BIOSYNTHESIS OF OPIUM ALKALOIDS. SUBSTRATE SPECIFICITY AND ABERRANT BIOSYNTHESIS: ATTEMPTED DETECTION OF ORIPAVINE IN *PAPAVER SOMNIFERUM*

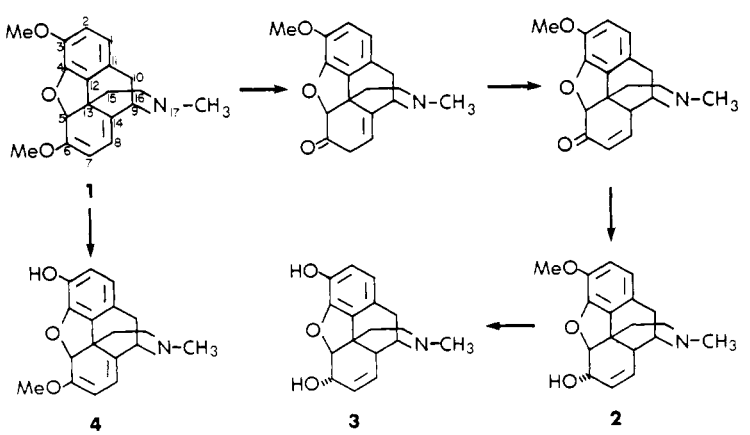
E. Brochmann-Hanssen and Y. Okamoto

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143

Abstract.—The unnatural thebaine analog, oripavine 3-ethyl ether, was efficiently metabolized to morphine 3-ethyl ether and morphine in the opium poppy. An attempt to detect oripavine by an isotope dilution experiment based on its presumed biosynthesis from reticuline was unsuccessful.

Metabolic reactions are in general enzymic and, therefore, show considerable selectivity. This selectivity becomes an important factor for biosynthetic studies of natural products because specific incorporation of a labeled substrate usually provides the first indication that the compound is a natural precursor. There are, however, examples in the literature of unnatural compounds being transformed by a plant to a natural product (1–3) and, also, of unnatural modified precursors being converted to correspondingly modified end products (3–7).

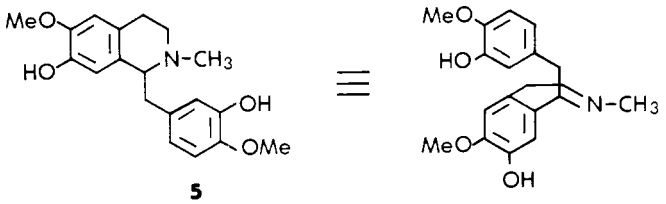
Kirby *et al* (6) have reported the demethylation of several unnatural codeine analogs to the corresponding morphine analogs by the opium poppy. Thebaine (**1**) is another opium alkaloid which, in contrast to codeine (**2**) and morphine (**3**), is produced by several members of the genus *Papaver*. Some of these species, such as *P. orientale* and *P. bracteatum*, are able to demethylate the phenolic ether to give oripavine (**4**), but lack the ability to demethylate the enolic ether in position 6 of thebaine (scheme 1). Although both demethylations appear to pro-



Scheme 1. Biotransformations of thebaine.

ceed by the same mechanism (8), they require different enzymes. *P. somniferum* has enzymes for both *O*-3 and *O*-6 demethylations and it is, therefore, conceivable that this species may contain oripavine as a genuine alkaloid. If present in low concentration compared to morphine and other phenolic alkaloids, it may hitherto

have escaped detection. This possibility was examined by an isotope dilution experiment based on its biosynthesis from reticuline (**5**), which is a precursor of thebaine (9–11). Synthetic oripavine was used as a cold carrier. The enolic ether demethylation of an unnatural thebaine analog, oripavine 3-ethyl ether, was also investigated as well as the 3-*O*-dealkylation of a higher ether homolog of codeine. This was part of an effort to study the specificity of enzymes involved in the biosynthesis of opium alkaloids. Oripavine (**4**) and labeled oripavine 3-ethyl ether (**11**) were synthesized as illustrated in scheme 2.



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RESULTS AND DISCUSSION

As shown in table 1, oripavine 3-ethyl ether is effectively incorporated into morphine 3-ethyl ether and into morphine. Within experimental error, all radioactivity resides in the *N*-methyl group of morphine indicating that incorporation was achieved without scrambling of the radioactive label. It may be concluded that oripavine 3-ethyl ether enters the same biosynthetic pathway as is operative for transformation of thebaine to codeine. There is no evidence that the ethyl group in position 3 in any way interferes with *O*–6 demethylation or with the reduction of the carbonyl group. The *O*–3 dealkylation of the aromatic ethyl ether group appears to be of approximately the same order of efficiency as normally observed for conversion of codeine to morphine (6). Therefore, the enzymes involved in these biotransformations are not sufficiently specific to reject the unnatural substrates modified by replacement of the methoxy group by an ethoxy group in position 3.

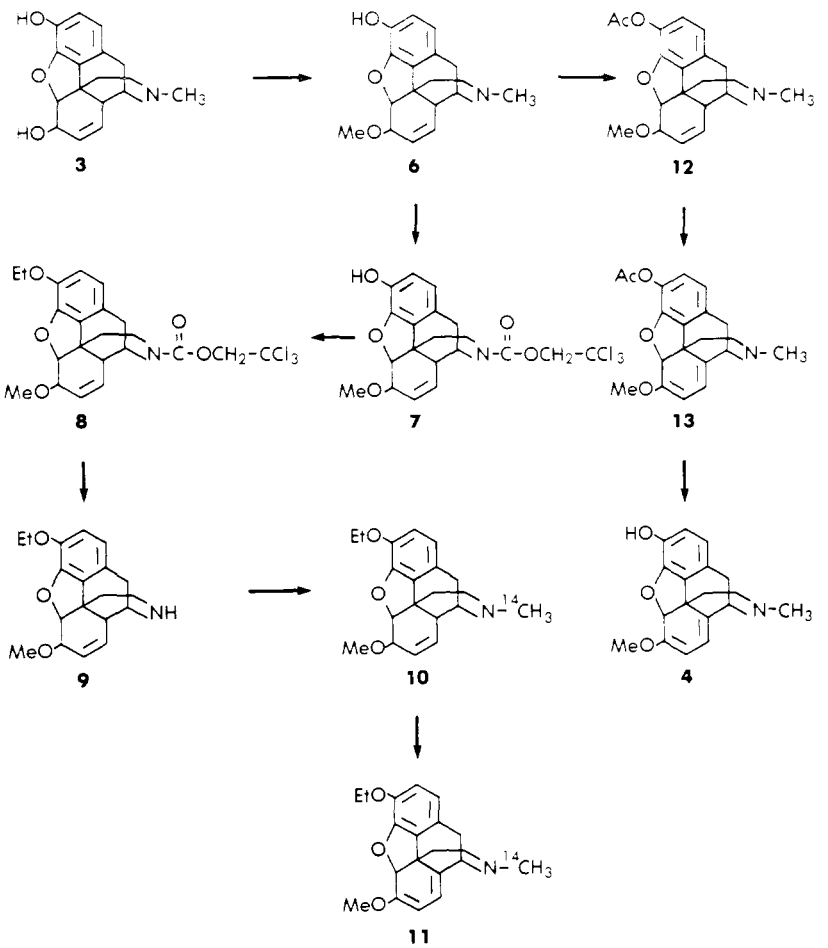
TABLE 1. Results of feeding experiments with *Papaver somniferum*.

Compound fed	Amt. fed mCi	Variety	No. of Plants	Percent Incorporation of ^{14}C Into			Rel. ^{14}C Amt. in *N-methyl* group
				Oripavine[a]	3-Ethyl-morph.[a]	Morphine	
Oripavine 3-ethyl ether[b]	0.011	Noordster	12	—	12.7	3.6	98.5
DL-Reticuline[b]	0.10	Ikkanshu	15	0.003	—	4.8	99.2

[a]Isolated by carrier dilution.
[b][*N-methyl*-^{14}C].

The incorporation of reticuline into oripavine is so small as to be insignificant. This can hardly be explained on the basis of different reaction rates for enolic and phenolic ether cleavage. Even vastly different reaction rates would give a measurable incorporation of radioactivity into oripavine in view of the large amount of radioactivity administered in the form of reticuline and the high incorporation

into morphine. Although different varieties of *P. somniferum* may show somewhat different alkaloid compositions, it is nevertheless reasonable to conclude from this experiment that oripavine is not a genuine alkaloid in the opium poppy. If oripavine is derived directly from thebaine by phenolic methyl ether cleavage, as is generally believed and supported by the work of Hodges *et al.* (12), the enzyme which converts codeine to morphine in *P. somniferum* is different from the enzyme demethylating thebaine to oripavine, the latter being absent from the opium poppy. However, it is also conceivable that thebaine may not be a precursor of oripavine, but the two alkaloids may be biosynthesized along different but parallel



SCHEME 2. Synthesis of oripavine and labeled oripavine 3-ethyl ether.

pathways. Irrespective of whether oripavine is derived from thebaine or not, reticuline is a reasonable precursor of both. Work is in progress to resolve these questions regarding the biosynthesis of oripavine.

EXPERIMENTAL

MATERIALS AND METHODS.—Two varieties of *Papaver somniferum* were used in these experiments, Ikkanshu and Noordster. The methods for cultivation of the plants, administration of labeled precursors, extraction, separation, purification and controlled degradation of alka-

loids, and determination of radioactivity have been described in previous communications (9, 10, 13, 14). In general, the crude alkaloid extracts were separated into weakly basic alkaloids by extraction of an acidic solution with chloroform, moderately basic nonphenolic alkaloids by extraction with chloroform at pH 13, and phenolic alkaloids by extraction with chloroform-2-propanol (3:1) at pH 9. Morphine 3-ethyl ether was isolated from the moderately basic nonphenolic fraction by column chromatography on alumina with benzene-chloroform (4:1) and purified by crystallization to constant radioactivity. Morphine and oripavine were separated from the phenolic fraction by column chromatography on silica gel with chloroform containing increasing amounts of methanol ranging from 1% to 20%. Morphine 3-ethyl ether used for carrier dilution was obtained from Merck & Co. The radioactive purity of labeled precursors was determined by radioactivity scanning with a Berthold radioscanner (Varian Aerograph Co.) of thin-layer chromatograms prepared with two different solvent systems. 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.

PREPARATION OF LABELED SUBSTRATES AND NONRADIOACTIVE CARRIERS.—*N-Methyl*-^{14}C-(±)-reticuline was synthesized as described previously (13): specific activity, 0.614 mCi/mmole. Morphine 6-methyl ether (heterocodeine) (**6**) was prepared from morphine by the method of Barber and Rapoport (15): yield 98%, mp 242–243° (lit. mp 242–243° (14)); ei mass spectrum *m/e* 299 (M^+); ^{1}H nmr δ 2.5 (s, 3H), 3.5 (s, 3H), 4.9 (d, 1H, J=3Hz), 5.5 (m, 2H), 6.5 (q, 2H, J=4Hz).

The method of demethylation was adapted from Montzka *et al.* (16). Heterocodeine (1.72 g) was dissolved in 150 ml chloroform, 11.0 g potassium bicarbonate, and 11.5 g of trichloroethylchloroformate were added and the mixture refluxed with magnetic stirring for 3 days. The chloroform solution was decanted, the residue dissolved in water, extracted with chloroform and the extracts combined with the original chloroform solution. Removal of the solvent gave a residue which was dissolved in 120 ml of methanol, and a solution of 4.2 g of potassium hydroxide and 7.4 g of potassium bicarbonate in 75 ml water was added. The mixture was stirred at room temperature for 24 hr, the pH was adjusted to 5 with hydrochloric acid, and the methanol was removed at reduced pressure. The crystals which separated out were filtered off and purified by column chromatography on silica gel with chloroform containing 2% methanol: yield of trichlorocarbethoxy normorphine 6-methyl ether (**7**), 84.4%; homogeneous on tlc. To a solution of compound **7** (1.68 g) in 100 ml acetone were added 0.5 g of potassium carbonate and 1 ml of ethyl iodide, and the mixture was heated at reflux with stirring for 24 hr. After evaporation of the solvent, the residue was extracted with chloroform and purified by column chromatography: yield of trichlorocarbethoxynormorphine 3-ethyl-6-methyl ether (**8**), 95%. Compound **8** (1.7 g) was dissolved in 50 ml of 90% acetic acid, 2 g of zinc powder was added and the mixture stirred overnight. After addition of water, the solution was filtered, and the filtrate was adjusted to pH 11–12 with ammonia and a little potassium hydroxide and extracted with chloroform. The combined extracts were washed with ammonia containing a little potassium hydroxide, then twice with water, dried and evaporated to dryness. The residue was purified by chromatography on silica gel with chloroform containing 2% methanol: yield of normorphine 3-ethyl-6-methyl ether (**9**), 62%, e.i. mass spectrum *m/e* 313 (M^+); ^{1}H nmr δ 1.36 (t, 3H, *J*=8 Hz), 3.52 (s, 3H), 4.08 (q, 2H, *J*=8 Hz), 6.45 (d, 1H, *J*=9 Hz), 6.63 (d, 1H, *J*=9 Hz).

N-Methyl-^{14}C-morphine 3-ethyl-6-methyl ether (**10**) was obtained by methylation with ^{14}C-paraformaldehyde and formic acid according to Andersen and Woods (17). The reaction product was purified by column chromatography on silica gel with ethyl acetate-methanol-ammonia (98:2:0.5) to give an oily liquid which crystallized on standing: mp 125°; homogeneous on tlc; yield 76%; ei mass spectrum *m/e* 327 (M^+); ^{1}H nmr δ 1.29 (t, 3H, *J*=8 Hz), 3.38 (s, 3H), 3.47 (s, 3H), 4.04 (q, 2H, *J*= 8 Hz), 6.49 (d, 2H, *J*=8 Hz), 6.58 (d, 2H, *J*=8 Hz).

N-Methyl-^{14}C-morphine 3-ethyl-6-methyl ether (**10**) was oxidized with activated manganese dioxide (18) as described by Barber and Rapoport for synthesis of thebaine (15). The reaction product (**11**) was crystallized from ethanol: yield 8.5%; mp 139–141°; homogeneous on tlc, ei mass spectrum *m/e* 325 (M^+); ^{1}H nmr δ 1.36 (t, 3H, *J*=8 Hz), 2.51 (s, 3H), 3.62 (s, 3H), 4.15 (q, 2H, *J*=8 Hz), 5.04 (d, 1H, *J*=8 Hz), 5.29 (s, 1H), 5.60 (d, 1H, *J*=8 Hz), 6.65 (d, 1H, *J*= 8 Hz), 6.67 (d, 1H, *J*=8 Hz). Specific activity 0.173 mCi/mmole.

Oripavine (**14**) was prepared *via* morphine-3-acetate 6-methyl ether (**12**) and oripavine 3-acetate (**13**) (15): total yield from morphine, 36%; mp 199–201°; ei mass spectrum *m/e* 297 (M^+); identical with authentic oripavine by tlc; ^{1}H nmr identical with that of thebaine minus the 3-methoxy group.

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