Opium Alkaloids XI: Biosynthesis of Aporphines in *Papaver somniferum*

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Abstract □ When specifically labeled (±)-reticuline was administered to opium poppies, it was incorporated into isoboldine to an extent of about 0.08%. No incorporation of reticuline could be observed in magnoflorine. Since reticuline, isoboldine, and magnoflorine are known to exist in the opium poppy, it may be concluded that aporphines with a 1,2,9,10-substitution pattern (isoboldine type) can be biosynthesized by direct phenol coupling (*ortho–para*), while this may not be the case for aporphines with substituents in positions 1,2,10,11 (corytuberine type). Because of steric factors, these aporphines are more likely to be produced *via* a dienone intermediate (proaporphine) followed by a dienone–phenol rearrangement.

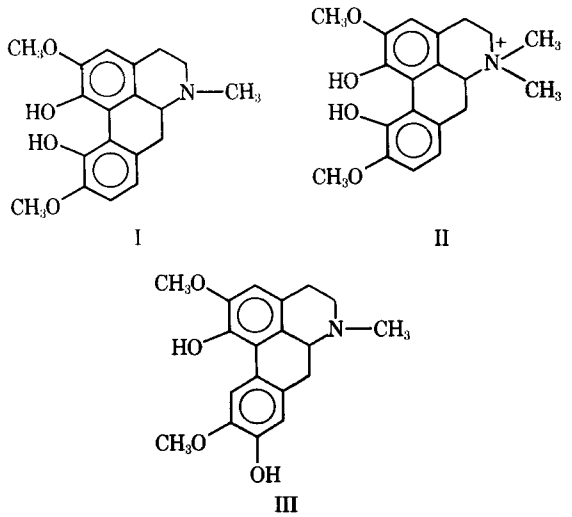
Keyphrases □ *Papaver somniferum*—biosynthesis of aporphines □ Aporphines—biosynthesis in *Papaver somniferum* □ (+)-Reticuline—incorporation into isoboldine, determination of aporphine biosynthetic pathways in *Papaver somniferum* □ Opium alkaloids—aporphine biosynthetic pathways in *Papaver somniferum*

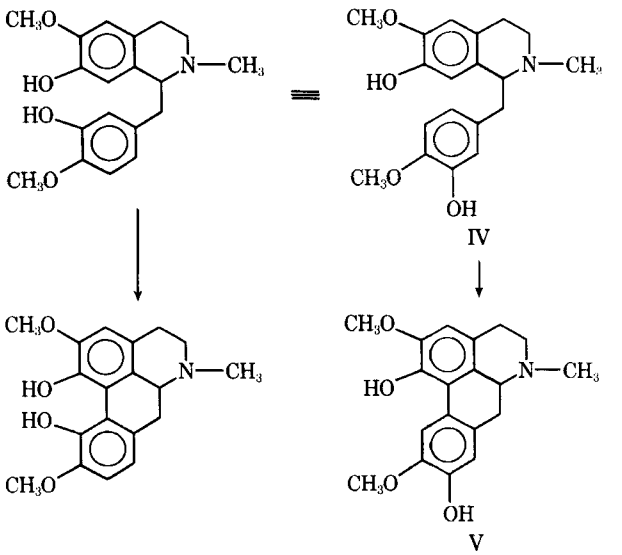
The aporphines have only recently joined the family of opium alkaloids. Nijland (1) reported the isolation of corytuberine (I) and magnoflorine (II) in 1965. Two years later, isoboldine (III) was added to the list (2). These alkaloids have the absolute configuration corresponding to the *S*-series, which relates them to (+)-reticuline, (+)-orientaline, and (−)-norlaudanosoline (2, 3).

In their famous paper on oxidative coupling of phenols, Barton and Cohen (4) proposed two biosynthetic pathways by which suitable, phenolic tetrahydroisoquinolines might be converted to aporphines, either by a direct coupling (Scheme I) or indirectly *via* an intermediate dienone which, in turn, could undergo rearrangements to a variety of aporphines (Scheme II). Although the biosynthetic pathways leading to aporphines are far from elucidated, there is today considerable evidence in support of both the direct coupling and the mechanisms that require the intermediate dienone. *In vitro* oxidation of reticuline (IV) by reagents that favor formation of free radicals has produced isoboldine (V) (5, 6). Even better yields were obtained when the nitrogen was quaternary (7, 8). However, never has a product been isolated from such reaction mixtures to indicate that a direct *ortho–ortho* coupling to aporphines takes place. Aporphines also have been synthesized by oxidative coupling to a dienone, reduction to the corresponding dienol, and rearrangement to the aporphine. The synthesis of isothebaine (IX) from orientaline (VI) *via* orientalinone (VII) was performed by Battersby and coworkers (9, 10) and is illustrated in Scheme III. Rearrangement of orientalinone without prior reduction has given rise to isocorytuberine (VIII) (11), an alkaloid not yet isolated from nature. Similar "biogenetic-type" syntheses of aporphines by dienone–phenol and dienol–benzene rearrangements were reported by several investigators (12–16).

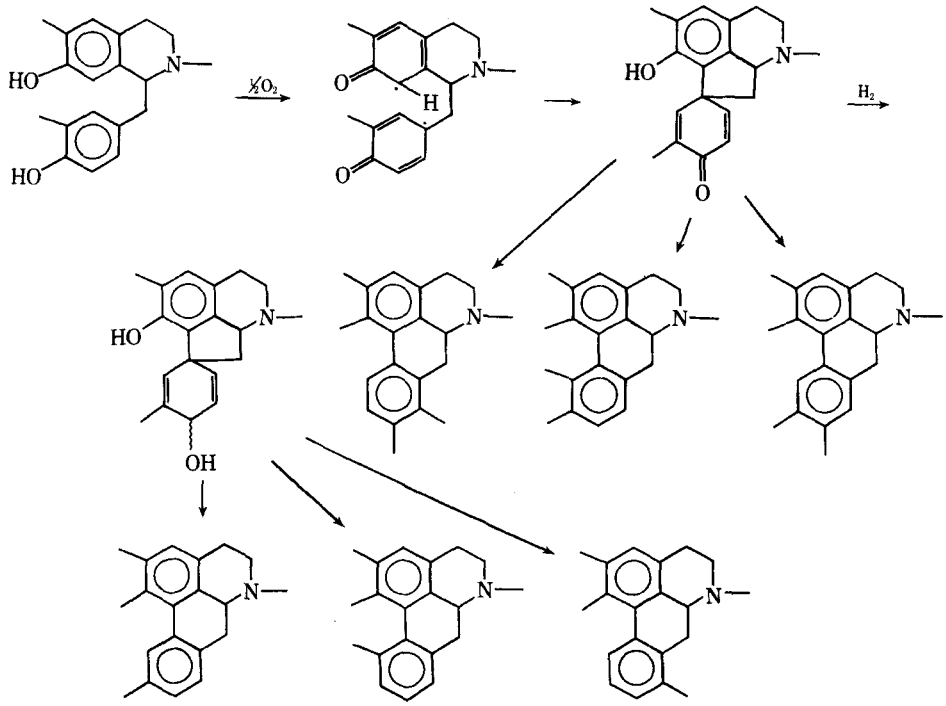
Orientalinone and related dienones are present in many plants and are generally referred to as proaporphines. Experimental evidence for the biosynthetic (*in vivo*) conversion of such compounds to aporphines by dienone–phenol and dienol–benzene rearrangements was furnished by Barton *et al.* (16), Haynes *et al.* (17), and Battersby *et al.* (18–20).

Blaschke (21, 22) reported good incorporation of reticuline (X) into bulbocapnine (XI) in *Corydalis cava* in a process that might be expected to involve corytuberine (XII) as an intermediate (Scheme IV). This represents the only experimental evidence in favor of a





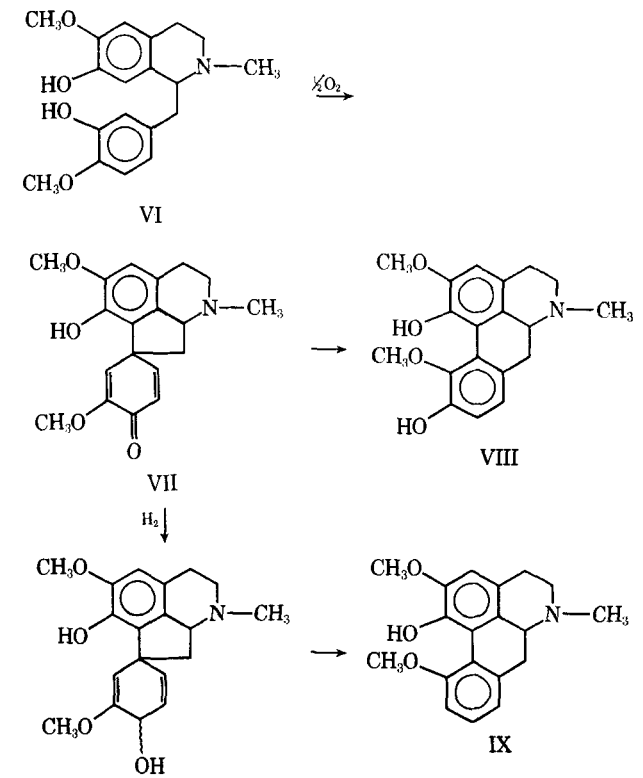
Scheme I—Proposed biosynthesis of aporphines by direct oxidative coupling of reticuline



Scheme II—Proposed biosynthetic pathways of aporphines via *a dienone intermediate*

direct *ortho–ortho* coupling in the biosynthesis of aporphines, although an *in vitro ortho–ortho* coupling to a dienone was achieved (11).

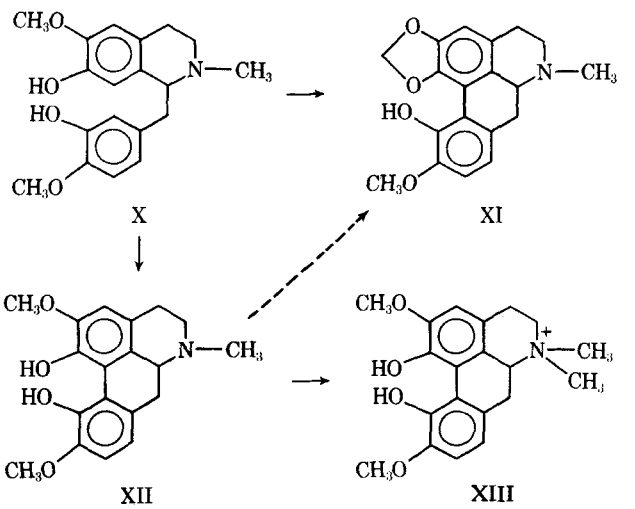
Since the opium poppy contains reticuline as well as diphenolic tetrasubstituted aporphines with both 1,2,9,10- and 1,2,10,11-substitution patterns, it seems to lend itself to an examination of aporphine biosynthesis. As a first step in this investigation, it was decided to study the possibility that reticuline might be the precursor of both types of aporphines by a direct oxidative coupling as illustrated in Scheme I. The amounts of aporphines present in the opium poppy are too small for direct isolation, and the reverse isotope dilution technique was used. Labeled reticuline was administered to the plants, and isoboldine and magnoflorine were added as "cold" carriers during the extraction. The alkaloids were isolated and purified to constant radioactivity.



Scheme III—Synthesis of isothebaine (IX) and isocorytuberine (VIII) from orientaline (VI) via *orientalinone (VII)*

RESULTS AND DISCUSSION

The amounts of labeled reticuline administered, the number and the variety of plants, and the results obtained are given in Table I. When (±)-reticuline-(3-^{14}C) was administered to opium poppies



Scheme IV—Proposed biosynthesis of bulbocapnine (XI), corytuberine (XII), and magnoflorine (XIII) from reticuline (X)

Table I—Radioactive Feeding Experiment with *Papaver somniferum* L.

Precursor	Amount Administered—μc.	Amount Administered—Number of Plants	Year	Variety	Alkaloid Added as Carrier	Incorporation, %	Degradation, Activity in Isolated Fragment, %
(±)-Reticuline-(3-^{14}C)	80.31	18	1968	Noordster	(±)-Isoboldine	0.073	—
(±)-Reticuline-(N-$^{14}CH_3$)	144.2	14	1969	Indra	(±)-Isoboldine	0.084	98.9
(±)-Reticuline-(N-$^{14}CH_3$)	144.2	14	1969	Indra	(+)-Magnoflorine iodide	0.001	—

(Noordster), it was incorporated into isoboldine to an extent of 0.073% [based on the amount of (±)-isoboldine added as cold carrier]. The amount of isoboldine remaining after purification to constant radioactivity was too small for controlled degradation and determination of the position of the label. The feeding experiment was, therelore, repeated with (±)-reticuline-(N-$^{14}CH_3$) (Indra), (±)-isoboldine, and (+)-magnoflorine being used as carriers. Again, isoboldine showed good incorporation of radioactivity (0.084%), whereas magnoflorine was inactive. The isolated and purified isoboldine was degraded by a selective Zeisel demethylation procedure. The *N*-methyl group contained all radioactivity (98.9%). This finding strongly suggests that isoboldine is biosynthesized by a direct oxidative *ortho–para* coupling of reticuline in the opium poppy. It is conceivable, however, that isoboldine may also be produced by an alternative pathway, involving orientalinone and a dienone–phenol rearrangement. Work is in progress to study this possibility.

The fact that reticuline was not incorporated into magnoflorine tends to indicate that 1,2,10,11-substituted aporphines may not be biosynthesized by a direct phenol coupling of reticuline in the opium poppy. This is in contrast to the results reported by Blaschke (21, 22) for bulbocapnine. From spectroscopic data as well as model considerations, it is evident that substituents in positions 1 and 11 cause considerable steric hindrance to coplanarity (3, 23–25), thereby making an *ortho–ortho* coupling of reticuline seem very difficult.

There is still a great deal of work to be done before the biosynthesis of aporphines can be fully understood. Based on the evidence available at present, one can only conclude that there are several pathways and mechanisms leading to natural aporphines. It is even possible that different plants may produce the same aporphine by different routes, depending on the precursors and enzyme systems available.

EXPERIMENTAL

The syntheses of (±)-reticuline-(3-^{14}C) and (±)-reticuline-(N-$^{14}CH_3$) and the methods for determination of radioactivity, cultivation of plants, and administration of labeled substances were described in a previous communication (26).

Alkaloids for Carrier Dilution—*Magnoflorine Iodide*—This was used as supplied[1].

(±)-Isoboldine—This was synthesized by a modified Pschorr ring closure reaction. 1-[3-(Benzyloxy-4-methoxy-6-aminobenzyl)]-6-methoxy - 7 - benzyloxy - *N* - methyl - 1,2,3,4 - tetrahydroisoquinoline (0.48 g.), prepared as described by Tomita and Kikkawa (27), was dissolved in a mixture of 1.5 ml. concentrated sulfuric acid, 15 ml. glacial acetic acid, and 15 ml. water. The solution was cooled in ice, and a solution of 0.12 g. of sodium nitrite in 4 ml. of water was added dropwise. After the addition was complete, the solution was stirred for 15 min. Concentrated sulfuric acid (20 ml.), 60 ml. of water, and 2.3 g. of zinc powder were added, and the mixture was refluxed for 1 hr. (28). The solution was filtered, and the filtrate was made basic with ammonia and extracted with chloroform. Evaporation of the chloroform gave a residue (256 mg.) which was purified by preparative TLC on silica gel with chloroform–methanol (9:1) and, finally, by crystallization from methanol, m.p. 122–123°. The product was compared with natural isoboldine by TLC on silica gel [chloroform–methanol (9:1), benzene–ethanol (8:2)], by GC of the free base and the trimethylsilyl derivative, and by NMR spectroscopy.

Extraction, Separation, and Purification of Alkaloids—The plants were extracted as described previously (26).

Magnoflorine—The aqueous acidic solution of total alkaloids was first extracted with chloroform to remove the weakly basic alkaloids, adjusted to pH 9 with ammonia, and then extracted with a mixture of chloroform and isopropyl alcohol (3:1). The aqueous phase was acidified with concentrated hydrochloric acid, and magnoflorine precipitated as the reineckate (29). The precipitate was washed with water, dried in a desiccator, and dissolved in acetone (200 ml.). A saturated solution of silver sulfate in water was added dropwise until no further precipitation occurred. The solution was filtered, and the filtrate concentrated to about 20 ml. The precipitate which formed during concentration was removed by filtration, and a saturated aqueous solution of potassium iodide was added. The solution was placed in a refrigerator to crystallize, and the crystals of magnoflorine iodide were recrystallized to constant radioactivity (9 d.p.m./mg.), m.p. 224–225° [lit. (29) 224–225°]. TLC on silica gel with a mixture of *n*-propanol, ammonium hydroxide, and water (4:1:1) gave the same R_f value as an authentic sample of magnoflorine iodide.

Isoboldine—The aqueous, acidic solution of total alkaloids from the plants to which (±)-isoboldine had been added as cold carrier was extracted with several portions of chloroform to remove the weakly basic alkaloids. The aqueous phase was basified to pH 8–9 with ammonia and extracted with chloroform. Evaporation of the chloroform left a dark residue, which was dissolved in 0.5 *N* hydrochloric acid. After addition of sodium hydroxide to pH 14, nonphenolic alkaloids were removed by extraction with chloroform. Ammonium chloride was added to bring the pH to about 9, and the solution was extracted with ether. Evaporation of the ether gave a dark-red residue, which was purified by column chromatography on silica gel with chloroform–methanol (97:3). The fractions containing isoboldine were combined and evaporated to dryness. The residue was dissolved in a small amount of ether, an excess of diazomethane in ether was added, and the mixture was set aside at room temperature for 2 days. After evaporation of the solvent, the residue was purified by preparative TLC on silica gel with chloroform–methanol (9:1). The resulting *O,O*-dimethylisoboldine (glaucine) was dissolved in ether (20 ml.), an excess of iodomethane was added, and the mixture was allowed to stand at room temperature for 12 hr. The crystals of glaucine methiodide were collected and recrystallized from a mixture of methanol and petroleum ether to constant radioactivity, 429 d.p.m./mg., corresponding to 634 d.p.m./mg. calculated on the basis of isoboldine.

The aqueous, acidic solution of total alkaloids from the plants to which (±)-isoboldine and magnoflorine had been added as cold carriers was first extracted as already described for magnoflorine. The chloroform–isopropyl alcohol extract was evaporated to dryness, and the residue was treated as described for isoboldine. The column fractions containing isoboldine were combined and evaporated to dryness, and the residue was crystallized from methanol to constant radioactivity, 900 d.p.m./mg., m.p. 122–123°. It was identified as isoboldine by TLC and GLC.

Degradation of Radioactive Isoboldine—Isoboldine isolated from the plants to which (±)-reticuline-(N-$^{14}CH_3$) had been administered was degraded by a selective Zeisel demethylation method as de-

[1] Supplied by Dr. Jack L. Beal.

scribed previously (30). The triethylmethylammonium iodide resulting from *N*-demethylation was crystallized from a mixture of ethanol and ether to constant radioactivity, 1128 d.p.m./mg.

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Liquid Crystalline Phases in Aerosol Formulations I: Phase Equilibria in Propellant Compositions

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Abstract □ Phase equilibria in a system of water, mixed emulsifiers, and liquid propellant are determined by visual observation of the number of phases after centrifugation. Three different liquid phases and one liquid crystalline phase are observed. A small increase in the amount of water gives rise to a complex phase pattern, which is explained by the interaction between the two emulsifiers and the water.

Keyphrases □ Propellant composition—determination of phase equilibria □ Phase equilibria, determination—water–emulsifier–propellant systems, by visual observation □ Emulsifier–water interaction—role in phase equilibria of water–emulsifier–propellant systems

The stability of emulsions has been treated for a long time as arising from the properties of the interface between two liquid phases, of which one is dispersed in the other (1–3). In 1969, evidence was presented that liquid crystals are present in simple systems of water, hydrocarbons, and a single emulsifier (4). Further research (5) showed how the presence of the liquid crystalline phase has a dominant effect on the stability of the emulsion.

Later, Saunders (6) suggested that the stability of aerosol emulsions and foams could be related to "molecular complexes" forming multilayers of liquid crystalline character at the interface. The fact that surfactants, water, and amphiphilic substances can form liquid crystalline phases when mixed in certain proportions (7, 8) was cited as proof of the suggestion.

Because of this suggestion and the increased importance of aerosol packings, an investigation of the presence of liquid crystalline phases in systems of water, liquified chlorofluorocarbons, and one emulsifier was of interest. This publication is the first report on this investigation in which the emulsifier system of octanoic acid and 1-aminooctane was used. The system earlier gave well-defined results (9) in connection with emulsion stability.