BIOSYNTHESIS OF ERGOT ALKALOIDS. INCORPORATION OF (17R)-[17-^{3}H]- AND (17S)-[17-^{3}H] CHANOCLAVINE-I INTO ELYMOCLAVINE BY *CLAVICEPS*

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It has previously been demonstrated that chanoclavine-I (**1**) is an efficient precursor of tetracyclic ergot alkaloids (1). Studies on the mechanism of the conversion of **1** into elymoclavine (**2**) showed that **1** is incorporated into **2** with loss of half of the tritium from C–17 and that essentially all the tritium is confined to C–7 of elymoclavine (2). This suggested the intermediacy of chanoclavine-I-aldehyde (**3**), which in fact was found to be twice as efficient as **1** as a precursor to elymoclavine (2). Degradation of the elymoclavine from a feeding experiment with [17-^{3}H]chanoclavine-I-aldehyde confirmed that the tritium was entirely located at C–7 of elymoclavine and that the incorporation of the aldehyde is specific (2). In a feeding experiment with [3'-$^2H_3$]mevalonate both methylene hydrogens at C–17 of **1** were found to be deuterated while only one hydrogen at C–7 of elymoclavine, the *pro-S* hydrogen, carried deuterium (Scheme I). Thus, clearly one of the two hydrogens at C–17 of chanoclavine-I is lost during the conversion into tetracyclic ergolines. We now report results that establish which heterotopic hydrogen is removed from C–17 during this conversion.

RESULTS AND DISCUSSION

To probe the stereochemistry of hydrogen removal from C–17 of chanoclavine-I we synthesized (17*S*)-[17-^{3}H]- and (17*R*)-[17-^{3}H]-**1**. [17-^{3}H]Chanoclavine-I (3) was oxidized with MnO_2 to [17-^{3}H] chanoclavine-I-aldehyde (2). Reduction of the tritiated aldehyde with horse liver alcohol dehydrogenase (LADH) and NADH (4) yielded chirally tritiated **1** (6.4% overall yield). Based on the stereochemistry observed when a variety of aldehydes are reduced by LADH (4), it can be safely assumed that the chanoclavine-I possesses the 17**S** configuration.Similarly, reduction of unlabeled chanoclavine-I-aldehyde with tritiated NADH (5) yielded chirally tritiated **1** of *17R* configuration (6.06% overall yield).

Each chirally tritiated **1** was then fed to two three-day old shake cultures of *Claviceps* strain SD 58. Nine days later the alkaloids were extracted from the culture filtrate. Elymoclavine was purified from the mixture and analyzed for its radioactivity. The results are summarized in table 1. In experiment 1 the isolated elymoclavine was purified by preparative thin-layer chromatography to constant specific radioactivity, and in experiment 2 the reported specific activity is that obtained after two purifications by preparative tlc.

The data show clearly that chanoclavine-I is incorporated into elymo-

TABLE 1. Incorporation of chirally tritiated chanoclavine-I into elymoclavine by *Claviceps sp.*, strain SD 58.

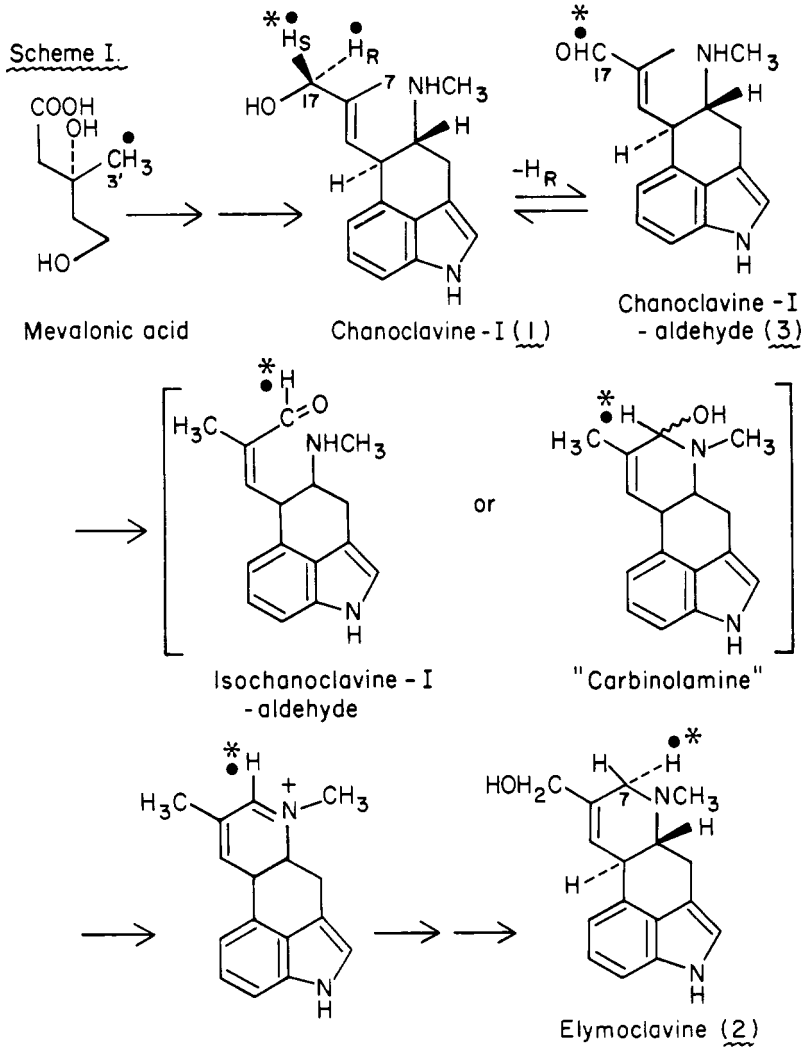
Expt.	Precursor fed	Total alkaloids, and amount of elymoclavine isolated (mg)	Radioactivity of elymoclavine	Incorporation (%)
1	(17S)-[17-^{3}H] Chanoclavine-I 3.03×10^{11} dpm/mmol, 2.60×10^6 dpm total	70.1, 51.0	6.36×10^5 dpm/mmol, 1.27×10^6 dpm total	48.8
2	(17*R*)-[17-^{3}H] Chanoclavine-I 1.33×10^9 dpm/mmol, 3.86×10^5 dpm total	80.0, 57.5	5.05×10^4 dpm/mmol, 1.11×10^4 dpm total	2.8 (max)

clavine with loss of the *pro-17R* and retention of the *pro-17S* hydrogen. They indicate that the stereochemistry for the hydrogen atom removal in the conversion of **1** into **2** is the same as for the yeast and liver alcohol dehydrogenases (4). All the tracer experiments are thus in agreement with the assumption (2) that **3** is an intermediate in the conversion of **1** into **2**. This notion is also supported by the recent isolation of a *Claviceps* mutant blocked in the synthesis of tetracyclic ergot alkaloids which accumulates **3** (6). Scheme I shows the most probable course of events in the conversion of chanoclavine-I to elymoclavine.

EXPERIMENTAL

MATERIALS.—Non-labeled chanoclavine-I was prepared chemically from elymoclavine by conversion to *N*-methyl-secoelymoclavine (7) followed by demethylation with diethyl azodicarboxylate (8). [17-^{3}H] chanoclavine-I had been prepared previously in our laboratory (3). Elymoclavine was a gift from Eli Lilly and Company. [^{3}H]$NaBH_4$ was obtained from Amersham. NAD^+, NADH and horse-liver alcohol dehydrogenase (ADH) were obtained from Sigma.

METHODS.—Oxidations of **1** to **3** were carried out as described by Floss *et al.* (2) and the reduction of NAD^+ with [^{3}H] $NaBH_4$ followed the procedure of Abou-Chaar *et al.* (5). For the enzymatic reduction of **3**, the aldehyde was incubated in potassium phosphate buffer (0.1 M, pH 6.95, 0.5 ml), Tween 80 (100 µl 1% aqueous solution), deionized water (2.5 ml) with NADH (non-labeled or tritiated, 1.6 mg, 2.2 µmol) and LADH (3 mg, 1.9 units/mg) for 24 hours at room temperature. The product was extracted with ether at an alkaline pH and purified by preparative tlc [ethyl acetate-methanol (11:4), 3 developments]. The feeding experiments with *Claviceps sp.*,



strain SD 58 and the isolation of elymoclavine were carried out as previously described (5).

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