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THE STRUCTURE OF CYCLOPENIN AND CYCLOPENOL, METABOLIC PRODUCTS FROM PENICILLIUM CYCLOPIUM WESTLING AND PENICILLIUM VIRIDICATUM WESTLING

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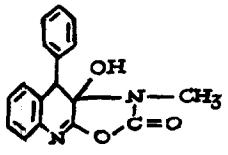
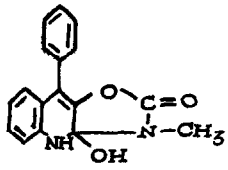
Bracken, Pocker and Raistrick[1] isolated in 1954 a nitrogen containing optically active product from a strain of Penicillium cyclopium Westling. It was given the name cyclopenin and the molecular formulae, $C_{17}H_{14}N_2O_3 \cdot \frac{1}{2}H_2O$ or $C_{17}H_{14}N_2O_3 \cdot 2H_2O$. On acid hydrolysis it gave methylamine, carbon dioxide and another product which after purification was identical with viridicatin, $C_{15}H_{11}NO_2$, (I; R=H), a metabolic product of other strains of Penicillium cyclopium and of Penicillium viridicatum[2]. On the basis of these hydrolytic products, cyclopenin was given either structure (II) or (III).

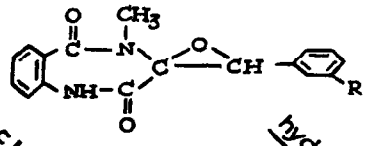
Preliminary chromatographic results obtained by Luckner and Mothes[3] showed cyclopenin to be a mixture of two closely related compounds. Accordingly, a chemical investigation was started at the London School of Hygiene and Tropical Medicine[4] and later continued at the Laboratories of Professor K. Mothes in Halle/S, D.D.R.



II



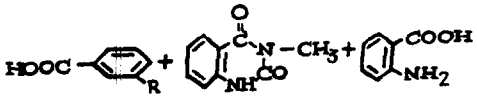
III



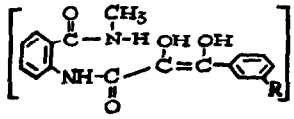
(IV)

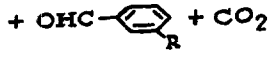
oxidation

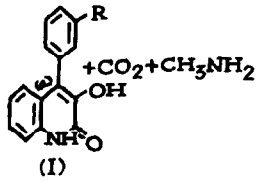
hydrolysis



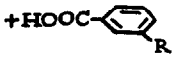
V







(I)



The metabolic product has been separated into two closely related compounds[5]. For one of these compounds, $C_{17}H_{14}N_2O_3$, m.p. 183-184°, $[\alpha]_{5461}^{20}$ -291°, the name cyclopenin was retained.

The I.R. spectrum of cyclopenin Nujol showed the following maxima, 3090 (NH); 1700, 1630 (-CO-NH-); 990 and 885 cm^{-1}. The second compound, $C_{17}H_{14}N_2O_4$, m.p. 215° (d), $[\alpha]_{5461}^{20}$ -309°, was given the name cyclopenol. Its I.R. spectrum in Nujol had the following maxima, 3300 (OH): 3100 (NH); 1680, 1630 (-CO-NH-); 990 and 880 cm^{-1}. On methylation with diazomethane, cyclopenin gave a monomethyl-derivative, $C_{18}H_{16}N_2O_3$, m.p. 206°, and its I.R. in Nujol had the following maxima, 1680, 1655 (-CO-NH-); 980 and 840 cm-1. Cyclopenol when similarly treated gave a dimethyl-derivative, $C_{19}H_{18}N_2O_4$, m.p. 167-169°, having one methoxyl group and its I.R. in Nujol had the following maxima, 1690, 1645 (-CO-NH-); 1600; 980 and 840 cm^{-1}.

In agreement with Bracken *et al*[1] our cyclopenin was hydrolysed by dilute acids to give viridicatin (I; R=H), methylamine and carbon dioxide all obtained in over 80% yield. However, a minor product, benzoic acid was also isolated from the acidic hydrolysate. On subjecting cyclopenol to the same treatment, it afforded viridicatol, $C_{15}H_{11}NO_3$, (I; R=OH), m.p. 280°, which was also isolated from the mycelium of *Penicillium viridicatum*[3] and was shown to be 3'-hydroxyviridicatin[5]. Other hydrolytic products are, methylamine, carbon dioxide, m-hydroxybenzoic acid and a small amount of a carbonyl compound which gave a 2:4 dinitrophenylhydrazone, $C_{14}H_{12}N_4O_6$, m.p. 245°, probably that of m-hydroxyphenylglycollic aldehyde. The oxidation of cyclopenin

with hydrogen peroxide in glacial acetic acid gave completely different products. The following compounds were either isolated or identified chromatographically;

(1) 3-N-Methyl-2:4-quinazolinedione (V), a highly stable compound isolated in 30% yield and compared with an authentic specimen[6].

(2) An acidic product which is mainly composed of benzoic acid and another gummy material.

(3) Benzaldehyde, a small amount identified as its 2:4-dinitrophenylhydrazone, m.p. 237°.

(4) Carbon dioxide, obtained in a yield of more than one mole.

(5) Anthranilic acid, identified chromatographically.

Similarly, the oxidation of cyclopenol gave 3-N-methyl-2:4-quinazolinedione (V), m-hydroxybenzoic acid, carbon dioxide and anthranilic acid. If the oxidation is carried out in 50% aqueous acetic acid, the hydrolysis products are obtained. The hydrolytic and oxidative degradations are represented as shown on page 4.

These oxidation products and the identification of benzoic acid and m-hydroxybenzoic acid among the hydrolysis products of cyclopenin (IV; R=H) and cyclopenol (IV; R=OH) are difficult to derive from the previously given structures (II) or (III). In the oxidation process, both the carbon and the nitrogen atoms which were evolved during the hydrolysis as carbon dioxide and methylamine are stabilised in 3-N-methyl-2:4-quinazolinedione (V) (proved by incorporating $^{14}COOH$ anthranilic acid into cyclopenin and its subsequent degradation by oxidation and hydrolysis*). This clearly indicates that the bond (a) in viridicatin (I; R=H) or viridicatol (I; R=OH) is not present in the parent compounds and is formed by rearrangement during hydrolysis. The simple transformation of cyclopenin (IV; R=H) and cyclopenol (IV; R=OH) to viridicatin (I; R=H)

and viridicatol (I; R=OH) would suggest that the former compounds are the biological precursors of the latter. Furthermore, an enzyme obtained from the mycelium of Penicillium viridicatum, was found to convert cyclopenin (IV; R=H) to viridicatin (I; R=H) with the evolution of carbon dioxide*. The present information would then give a structural skeleton for cyclopenin (IV; R=H) and cyclopenol (IV; R=OH) as a seven membered cyclic peptide formed from anthranilic acid and phenylalanine with the N-Me group probably coming from methionine. The biosynthetic results ^{3} obtained by incorporating phenylalanine labelled with ^{14}C in different positions into viridicatin (I;R=H) also support this formulation. The only atomic species left undefined in the molecular structure are an oxygen and a hydrogen atom. For the oxygen atom which is not present as a hydroxyl group or as a carbonyl function we suggest an epoxide linkage as shown in the formula (IV) with the hydrogen atom attached to the only left free valency at C_4. The N.M.R. spectra of cyclopenin (IV; R=H) and the dimethylcyclopenol showed a single peak for a tertiary unsplit proton at τ=4.04 p.p.m. consistent with it being in the environment shown in the formula (IV). Further chemical and biosynthetic studies are now in progress.

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* Unpublished results.