BIOSYNTHESIS OF HYDROPHENANTHRENE ALKALOIDS IN *PAPAVER ORIENTALE*

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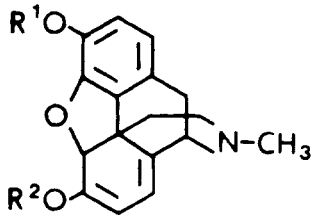
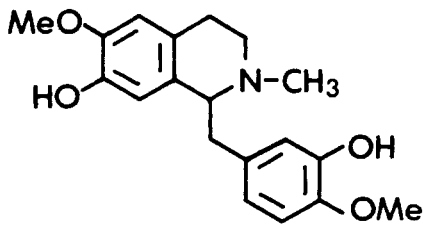
ABSTRACT.—Feeding experiments in *Papaver orientale* with radioactively labeled reticuline and thebaine have demonstrated that oripavine is derived from reticuline *via* thebaine. Reticuline undergoes racemization in this plant as has been earlier shown for *P. somniferum* and *P. bracteatum*.

The close relationship between *P. orientale*, *P. pseudo-orientale* and *P. bracteatum* has made species identification difficult and caused contradictory reports and uncertainties with regard to their alkaloid content. They belong to section oxytona and have been classified according to their haploid chromosome number (1). Differentiation may be made by cytological and morphological examination (2) and—with perhaps less certainty—by color reactions of the plant latex with Fröhde's reagent (3). Lalezari *et al.* (4–7) have performed thorough examinations of several members of the genus *Papaver* and have reported on the alkaloids of the three species referred to above after proper identification. Although different geographical areas have been shown to produce different chemotypes of *P. orientale*, they all contain oripavine (**1a**) either as the sole alkaloid or as a major alkaloid together with thebaine (**1b**) (7). *Papaver bracteatum*, in which thebaine is the principal alkaloid, has also been reported to contain oripavine, but only in trace amounts (8, 9), while *P. pseudo-orientale* appears to lack both of these alkaloids (6).



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

Several investigators have shown that reticuline (**2**) is a precursor of thebaine in *P. orientale* (10) and in *P. bracteatum* (9, 11). The origin of oripavine is not as well established.



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Using seeds of a "mixed" commercial variety of *P. orientale*, Stermitz and Rapoport (12) fed randomly labeled thebaine to mature plants and reported incorporation into two substances whose uv absorption spectra and R_f values by paper chromatography were such as to cause them to suspect oripavine although

no positive identification could be made. More recently, Rapoport *et al.* (9) studied the metabolism of thebaine in *P. bracteatum*. They found that fed 16-^{3}H-thebaine was substantially metabolized in this plant, but not by pathways that involved demethylation to either oripavine or northebaine. Nevertheless, incorporation of thebaine into oripavine of 0.06% and 0.31% in two separate experiments suggest that thebaine may indeed be a precursor of oripavine. Since uncertainties still prevail regarding the biosynthetic pathway leading to oripavine, it appeared to be of interest to study its biosynthesis in *P. orientale* of known identity and origin. For this purpose N-*methyl*-^{14}C-1-^{3}H,(±)-reticuline and 2-^{3}H-thebaine were used as precursors.

RESULTS AND DISCUSSION

The results of the feeding experiments are recorded in table 1. Reticuline was incorporated into both thebaine and oripavine. Controlled chemical degradation of oripavine showed that, within experimental error, all ^{14}C activity resided in the *N*-methyl group. The loss of ^{3}H from the asymmetric centers of thebaine and oripavine, as indicated by the decrease in the ^{3}H:^{14}C ratio, shows that *P. orientale* is capable of racemizing reticuline as has earlier been demonstrated for *P. somniferum* and *P. bracteatum* (11, 13). The low incorporation of radioactivity into thebaine as compared to oripavine suggests a fast turn-over rate of thebaine in the plant.

The incorporation of 2-^{3}H-thebaine into oripavine without scrambling of the radioactive label is significant and clearly establishes thebaine as a precursor of oripavine. The difficulty of making manual injections into the hard hypocotyl and some leakage of the feeding solution from the injection sites, together with possible problems of transport to sites of active biosynthesis, may at least in part be responsible for lower incorporations that might have been achieved with more efficient feeding techniques. Nevertheless, it is not unreasonable to surmise that the metabolism of thebaine in *P. orientale* is not confined solely to its conversion to oripavine. This would be consistent with the observations by Hodges, Horn and Rapoport on the metabolism of thebaine in *P. bracteatum* (9).

TABLE 1. Results of feeding experiment with *Papaver orientale*.

Compound fed	Amt. fed mCi	No. of plants	Alkaloids isolated (mg) and incorporation of radioactivity (%)		Rel. Amt. of radioactivity in fragment
			Thebaine	Oripavine	
(±)-Reticuline[1]	0.024	8	109 mg[3], 0.05%	98 mg[4], 0.31%	98.2[5]
Thebaine[2]	0.031	15	— —	203 mg, 0.17%	98.0[6]

[1]N-*methyl*-^{14}C[1-^{3}H], ^{3}H: ^{14}C=1.53. [2][2-^{3}H]. [3]52% ^{3}H loss. [4]53% ^{3}H loss. [5]Benzyltrimethylammonium bromide. [6] ^{3}H loss after proton exchange.

EXPERIMENTAL

PREPARATION OF LABELED PRECURSORS.—The synthesis of *N-methyl*-^{14}C-1-^{3}H, (±)-reticuline has already been described (11). Specific activity: ^{14}C, 1.35 mCi/mmole; ^{3}H, 2.06 mCi/mmole; ^{3}H: ^{14}C=1.53. 2-^{3}H-Thebaine was synthesized according to Barber and Rapoport (14) from 2-^{3}H-morphine (15); identical with authentic thebaine by tlc and glc. Specific activity: 0.18 mCi/mmole.

CULTIVATION OF PLANTS AND ADMINISTRATION OF PRECURSORS.—The seeds of *P. orientale* were obtained from Iran after authentic species determination (1,7). The plants were grown in flower pots in a greenhouse. The feeding was done to blooming and budding two-year old plants. The radioactive precursor was dissolved in an equivalent amount of 0.1*N* sulfuric acid and diluted with water to a concentration of 2 mg/ml. One-half ml was injected into the hypocotyl at one- to two-day intervals. The plants were harvested after 10 days and placed in a freezer until they were required for extraction. The whole plants were cut into small pieces and macerated with methanol in a high-speed blender. The suspension was poured into

a glass percolator and percolated with methanol until the extract gave negative tests for alkaloids. The extract was concentrated to a final volume of 1 liter in a rotary vacuum, evaporated at 38°, and chlorophyll was removed by extraction with ethyl acetate. The combined ethyl acetate extracts were washed with 3x50 ml of 0.5*N* hydrochloric acid, and the washings were combined with the original aqueous solution (total alkaloids). The total alkaloids were separated into a nonphenolic fraction by extraction with chloroform of pH 13 and a phenolic fraction by extraction with chloroform-2-propanol (3:1) after adjustment to pH 9 with solid ammonium chloride. The nonphenolic fraction was mainly thebaine which was purified by column chromatography, first on neutral alumina (activity grade III) with chloroform, then on silica gel (activity grade II) with chloroform containing 2% methanol. Chromatographically pure thebaine obtained in this way was crystallized from benzene-hexane (1:1) to constant radioactivity.

The phenolic fraction was almost pure oripavine, identified by comparison with an authentic sample by tlc and glc. It was further purified by column chromatography on silica gel with chloroform containing increasing amounts of methanol (0–5%), and finally crystallized from aqueous ethanol to constant radioactivity.

DETERMINATION OF THE POSITIONS OF THE LABELS.—Radioactive oripavine isolated from *P. orientale* plants fed N-*methyl*-^{14}C-1-^{3}H,(±) reticuline was methylated with trimethylanilinium sulfate (15). The resulting thebaine was degraded as described previously (11), and the *N*-methyl group was isolated as benzyltrimethyl ammonium bromide.

Radioactive oripavine isolated from plants fed 2-^{3}H-thebaine was subjected to proton exchange as described for morphine (15).

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