Biosynthesis of Ergot Alkaloids. Synthesis of Chanoclavine-I-aldehyde and its Incorporation into Elymoclavine by *Claviceps*

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Summary Chanoclavine-I-aldehyde has been synthesized from chanoclavine-I and its conversion into elymoclavine by *Claviceps* strain SD58 has been demonstrated

BOTH Arigoni s[1] and our group[2] have independently shown that in the biosynthesis of ergot alkaloids from tryptophan and mevalonic acid,[3] chanoclavine-I is cyclized to the tetracyclic ergolines with concomitant *cis–trans* isomerization at the allylic double bond Further results suggested[4] that one of the methylene hydrogens at C-17 of chanoclavine-I is eliminated during the conversion To substantiate this observation we first prepared [17-T]chanoclavine-I (0 6 Ci/mmole) by the reaction sequence chanoclavine-I → *N*-acetylchanoclavine-I → *N*-acetylchanoclavine-I-aldehyde[5] → [17-T]-*N*-acetylchanoclavine → [17-T]chanoclavine-I (I),[6] using tritiated sodium borohydride in the reduction of the aldehyde The tritiated material was then mixed with ^{14}C-chanoclavine obtained biosynthetically from ^{14}C-tryptophan[7] and fed (1 19 10^6 d p m ^{14}C, T : ^{14}C = 5·15) to two three-day old cultures of *Claviceps* strain SD58 Nine days later the alkaloids (141 mg) were extracted from the culture filtrate and elymoclavine was isolated from the mixture and purified to constant specific radioactivity (T : ^{14}C = 2 72, ^{14}C-incorporation 17 6%) The tritium retention of 53% confirms the loss of one of the two labelled hydrogens from C-17 of chanoclavine-I in the conversion, suggesting a compound with only one hydrogen at this carbon atom as a possible intermediate

This result prompted us to synthesize chanoclavine-I-aldehyde Attempted oxidation of chanoclavine-I with MnO_2[8] in acetone at room temperature was unsuccessful, however, on refluxing this mixture for 45 min the aldehyde was obtained in yields of 50–60% It was purified by preparative t l c on silica gel G, could be reconverted into chanoclavine-I on treatment with $NaBH_4$, and was further characterized on the basis of the following data The mass spectrum of chanoclavine-I-aldehyde showed a molecular ion at *m/e* 254·1403 confirming the molecular formula of

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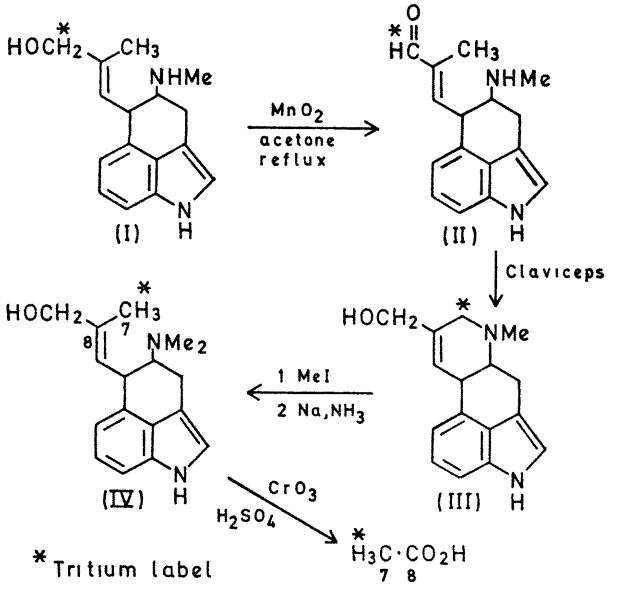
$C_{16}H_{18}N_2O$ (calculated 254·1419) A prominent ion at *m/e* 154·0654 ($C_{11}H_8N$) is typical of tricyclic and tetracyclic ergolines [9] The i r spectrum of the aldehyde ($CHCl_3$) had a strong absorption at 5 92 μm, which is typical for the $\alpha\beta$-unsaturated aldehyde moiety, and compared favourably with the absorption at 5 90 μm for *N*-acetylchanoclavine-I-aldehyde as reported by Fehr [5] Comparison of the n m r spectrum of the aldehyde in C_5D_5N with that of chanoclavine-I[10] further confirmed the structural assignment The signals for the *N*-methyl and vinyl methyl groups in the alcohol and aldehyde were unchanged, both appearing at δ 2 43 and 2 0, respectively The indole N–H also appeared at δ 11 6 in both compounds The broad singlet which appeared at δ 4 45 in the spectrum of chanoclavine-I ($-CH_2-OH$) was absent in the aldehyde spectrum which showed absorption at δ 9 6, typical of an aldehyde proton Finally, the vinyl proton signal which appeared at about δ 5·95 in chanoclavine-I was absent in the spectrum of the aldehyde, in line with the expected downfield shift of a vinyl proton β to the aldehyde group



[17-T]Chanoclavine-I-aldehyde (II, 5 mC/mmole) was prepared by oxidation of [17-T]chanoclavine-I (I) This material ($1·01·10^7$ d p m) was added to two four-day-old cultures of *Claviceps* strain SD58, while two parallel cultures received [17-T]chanoclavine-I ($4\ 88\ 10^6$ d p m). Thirteen days later, elymoclavine (III) was isolated from the crude alkaloid mixture (142 mg and 141 mg, respectively) and purified to constant specific radioactivity Tritium from the aldehyde was found to be incorporated into elymoclavine to the extent of 40%, compared to 9·9% incorporation from chanoclavine-I The elymoclavine obtained from the aldehyde experiment was diluted with carrier material (final specific radioactivity 208 d p m./μmole) and subjected to Emde–Birch reduction [11] Kuhn–Roth oxidation of the resulting *N*-methyl-seco-elymoclavine (IV) gave acetic acid (205 d p m /μmole) from C-7 and C-8, showing that all the tritium was confined to C-7 of the alkaloid A complete repetition of this feeding experiment gave essentially the same data In another experiment, a sample of [^{14}C-17-T]chanoclavine-I-aldehyde (T : ^{14}C = 1·78) was prepared from [^{14}C-17-T]chanoclavine-I (T : ^{14}C = 3 50) and upon feeding to *Claviceps* strain SD58 produced elymoclavine of T : ^{14}C = 1 61, indicating that at least the majority of the tritium at C-17 is retained in the conversion of the aldehyde into elymoclavine

These data show quite clearly that chanoclavine-I-aldehyde is specifically converted into elymoclavine by the ergot fungus To determine whether the compound is also a natural intermediate in the biosynthesis of the tetracyclic ergolines requires further study, but the high efficiency of its conversion and the fact that chanoclavine-I loses one hydrogen from C-17 in its transformation to elymoclavine would be in line with this assumption

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