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NATURAL OCCURENCE OF 4-[4-HYDROXY-3-METHYL-Δ^2-BUTENYL]-TRYPTOPHAN IN CLAVICEPS PURPUREA PRL 1980

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The role of 4-dimethylallyltryptophan as the first intermediate in the pathway of clavine alkaloid biosynthesis appears to be well established (1,2). Hydroxylation of one of the methyl groups of 4-dimethylallyltryptophan has been proposed as the next step in the biosynthetic pathway. 4-[E-4-hydroxy-3-methyl-Δ^2-butenyl-tryptophan (E-HODMAT) was incorporated into elymoclavine (3) but not into agroclavine (H. Plieninger, personal communication). Because of the possible role of HODMAT in the pathway of clavine alkaloid biosynthesis, the presence of the compound in cultures of Claviceps purpurea PRL 1980 was investigated.

EXPERIMENTAL. Cultures of Claviceps purpurea PRL 1980 were grown on a mannitol-tryptophan-succinic acid medium (4) supplemented with 3mM niacinamide per liter. Cultures were harvested after three to four days. The mother liquor from ten liters of culture was fractionated successively with Dowex 50(H) cation exchange resin (5), by Sephadex G-10 column chromatography (6), preparative TLC on silica gel G (developing solvent methyl acetate:isopropanol:ammonia, 45:35:20), and preparative TLC on Cheng-Chin polyamide sheets (developing solvent 80% formic acid:water, 1:2). Throughout the purification HODMAT was located by comparison with reference E-HODMAT. Reference and sample were then methylated and trifluoroacetylated (7). The derivative was added directly to the probe of the Varian Mat 311 Mass Spectrometer and the mass spectrum obtained at 150° and 20EV.

RESULTS. The same peaks with the same relative intensities were obtained for the mass spectra of sample and reference HODMAT. The spectrum of derivatized HODMAT is shown in Fig. 1. The mass spectrum is consistant with the structure 4-[4-methoxy-3-methyl-Δ^2-butenyl]-N,N-di(trifluoroacetyl)tryptophan methyl ester for the product of methylation and trifluoroacetylation of HODMAT. A parent peak for this compound (MW=508) was not observed. This is due to stabilization of the

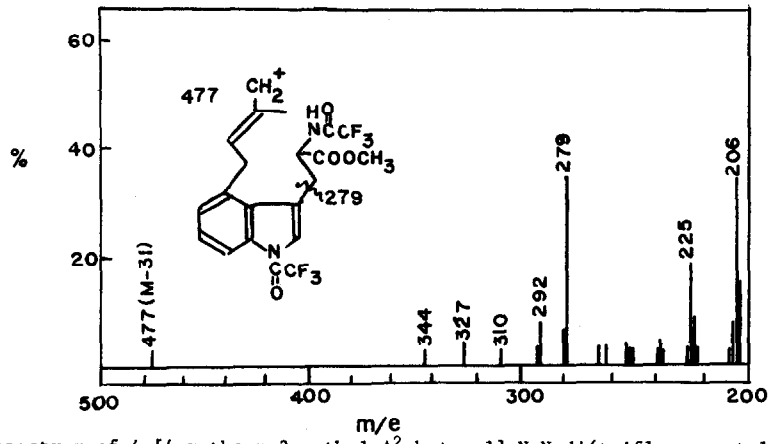

Fig. 1. Mass spectrum of 4-[4-methoxy-3-methyl-Δ^2-butenyl]-N,N-di(trifluoroacetyl)tryptophan methyl ester.

allylic carbonium ion formed after elimination of the methoxyl group (MW-31=477). The prominant peak at m/e 279 is due to loss of the N-trifluoroacetylalanine methyl ester sidechain as well as the methoxy group (MW-31-198). The small peak at m/e 310 is due to loss of the derivatized alanyl sidechain but retention of the methoxy group (MW-198).

The configuration of the HODMAT isolated from cultures of Claviceps purpurea PRL 1980 remains to be established. However, the natural occurence of HODMAT in cultures of Claviceps sp. and the incorporation of the E-isomer into elymoclavine (3) suggest that one or both isomers may have an important role in the biosynthesis of the clavine alkaloids.

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