

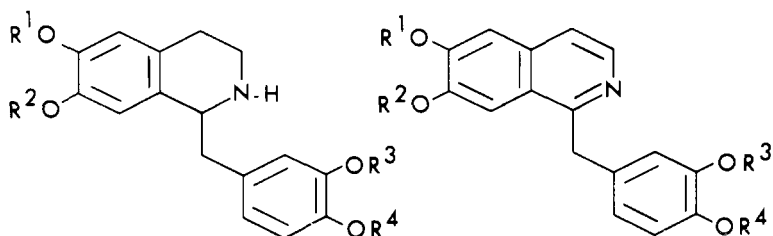
# BIOSYNTHESIS OF UNNATURAL PAPAVERINE DERIVATIVES IN *PAPAVER SOMNIFERUM*

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**ABSTRACT.**—Dehydrogenation of various tetrahydropapaverine analogs to the corresponding, unnatural analogs of papaverine has been studied in *Papaver somniferum*. The efficiencies of the unnatural process have been compared with the natural conversion of tetrahydropapaverine to papaverine in an attempt to gain information regarding the specificity of the enzyme(s) and the mechanism of the aromatization reaction.

Papaverine (6) is produced in the opium poppy by dehydrogenation of tetrahydropapaverine (THP) (1). The reaction is stereospecific involving only the L(S)-isomer, but its substrate specificity is less well defined (1). We now report the biosynthetic conversion of some modified THP analogs to the correspondingly modified papaverine analogs. The modifications consisted of the replacement of one of the methoxy substituents by an ethoxy group to give four analogs of THP represented by structures 2-5. These compounds were synthesized with a  $^{14}\text{C}$  label in position 3. The corresponding papaverine analogs (7-10) were synthesized for use as carriers in the isotope dilution method.



- 1; R<sup>1</sup>=R<sup>2</sup>=R<sup>3</sup>=R<sup>4</sup>=Me
- 2; R<sup>1</sup>=Et, R<sup>2</sup>=R<sup>3</sup>=R<sup>4</sup>=Me
- 3; R<sup>2</sup>=Et, R<sup>1</sup>=R<sup>3</sup>=R<sup>4</sup>=Me
- 4; R<sup>3</sup>=Et, R<sup>1</sup>=R<sup>2</sup>=R<sup>4</sup>=Me
- 5; R<sup>4</sup>=Et, R<sup>1</sup>=R<sup>2</sup>=R<sup>3</sup>=Me

- 6; R<sup>1</sup>=R<sup>2</sup>=R<sup>3</sup>=R<sup>4</sup>=Me
- 7; R<sup>1</sup>=Et, R<sup>2</sup>=R<sup>3</sup>=R<sup>4</sup>=Me
- 8; R<sup>2</sup>=Et, R<sup>1</sup>=R<sup>3</sup>=R<sup>4</sup>=Me
- 9; R<sup>3</sup>=Et, R<sup>1</sup>=R<sup>2</sup>=R<sup>4</sup>=Me
- 10; R<sup>4</sup>=Et, R<sup>1</sup>=R<sup>2</sup>=R<sup>3</sup>=Me

## EXPERIMENTAL

1-Benzyl-isoquinolines and -tetrahydroisoquinolines were synthesized as described previously (2-4). The radioactive purity of the labeled substrates was determined by radioactivity scanning with a Berthold radio scanner (Varian Aerograph Co.) of thin layer chromatograms developed with two different solvent systems. Physical measurements and spectroscopic data were obtained with nonradioactive samples synthesized by the same methods as the labeled compounds and their identity established by chromatography (glc, tlc).  $^1\text{H}$  Nmr spectra were measured in deuteriochloroform with tetramethylsilane as internal standard (table 1).

*Papaver somniferum*, Noordster variety, was used throughout. The methods for cultivation of the plants, administration of labeled substrates, extraction, separation, purification and controlled degradation of the alkaloids have been described in previous communications (4, 5). In general, the weakly basic papaverine analogs were separated from the total alkaloid extract by extraction with chloroform at pH 1-2 and chromatographed on a column of silica gel with chloroform containing 2% methanol and 1% glacial acetic acid. The fractions containing papaverine and its ethoxy analog were combined and subjected to preparative tlc on silica gel with the following solvent systems: (1) chloroform-methanol (9:1), (2) benzene-ethanol (8:2) and (3) ethyl acetate-methanol (96:4). Finally, the papaverine analogs were crystallized from ether to constant radioactivity.

TABLE 1. Spectroscopic data of substrates and carrier compounds.

Compound No.	$^1\text{H-nmr}$ ( $\text{CDCl}_3$ ), $\delta$					MS(EI) $m/e$
	EtO-	6-OMe	7-OMe	3'-OMe	4'-OMe	
1	—	3.86	3.85	3.81	3.85	343(M <sup>-</sup> ) 192(M-151) <sup>+</sup>
2	1.46(t,3H) 4.12(q,2H)	—	3.89	3.84	3.87	357(M <sup>+</sup> ) 206(M-151) <sup>-</sup>
3	1.41(t,3H) 4.00(q,2H)	3.88	—	3.85(s,6H)	—	357(M <sup>-</sup> ) 206(M-151) <sup>-</sup>
4	1.45(t,3H) 4.10(q,2H)	3.89(s,6H)	—	—	3.86	357(M <sup>-</sup> ) 192(M-165) <sup>-</sup>
5	1.44(t,3H) 4.10(q,2H)	3.88(s,6H)	—	3.84	—	357(M <sup>-</sup> ) 192(M-165) <sup>-</sup>
6	—	3.99	3.90	3.77	3.82	
7	1.53(t,3H) 4.25(q,2H)	—	3.92	3.80	3.84	
8	1.48(t,3H) 4.16(q,2H)	4.01	—	3.80	3.84	
9	1.38(t,3H) 4.04(q,2H)	4.03	3.94	—	3.84	
10	1.41(t,3H) 4.06(q,2H)	4.00	3.91	3.78	—	

## RESULTS AND DISCUSSION

As shown in table 2, all four THP analogs were aromatized to the corresponding papaverine analogs with the  $^{14}\text{C}$  label intact, but the efficiency of the incorporations varied with the position of the ethoxy group. When the modification involved the A-ring of the tetrahydroisoquinoline moiety, dehydrogenation took place with about the same degree of efficiency as with THP itself (1). It was considerably less efficient, however, when the ethoxy group resided in the benzylic portion of the molecule, indicating that the increased bulkiness prevented optimum interaction with the enzyme. In an earlier communication, we have reported that

TABLE 2. Results of feeding experiments with *Papaver somniferum*.

Precursor Compound	Percent incorp. of $^{14}$ into compd.				Rel. activity in isolated fragment
	7	8	9	10	
2	11.25				97.8
3		10.00			98.6
4			1.91		95.8
5				1.50	98.1

aromatization can occur to an extent comparable to that of THP when one of the methoxy groups is demethylated irrespective of whether the phenolic hydroxyl group is in ring A or in ring C.

The mechanism of the reaction is still not known in all details, but the size of the substituents of ring C and the orientation of this ring in space as determined by the chiral center at position 1 are determinant factors. Battersby *et al.* (6) have proposed a stepwise mechanism by which the first double bond is introduced between the nitrogen and C-3 by a stereospecific loss of hydrogen at C-3 followed by a nonstereospecific isomerization of the imine to the enamine (1,2-dihydro-papaverine).

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#### LITERATURE CITED

1. E. Brochmann-Hanssen, C-H. Chen, C. R. Chen, H-C. Chiang, A. Y. Leung and K. McMurtrey, *J. Chem. Soc., Perkin I*, 1531 (1975).
2. E. Brochmann-Hanssen and K. Hirai, *J. Pharm. Sci.*, **57**, 940 (1968).
3. A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin and H. Ramuz, *J. Chem. Soc.*, 3600 (1964).
4. E. Brochmann-Hanssen, C-C. Fu and G. Zanati, *J. Pharm. Sci.*, **60**, 873 (1971).
5. E. Brochmann-Hanssen, C-C. Fu, A. Y. Leung and G. Zanati, *J. Pharm. Sci.*, **60**, 1672 (1971).
6. A. R. Battersby, P. W. Sheldrake, J. Staunton and M. C. Summers, *Bioorg. Chem.*, **6**, 43 (1977).