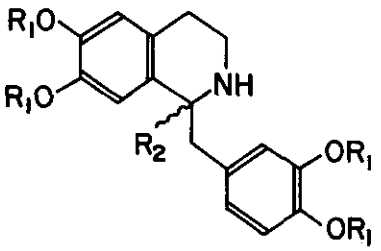

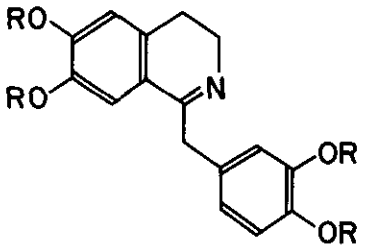

3: R_1=H, R_2=COOH

4: R_1=R_2=H

5: R=H

8: R=Me

6: R_1=Me, R_2=COOMe

7: R_1=Me, R_2=H

The availability of cell-free methodology in the Papaver series will allow development of the enzymology of codeine and morphine biosynthesis.[14]

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