α-Acetyl-γ-(β-indolyl)methyltetramic Acid. A Biosynthetic Intermediate of Cyclopiazonic Acid and of Bis-secodehydrocyclopiazonic Acid

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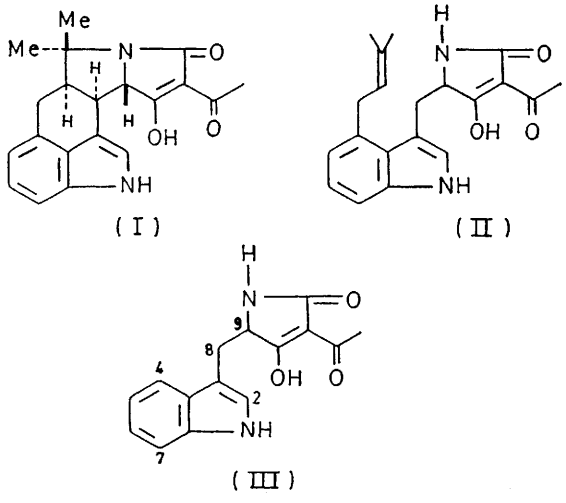
Summary It has been shown that tryptophan is not utilized in a cell-free system of *Penicillium cyclopium*; α-acetyl-γ-(β-indolyl)methyltetramic acid, a biosynthetic intermediate of the cyclopiazonic acids, has been identified, and the L-configuration at the asymmetric centre of the new indole established.

INVESTIGATIONS on the biosynthesis of cyclopiazonic acid (I) by *Penicillium cyclopium* Westling have shown that mevalonic acid, acetic acid, and tryptophan (Trp) are precursors of this metabolite.[1] Schabort[2] established that bis-secodehydrocyclopiazonic acid (II) is a direct intermediate of (I). Very little is, however, known of the sequence of events leading to the construction of (II).

Experiments with growing cultures of *P. cyclopium* showed that Trp is a very efficient precursor of (I) as 24·7% of the added DL-Trp-benzene ring-^{14}C was incorporated.[1] Trp was, however, not incorporated into (I) or (II) by cell-free extracts of the organism. The cell-free extracts were prepared from the mycelium of 4 day old cultures of *P. cyclopium* by extruding the mycelium through an ice shear press[3] at −25°. The extruded material was extracted by stirring at 0° with a Tris–chloride buffer solution pH 7·9, 0·1 *I* (ionic strength), and centrifuged (74,000 g, 2 h at 2°) to furnish the crude extract. An incubation mixture consisting of the crude extract (5 ml), L-[G-^{3}H]Trp, $3{\cdot}5 \times 10^5$ d.p.m., 1 μmol, and [1-^{14}C]DMA-PP, $3{\cdot}5 \times 10^5$ d.p.m., 1·25 μmol gave (I) and (II) of constant radioactivity (8% incorporation in total) containing only ^{14}C. The result suggested that Trp is converted into a new metabolite in whole cells of the organism which is then employed as a substrate by the enzyme that incorporates the DMA unit into (II).



A probe was developed using a cell-free system which synthesised (II) from [1-^{14}C]DMA-PP and the Trp derivative. The enzymatic assay indicated that maximal concentrations of the derivative were present in 4 day old cultures of the organism. Solvent extraction of the culture at pH 4·5 permitted the isolation of the new Ehrlich positive metabolite (yield, *ca.* 5 mg/l). The crystalline metabolite (III) could be converted by the above cell-free

system and [1-^{14}C]DMA-PP into radioactive (II) (purified by SiO_2 t.l.c.). The compound (III), $C_{15}H_{14}N_2O_3$, crystallised from benzene, m.p. 164—165°, had λ_{max} (MeOH) 222, 272, 279, and 290 nm (log ϵ 4·60, 4·24, 4·33, and 4·19, respectively); ν_{max} ($CHCl_3$) 3480, 3430, 1710, 1660, and 1620 cm^{-1}; δ($CHCl_3$) 8·4 (1H, s, N*H*), 6·8—7·6 (4H, m, Ar*H*), 4·05 (1H, X part of an ABX system, J_{AX} <1, J_{BX} 4·5 Hz, 9-H), 3·32 and 2·78 (2H, AB part of ABX system, J_{AX} <1, J_{BX} 4·5, J_{AB} 15·0 Hz, 8H_2), and 2·38 (3H, s, COCH_3); c.d. (MeOH): $\Delta\epsilon$ (370 nm) 0, (320 nm −1·8, (280 nm) −5·3, (263 nm) 0, (242 nm) −3·5; mass spectrum *m/e* 270 (M^+, $C_{15}H_{14}N_2O_3$) (10%) and *m/e* 130 (C_9H_8N) (100%). A direct comparison [m.p., i.r., mass spectra, and partition chromatography on SiO_2 t.l.c. and $HCONH_2$:$(CO_2H)_2$, 50:3 impregnated Whatman No. 1 paper] of this compound with authentic α-acetyl-γ-(β-indolyl)methyltetramic acid established the identity of the two compounds.

The distinct similarity in the c.d. Cotton effects[4] of tenuazonic acid $\Delta\epsilon$ (320 nm) −2·34, (280 nm) −2·8, and (240 nm) −2·34 and the acid (II) $\Delta\epsilon$ (325 nm) −1·0 and (280 nm) −5·0 with those of compound (III) establishes the 9L-configuration for the natural product (III).

The results of the experiments support the introduction of the DMA unit into the C-4 position of Trp after it is converted into a tetramic acid by the addition of an acetoacetyl group. The biogenetic sequence resembles that of echinulin[5] where the DMA unit is transferred to *cyclo*-L-alanyl-L-tryptophanyl.

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