Opium Alkaloids XIV: Biosynthesis of Aporphines—Detection of Orientaline in Opium Poppy

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Abstract □ Orientaline was detected in the opium poppy by an isotope dilution method based on its biosynthesis from norlaudanosoline. Administration of labeled orientaline revealed that this alkaloid was not a precursor of isoboldine, and the experimental results provided no evidence for a pathway involving norprotosinomenine. No conclusion was possible relative to the origin of magnoflorine in the opium poppy.

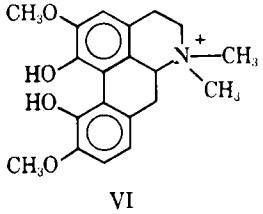
Keyphrases □ Opium alkaloids—biosynthesis of aporphines, detection of orientaline in opium poppy □ Orientaline—detection in opium poppy by isotope dilution method, biosynthesis from norlaudanosoline □ Biosynthesis of opium alkaloids—orientaline from norlaudanosoline □ Aporphine biosynthesis—detection of orientaline in opium poppy □ Isotope dilution technique—determination, orientaline in opium poppy

An earlier paper in this series (1) reported that isoboldine (III) is biosynthesized in the opium poppy by direct oxidative coupling (*ortho–para*) of reticuline (II), whereas a direct pathway could not be ascertained for magnoflorine (VI). At the same time, it was pointed out that an alternative pathway *via* orientaline (IV) and orientalinone (V) might also exist for isoboldine (Scheme I). Orientaline and orientalinone are present in *Papaver orientale* and *P. bracteatum* (2, 3), where they are involved in the biosynthesis of the aporphine isothebaine (2, 4, 5), but they have not yet been isolated from opium or the opium poppy. Biosynthesis of aporphines by direct *ortho–ortho* coupling of reticuline has been demonstrated for bulbocapnine in *Corydalis cava* (6, 7) and for magnoflorine in Columbine plants (aquilegia, McKana hybrid) (8). Battersby *et al.* (9) were unable to show either type of direct coupling for the biosynthesis of corydine (X), glaucine (XI), and dicentrine (XII) in *Dicentra eximia*. Instead, they found evidence of an indirect mechanism involving norprotosinomenine (VII) and the dienones VIII and IX. This opened a new possibility for the biosynthesis of isoboldine and magnoflorine in the opium poppy.

The present study was undertaken to discover alternative pathways for the biosynthesis of these alkaloids.

RESULTS AND DISCUSSION

Enzymes catalyzing biosynthetic reactions show a great deal of configurational specificity. Yet, they will often interact with substances foreign to the organism and transform them along normal metabolic routes, provided these foreign compounds are sufficiently similar to the normal metabolites. Thus, codeine 6-methyl ether has been converted to codeine by the opium poppy (10) and 1,3-dimethyl-1-pyrrolinium chloride to 3′-methylnicotine by the tobacco plant (11). Incorporation of a potential precursor into an alkaloid is, therefore, not sufficient evidence of its participation in the normal biosynthesis. It is also necessary to demonstrate that the proposed precursor actually exists in the living plant.

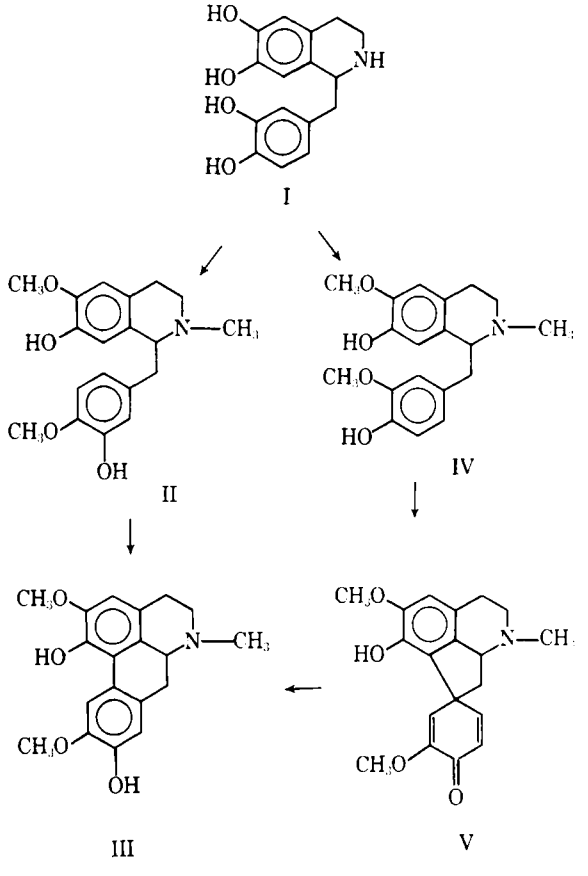


VI

In the anticipation of an alternative pathway for biosynthesis of isoboldine by way of orientaline, a scheme was developed for detection of orientaline based on its biosynthesis from norlaudanosoline (I). (±)-Norlaudanosoline-3-^{14}C was administered to opium poppies, and (±)-orientaline was added as cold carrier during the extraction. Isolation and purification of orientaline, first as the perchlorate and then as *O,O*-diacetylorientaline picrate, gave a radioactive compound. Based on the amount of orientaline added as a carrier, the incorporation of radioactivity amounted to 0.54%.

Orientaline (IV) and reticuline (II) are position isomers and difficult to separate. It was, therefore, conceivable that the radioactivity might be due to a small amount of reticuline present as an impurity. This possibility was tested by diluting a sample of the purified, radioactive diacetylorientaline picrate with 2.5 times its weight of diacetylreticuline picrate. Fractional crystallization of the mixture gave diacetylreticuline picrate of negligible specific activity.

Feeding experiments with (±)-orientaline-*N*-$^{14}CH_3$ gave no incorporation of radioactivity into isoboldine. In view of the apparent role of norprotosinomenine in the biosynthesis of apor-



Scheme I—Potential pathways for biosynthesis of isoboldine

phines (9), (±)-norprotosinomenine-3-^{14}C-7-*O*-methyl-^{3}H was administered to opium poppies, and isoboldine and magnoflorine were isolated after dilution with "cold" carriers. Neither alkaloid showed any incorporation of radioactivity.

It may be concluded that orientaline is a normal alkaloid constituent of the opium poppy. However, it does not participate in the biosynthesis of isoboldine. No definite conclusions may be drawn from these experiments about the biosynthesis of magnoflorine in the opium poppy, since both reticuline and norprotosinomenine have given negative results. There are great quantitative variations in the alkaloid composition of different varieties of the opium poppy. It is, therefore, quite possible that the variety used in these experiments (Noordster) may not have contained detectable amounts of magnoflorine.

EXPERIMENTAL

The methods used for the determination of radioactivity, cultivation of plants, and administration of labeled precursors were described previously (12). *Papaver somniferum*, Noordster variety, was used in these experiments. The radioactive purity of labeled, potential precursors was determined by radioactivity scanning of thin-layer chromatograms[1].

Alkaloids for Carrier Dilution—*Magnoflorine Iodide*—This alkaloid was used as supplied.

(±)-*Isoboldine*—This compound was synthesized as described by Kametani *et al.* (13). The product was identified by TLC and by comparison of its UV and NMR spectra with those of natural isoboldine (14).

(±)-*Orientaline*—This compound was synthesized by conventional methods (15, 16). It was purified as the perchlorate, m.p. 127° [lit. (16) m.p. 127°]. The base was further characterized by NMR (17) and mass spectrometry (18).

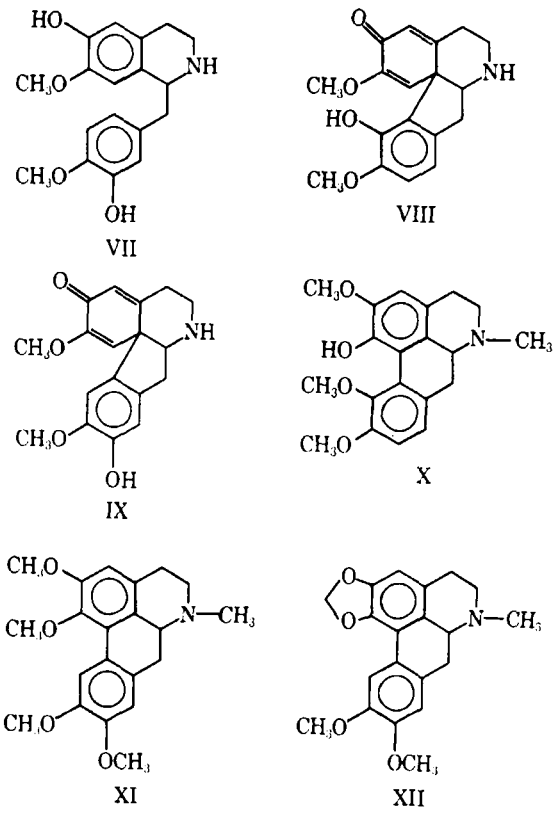
Synthesis of Labeled Precursors—(±)-*Norlaudanosoline-3-^{14}C Hydrochloride*—This compound was prepared by demethylation of (±)-tetrahydropapaverine-3-^{14}C as described by Battersby *et al.* (19), m.p. 278–281° [lit. (19) m.p. 278–281°], specific activity 0.36 mc./mmole.

(±)-*Orientaline*-N-^{14}*CH*$_3$—This compound was synthesized in the same way as the "cold" material, except that radioactive methyl iodide was distilled into the reaction vessel in a vacuum manifold system as described previously for (±)-reticuline-*N*-^{14}CH$_3$ (12). The intermediates in the synthesis and the final product were compared with the corresponding nonradioactive compounds by TLC on silica gel with two solvent systems (20) and by GLC on silicon rubber OV-1 and OV-225. The specific activity of (±)-orientaline-*N*-^{14}CH$_3$ was 1.68 mc./mmole.

(±)-*Norprotosinomenine-3-^{14}C-7-O-methyl-^{3}H Hydrochloride*—

1. (±)-*O,O*-Dibenzylnorprotosinomenine-3-^{14}C was prepared by standard methods as described for (±)-*O,O*-dibenzylnorreticuline-3-^{14}C (21), specific activity 0.24 mc./mmole.

2. (±)-*O,O*-Dibenzylnorprotosinomenine-7-*O*-methyl-^{3}H was prepared by dissolving catechol (55 g.) and benzyl chloride (63.3 g.) in 120 ml. of absolute ethanol. A solution of 11.5 g. of sodium in 170 ml. of absolute ethanol was added, and the mixture was refluxed for 2 hr. under a stream of nitrogen. The reaction mixture was allowed to cool and was then acidified with hydrochloric acid and placed in a refrigerator overnight. Sodium chloride and dibenzylcatechol crystallized out and were removed by filtration. The filtrate was evaporated to an oil, which was extracted with ether. The combined ether extracts were washed with water and evaporated to dryness. The oily residue was distilled under reduced pressure (10 mm. Hg). Unchanged catechol distilled over first, followed by the monobenzyl ether as a pale-yellow oil, boiling range 168–173°. To a well-stirred solution of *O*-benzylcatechol (5 g.) and potassium hydroxide (9 g.) in ethanol (40 ml.) and water (20 ml.) was added dropwise 10 ml. of chloroform while the solution was kept under an atmosphere of nitrogen. After 2 hr. at room temperature, the reaction mixture was refluxed for 30 min. Ethanol and unreacted chloroform were removed under reduced pressure. The residue was acidified with hydrochloric acid and extracted with ether. The combined ether extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The brown,



oily residue was chromatographed on silica gel[2], first with chloroform and then with chloroform containing 3% of methanol. The fractions containing 3-benzyloxy-4-hydroxybenzaldehyde were combined, evaporated to dryness, and crystallized from benzene, m.p. 113–114°, yield 0.288 g. NMR data agreed well with the structure. Mixed melting point with 4-benzyloxy-3-hydroxybenzaldehyde (m.p. 121–123°) showed great melting-point depression.

3-Benzyloxy-4-hydroxybenzaldehyde (22.8 mg., 0.1 mmole) was dissolved in 3 ml. of anhydous benzene, and 0.095 mmole of freshly prepared sodium ethoxide solution in 0.5 ml. of absolute ethanol was added. Radioactive iodomethane-^{3}H (10 mc., 0.01 mmole)[3] was distilled into the solution in a vacuum transfer system (21). The tube containing the reaction mixture was sealed and left at room temperature for 3 days with frequent shaking. The contents were transferred to a flask and evaporated to dryness. The residue was mixed with 330 mg. of 3-benzyloxy-4-methoxybenzaldehyde (*O*-benzylisovanillin) and dissolved in chloroform; the solution was washed with 10% potassium hydroxide solution (2 × 10 ml.) and then with water, dried over potassium carbonate, and evaporated to dryness. The residue was crystallized from ethanol.

O-Benzylisovanillin-*O*-methyl-^{3}H was converted to *O,O*-dibenzylnorprotosinomenine-7-*O*-methyl-^{3}H by conventional methods (21), specific activity 4.45 μc./mmole.

A mixture of 100 mg. of (±)-*O,O*-dibenzylnorprotosinomenine-3-^{14}C and 200 mg. of (±)-*O,O*-dibenzylnorprotosinomenine-7-*O*-methyl-^{3}H was debenzylated with hydrochloric acid (12) and purified by crystallization of the hydrochloride. Specific activity: ^{14}C, 80 μc./mmole; ^{3}H, 2.97 μc./mmole; and a ratio ^{14}C/^{3}H = 26.9.

Detection of Orientaline—(±)-Norlaudanosoline-3-^{14}C hydrochloride (10 mg.) was dissolved in 5 ml. of water, and the solution was administered to 10 plants. Two weeks later the plants were extracted by maceration with methanol in a high-speed blender[4]. (±)-Orientaline perchlorate (265 mg.) was dissolved in 100 ml. of 70% methanol and mixed with the plant material. The suspension was poured into a glass percolator and extracted with methanol until the extract gave negative tests for alkaloids. The extract was concentrated to about 500 ml. in a rotary vacuum evaporator at a temperature maintained below 40° and shaken in a separator with

[1] Berthold Radio Scanner, Varian Aerograph Co.

[2] Woelm, Activity I.
[3] Most of the iodomethane decomposed before use.
[4] Waring Products Co.

2 × 150 ml. of ethyl acetate. The ethyl acetate extract was back-extracted with 3 × 50 ml. of 0.5 *N* hydrochloric acid, and the washings were combined with the original aqueous solution (total alkaloids). The aqueous, acidic solution of total alkaloids was extracted with chloroform to remove weakly basic alkaloids, the pH was adjusted to 9 with ammonium hydroxide, and the solution was extracted with a mixture of chloroform and isopropyl alcohol (3:1). The extract was evaporated to dryness under reduced pressure, and the residue (2.2 g.) was chromatographed on a column of silica gel[5] with a mixture of chloroform and methanol (97:3). The fractions containing orientaline, as indicated by micro-TLC, were combined and evaporated to dryness. The residue (63 mg.) was converted to the perchlorate (16) and crystallized from ethanol. The specific activity decreased slowly with each recrystallization and was not yet constant after six crystallizations (930 d.p.m./mg.). The remainder of the sample (about 30 mg.) was converted to the base, dissolved in about 1 ml. of a mixture of pyridine and acetic anhydride (1:1), and left at room temperature. After 2 days, 1.05-mole equivalents of picric acid in a minimum volume of absolute ethanol were added gradually to the reaction mixture and the picrate was allowed to crystallize in a refrigerator. The crystals were collected and recrystallized from 75% ethanol to constant radioactivity, 338 d.p.m./mg., m.p. 216–217°.

To prove that the radioactivity was not due to an impurity of reticuline, 3 mg. was mixed with 7.5 mg. of *O,O*-diacetylreticuline picrate and the mixture was crystallized from 75% ethanol. The crystals, consisting mainly of *O,O*-diacetylreticuline picrate, had a specific activity that was considerably lower than that calculated for the mixture, and it decreased with each recrystallization. After six recrystallizations, when the sample was exhausted, the specific activity was 13 d.p.m./mg.

Isolation of Isoboldine and Magnoflorine—The aqueous, acidic solution of total alkaloids from the plants to which (±)-isoboldine and magnoflorine iodide had been added as cold carriers was extracted with chloroform to remove the weakly basic alkaloids. The aqueous phase was basified to pH 8–9 with sodium bicarbonate and extracted with several portions of ether. The combined ether extracts were washed with water and evaporated to dryness. The residue was chromatographed on silica gel[2] with chloroform–methanol (100:1). The fractions containing isoboldine were combined, evaporated to dryness, and crystallized from methanol to constant radioactivity; (±)-orientaline-*N*-$^{14}CH_3$ feeding: 3 d.p.m./mg.; norprotosinomenine feeding: 5 d.p.m./mg.

The aqueous, alkaline solution remaining after extraction with ether was acidified with glacial acetic acid. Magnoflorine was isolated as the iodide as described by Doskotch and Knapp (22) and crystallized from methanol to constant radioactivity; norprotosinomenine feeding: 2 d.p.m./mg.

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[5] Woelm, Activity II.