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BIOSYNTHESIS OF A NARCOTIC ANTAGONIST: CONVERSION OF *N*-ALLYLNORRETICULINE TO *N*-ALLYLNORMORPHINE IN *PAPAVER SOMNIFERUM*

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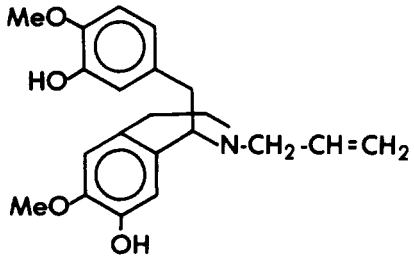
In a recent communication, we reported that the biosynthetic conversion of reticuline to morphinan alkaloids is stereoselective but not very substrate specific, and that minor modifications of the reticuline structure do not seriously affect the reactions (1). The biosynthesis of *N*-ethyl analogues of codeine and morphine from *N*-ethylnorreticuline suggested that it might be possible to produce a narcotic antagonist in this way. To test this possibility, *N*-allylnorreticuline (**1**) labeled with ^{14}C in position 3 was administered to intact opium poppies. After an appropriate period of time, the plants were harvested and extracted, and *N*-allylnormorphine (nalorphine) (**2**) was isolated by means of carrier dilution.



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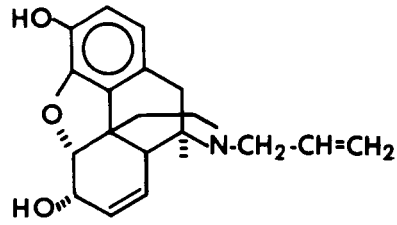

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RESULTS AND DISCUSSION

As shown in Table 1, (±)-[3-^{14}C]-*N*-allylnorreticuline was converted in the plant to nalorphine with all radioactivity in the appropriate position (C-16). Several steps are involved in the biosynthesis of morphine analogues from reticuline analogues (2-4). It is clear from our earlier studies (1) and from the work reported here that the rate of the enzymic reactions decreases as the size of the *N*-substituent increases from methyl to ethyl to allyl. Nevertheless, the incorporation of *N*-allylnorreticuline into nalorphine is appreciable. The great substrate specificity of the enzyme(s) involved in the racemization of reticuline in the opium poppy has been demonstrated (1,5). It appears that the *N*-methyl group is a prerequisite for racemization and participates in the reaction (6). It is, therefore, reasonable to assume that *N*-allylnorreticuline, in the same way as norreticuline and *N*-ethylnorreticuline, is not racemized. On this basis, the incorporation is actually twice as great as indicated in the table in which the incorporation was calculated from the racemic mixture administered to the plants.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The Noordster variety of *Papaver somniferum* L. was used in this experiment. The methods for cultivation of the plants and administration of the labeled substrate have been described (7,8). The plants were harvested 10 to 14 days after administration of the labeled precursor and were stored in a freezer until they could be extracted. All melting points were determined with the Thomas-Hoover melting point apparatus and are uncorrected. The ^1H-nmr spectra were determined in $CDCl_3$ with TMS as internal standard.

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

Compound fed	Amount fed ^{14}C (μCi)	Nalorphine isolated[a] specific activity (dpm/mg)	Incorporation of ^{14}C (%)	Relative ^{14}C activity at C-16 (%)
(±)-*N*-Allylnorreticuline[b] . . .	24.8	440	0.32	98.7

[a]Isolated by carrier dilution.
[b][3-^{14}C].

Radioactivity was determined by means of a Beckman LS 7800 liquid scintillation counter. The method for controlled degradation of nalorphine and isolation of the carbon atom at C-16 has been reported (1).

SYNTHESIS OF (±)-[3-^{14}C]-N-ALLYLNORRETICULINE.—The synthesis was carried out with nonradioactive materials prior to the synthesis of the labeled compound. The nonradioactive intermediates and final product were characterized by spectroscopic methods and by melting points. The radioactive compounds were identified by comparison with the nonradioactive compounds by tlc and glc.

(±)-[3-^{14}C]-*O,O*-Dibenzylnorreticuline (117 mg, specific activity 0.47 mCi/mmole), prepared as described previously (8), was dissolved in 8 ml DMF. Next, 30 mg of $NaHCO_3$ and 50 mg of allyl bromide were added, and the solution was refluxed under N_2 for 4 h. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$, washed with H_2O, dried, and evaporated to dryness. The crude product obtained in this way (113 mg) was purified by column chromatography on silica gel with $CHCl_3$ containing from 0 to 0.5% MeOH to give 50.5 mg of a pure compound which was identical with nonradioactive (±)-*N*-allyl-*O,O*-dibenzylnorreticuline; ms (ei) *m/z* 535 M^+ ($C_{35}H_{37}O_4N$). (±)-[3-^{14}C]-*N*-Allyl-*O,O*-dibenzynorreticuline (50 mg) was dissolved in 1 ml of concentrated HCl and 1 ml of C_6H_6, and the mixture was stirred under N_2 at room temperature overnight. The aqueous layer was separated from the C_6H_6 layer which was extracted with 1 N HCl. The acid solutions were combined, cooled in ice-water, adjusted to pH 13 with NaOH, and extracted with $CHCl_3$. Solid NH_4Cl was added to pH 8.5 and the solution extracted with $CHCl_3$ to give 25 mg of an almost pure compound, which was further purified by preparative tlc on silica gel (0.5 mm) with $CHCl_3$-MeOH (8:2). The product (15.8 mg, specific activity 0.47 mCi/mmole) gave only a single spot on tlc in several solvent systems and was identical with nonradioactive (±)-*N*-allylnorreticuline; ms (ei) 353 M-2$^+$ (1), ^{1}H-nmr, δ 2.3-3.2 (m, 6H, benzylic H's and H's on C-3), 3.30 (d, 2H, N-C*H*$_2$CH=CH$_2$, $J_{1,2}$= 6 Hz), 3.86 (s, 6H, OC*H*$_3$), 5.0-5.25 (2dd, NCH$_2$CH=C*H*$_2$), 5.80 (m, 1H, NCH$_2$C*H*=CH$_2$), 6.2-6.7 (m, arom. H's).

EXTRACTION, SEPARATION, AND PURIFICATION OF ALKALOIDS.—The extraction was carried out as described previously (1). *N*-Allylnorreticuline (400 mg) was added as a carrier at the time of extraction. The aqueous solution of total alkaloids was adjusted to pH 13, and nonphenolic alkaloids were removed by extraction with $CHCl_3$. Solid NH_4Cl was added to pH 9, and phenolic alkaloids were extracted with $CHCl_3$-*i*-PrOH (3:1). Nalorphine was isolated by preparative tlc on silica gel, first with $CHCl_3$-MeOH (8:2), then twice with EtOAc-MeOH-NH_3 (85:10:5), and crystallized from Et_2O-MeOH to constant radioactivity.

ACKNOWLEDGMENTS

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