

CLAVICIPITIC ACID, A NEW 4-SUBSTITUTED INDOLIC AMINO ACID
OBTAINED FROM SUBMERGED CULTURES OF THE ERGOT FUNGUS

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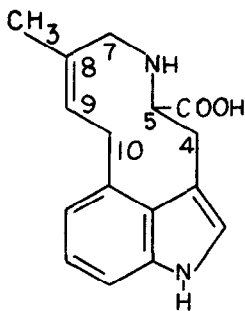
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In our studies of ergot alkaloid biosynthesis we have attempted to inhibit reactions within the biosynthetic pathway which we hoped would result in the accumulation of heretofore undetected biosynthetic intermediates. One of the reactions which seemed appropriate for this kind of experimentation was the N-methylation of the nitrogen atom derived from the α -amino group of tryptophan. It has been shown that this methylation is effected by an active methyl group which derives from methionine (1). In experiments which included a wide variety of organisms it has been shown that ethionine is a methionine antagonist and recently the accumulation of 4-dimethylallyltryptophan by the ergot fungus was demonstrated when the organism was subjected to the inhibitory effects of ethionine (2).

In the course of our inhibition experiments using ethionine a previously undescribed 4-substituted indolic amino acid was isolated and we have named it, Clavicipitic Acid. On the basis of the data described herewith we propose the following structure for this compound:



Clavicipitic acid was obtained from submerged cultures of Claviceps species, strain SD-58. The organism was grown on a yeast extract-mannitol culture medium (3). On the sixth day 40 μ moles/100 ml culture medium of DL-ethionine was added to the cultures which were then incubated for an additional 8 days. The cultures were harvested by homogenizing the mycelium with the culture medium and then removing the mycelium by filtration. The culture filtrate

was passed through a column of Dowex 50 (H^+) and the alkaloids and amino acids were removed from the resin by eluting with 5% ammonia. The alkaloids were removed from the eluate by partitioning with chloroform. By using column chromatography on silica gel and eluting with 30% ethanol in chloroform, clavicipitic acid was isolated from the aqueous phase. Recrystallization from ethanol produced colorless plates that decompose at 262° . The molecular formula determined from the high resolution mass spectrum is $C_{16}H_{18}N_2O_2$.

A violet-blue color with Ehrlich's reagent and ultraviolet absorption maxima at λ ethanol 288 $m\mu$ ($\log \epsilon$ max. 3.81) and 225 $m\mu$ ($\log \epsilon$ max. 4.58) are in agreement with an indole structure.

Retention on an anionic exchange resin, a strong absorption band at 1630 cm^{-1} in the IR and a loss of m/e 44 from the M-1 ion in the mass spectrum, support the presence of a carboxyl group in the molecule.

It was found that DL-mevalonic acid 2- ^{14}C and DL-tryptophan (alanine 3- ^{14}C) were incorporated into the compound giving support to a structure analogous to the ergoline alkaloids. The mass spectrum showed an abundant ion at m/e 154 which is due to a tricyclic stable ion (4). This ion is found in the spectra of all ergot alkaloids and is indicative of substitution in position four of the indole nucleus.

The NMR spectrum of a trimethyl derivative of clavicipitic acid in $CDCl_3$ showed a signal for one vinyl proton at δ 5.7 suggesting the presence of an 8,9 or 9,10 double bond (5). The absence of fluorescence in UV light and the UV absorption spectrum support the 8,9 position.

Using radioactively labeled isotopes the remainder of the structure was clarified. The non-incorporation of the methyl group of methionine indicated the absence of a N-methyl group (6). Without this N-methyl group and because of the analogous structure to the ergolines an additional exocyclic carbon atom had to be accounted for. The incorporation of DL-tryptophan (carboxyl- ^{14}C) into clavicipitic acid indicates that this carbon atom is in a carboxyl group which must be attached at position five. Tritium retention experiments using DL-tryptophan (alanine-2- 3H -carboxyl- ^{14}C) and DL-mevalonic acid (2- ^{14}C -5 3H) give evidence that the α hydrogen of tryptophan and the two hydrogens in position five of mevalonic acid are retained in clavicipitic acid. This means the ring is not closed between carbon five and carbon ten leaving the molecule with a ten-membered ring.

Clavicipitic acid is a naturally occurring metabolite, since it is present in lesser amounts in ergot cultures which have not been treated with ethionine.

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