ABERRANT BIOSYNTHESIS OF OPIUM ALKALOIDS: BIOSYNTHETIC CONVERSION OF THE 6-ETHYL ANALOG OF THEBAINE TO CODEINE AND MORPHINE

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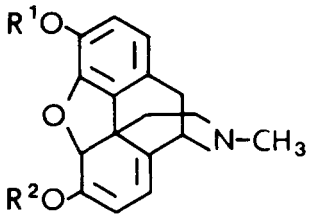
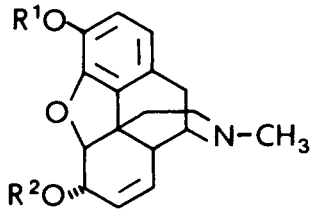
ABSTRACT.—When the unnatural 6-ethyl analog of thebaine was fed to *Papaver somniferum* plants, it was very efficiently dealkylated to codeinone as evidenced by high incorporations into codeine and morphine. This indicates that the enzyme involved in the *O*-6 dealkylation of thebaine is not specific for the methyl ether. It also lends support to an oxidative mechanism of dealkylation rather than a hydrolytic mechanism or one based on S_N2 displacement by nucleophilic attack at the alkyl group.

The mechanism as well as the specificity of enzymic dealkylations have been studied extensively in both plant and animal organisms (1–3). Our interest in alkaloid biosynthesis has led us to explore these questions in the opium poppy and in other members of the genus *Papaver*, especially as they relate to *O*–3 and *O*–6 demethylations of hydrophenanthrene alkaloids. It was suggested by Horn, Paul and Rapoport (4) that *O*–6 demethylation of the enol ether of thebaine (**1a**) in *P. somniferum* proceeds by an oxidative mechanism similar to that proposed for demethylation of aromatic methyl ethers (5). Although the mechanism may be the same in both reactions, the enzymes involved are different and require different substrates. Furthermore, the enzyme responsible for *O*–3 demethylation of codeine (**2a**) to morphine (**2b**) in *P. somniferum* is different from the *O*–3 demethylating enzyme converting thebaine to oripavine (**1b**) in *P. orientale* and *P. bracteatum*. This conclusion is based on the following observations: The opium poppy appears to be unable to demethylate thebaine to oripavine (6). Only after *O*–6 demethylation and reduction to codeine does *O*–3-demethylation take place. *P. orientale* and *P. bracteatum*, on the other hand, cannot perform demethylation of the enolic ether of thebaine to produce codeinone. However, if codeinone is administered to the plant, rapid and efficient reduction to codeine takes place, but no demethylation of this artificially produced codeine to morphine can be observed (7, 8). It would, therefore, appear that three different enzymes may be involved in these closely related reactions; one (*I*) affecting *O*–6 demethylation of thebaine, the other (*II*) *O*–3 demethylation of codeine in the opium poppy while a third enzyme (*III*) demethylates thebaine to oripavine in the two perennial poppy species. Enzymes *I* and *II* are not present in *P. orientale* and *P. bracteatum*, while enzyme *III* is missing in *P. somniferum*. Although enzyme *II* does not seem to act on thebaine, there are several reports in the literature that it is capable of demethylating a number of closely related but unnatural aromatic ethers. Kirby *et al* (9) have reported efficient *O*–3 demethylation of several codeine analogs to the corresponding morphine analogs. In an earlier report (6) we have described the biotransformation of the unnatural 3-ethyl analog of thebaine, oripavine 3-ethyl ether (**1c**), in the opium poppy. This modification of the thebaine molecule did not seem to interfere with the normal biosynthetic reactions, i.e., *O*–6 demethylation, reduction of the codeine analog and *O*–3 dealkylation took place with about the same efficacy as is normally observed in the biosynthesis of morphine.

As a logical sequel to the previous experiment with oripavine 3-ethyl ether, it was of interest to study the dealkylation of the enol ether of thebaine and the effect of using a higher homolog at the 6-position as a substrate. We now report the biotransformation of the 6-ethyl analog of thebaine (**1d**) in the opium poppy.



1a: $R^1=R^2=Me$
1b: $R^1=H$, $R^2=Me$
1c: $R^1=Et$, $R^2=Me$
1d: $R^1=Me$, $R^2=Et$



2a: $R^1=Me$, $R^2=H$
2b: $R^1=H$, $R^2=H$

RESULTS AND DISCUSSION

As shown in table 1, the 6-ethyl analog of thebaine was effectively incorporated into codeine and morphine. Within experimental error the tritium label remained at position 2 throughout the biotransformations to morphine. It may be concluded that *O*–6 dealkylation of the 6-ethyl analog takes place with at least the same efficiency as with thebaine itself. This lends considerable support to the suggestion (4) that the dealkylation is an oxidative rather than a hydrolytic reaction in which case one might have expected a reduced rate with an increase in the size of the alkyl group. An alternate S_N2 displacement by nucleophilic attack at the alkyl group (4) is also inconsistent with this unchanged rate for de-ethylation.

TABLE 1. Results of feeding experiments with *Papaver somniferum*.

Compound fed	Amt. fed. mCi	No. of plants	Alkaloids isolated	Percent Incorporation of ^3H	Radioactivity after proton exchange
6-Ethyl analog of 2-^{3}H-thebaine.....	0.024	10	Codeine, 65 mg Morphine, 175 mg	8.9 9.4	— 0

EXPERIMENTAL

MATERIALS AND METHODS.—The Noordster variety of *Papaver somniferum* was used in this experiment. The methods for cultivation of the plants, administration of the labeled precursor, extraction, separation, purification of alkaloids, determination of radioactivity and determination of the position of the tritium label of morphine have been described in previous communications (10–13). The crude alkaloid extract, after removal of chlorophyll, was separated into weakly basic alkaloids by extraction of an acidic solution with chloroform, moderately basic nonphenolic alkaloids by extraction with chloroform of pH 13 and phenolic alkaloids by extraction with chloroform-2-propanol (3:1) at pH 9. Codeine was isolated from the moderately basic nonphenolic fraction by preparative tlc on silica gel (0.5 mm) with chloroform-methanol (9:1), and purified, first by column chromatography on neutral alumina (activity grade IV) with benzene containing increasing amounts of methanol (20% to 80%), then by preparative tlc again on silica gel (0.25 mm) with the same solvent system as before. Finally, the codeine fraction was crystallized from benzene-hexane (1:1) to constant radioactivity.

The phenolic fraction was dissolved in 0.2 *N* sodium hydroxide (25 ml) in a 125 ml glass-stoppered flask. Fifty ml of ether was added, and the flask shaken vigorously. Enough solid ammonium chloride was added to pH 9.0 and the mixture shaken again for about 2 min and placed in a refrigerator overnight. Morphine crystallized and was recrystallized from aqueous methanol to constant radioactivity.

PREPARATION OF LABELED SUBSTRATE.—Spectroscopic measurements were made with nonradioactive samples synthesized by the same methods as the labeled compounds, and their identity was established by chromatographic methods (glc, tlc). Nmr spectra were taken in deuteriochloroform. The synthesis was adapted from the synthesis of thebaine as described by Barber and Rapoport (14).

2-^{3}H-Morphine was prepared by base-catalyzed proton exchange with tritiated water in anhydrous dimethylformamide and methylated to 2-^{3}H-codeine with trimethylanilinium sulfate at 125–130° (13).

2-^{3}H-codeine 6-ethyl ether. An excess of potassium hydride (300 mol %, 22% dispersion in oil) was washed with hexane (3 x 6 ml, distilled from CaH_2) and suspended in anhydrous tetrahydrofuran (10 ml). With stirring and under a nitrogen atmosphere, a solution of 2-^{3}H-codeine (170 mg, 0.57 mmole) in 4 ml of THF was added to the KH suspension over a period of 30 min and the mixture stirred for an additional 30 min. Ethyl iodide (0.15 ml, 2.3 mmole) was added rapidly and the reaction quenched after 2 min with 4 ml of 1 N sodium bicarbonate in anhydrous methanol. Water (15 ml) was added, the organic solvents evaporated and the aqueous mixture extracted with chloroform. The chloroform extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to give a residue of 197 mg, identical with a cold sample of codeine 6-ethyl ether by tlc. ^{1}H nmr δ 1.26 (t, OCH_2-CH_3, $J_{1,2}$=7 Hz), 2.44 (s, NCH_3), 3.70 (q, OCH_2-CH_3, $J_{1,2}$=7 Hz), 3.82 (s, OCH_3), 6.46 and 6.62 (2d, H-1 and H-2, $J_{1,2}$=8.2 Hz).

6-Ethyl analog of 2-^{3}H-thebaine. The 2-^{3}H-codeine 6-methyl ether obtained above was dissolved in anhydrous THF (10 ml), and the solution was shaken vigorously with activated manganese (IV) oxide[1] (250 mg) in a nitrogen atmosphere at room temperature. Further portions of MnO_2 (250 mg) were added at intervals of 1,3,5 and 10 hrs. After 48 hrs the black mixture was filtered through a fine sintered glass filter and the residue washed with THF (4 x 30 ml) and then with methanol (4 x 20 ml). The combined filtrate and washings were evaporated to give 137 mg of crude product which was purified by column chromatography on silica gel. The pure product was identical with a cold sample by tlc and glc. Specific activity 1,317,285 dpm/mg. ^{1}H nmr δ 1.33 (t, 6-O-CH_2-CH_3, $J_{1,2}$=7.0 Hz), 2.46 (s, NCH_3), 3.69 (q, 6-O-CH_2-CH_3), $J_{1,2}$=7.0 Hz), 3.85 (3-OCH_3), 5.00 (d, H-7, $J_{1,2}$=6.4 Hz) 5.54 (d, H-8, $J_{1,2}$=6.4 Hz), 5.26 (s, H-5), 6.60 and 6.62 (2s, H-1 and H-2).

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