Tetrahedron Letters No. 3, pp 307 - 308, 1978. Pergamon Press. Printed in Great Britain.

A NEW NATURAL OPHIOBOLIN

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(Received in UK 14 November 1977; accepted for publication 24 November 1977)

In previous papers it has been shown that culture filtrates of the phytotoxic fungus Cochliobolus Miyabeanus contain, besides Ophiobolins (1), Cochlioquinones A and B (2).

To study the biosynthesis of the latter compounds, suitable fermentations were performed as follows.

Fermentation was carried out in a rotary shaker (180 rpm) inside a lighted thermostatic room (24°C, 100 lux) inoculating aqueous suspensions from slant cultures (glucose 2%, pH 7.0) into three 500 ml Erlenmayer flasks containing 100 ml of potato broth. After one week the contents of the Erlenmayer were recombined and inoculated into a 90 l thermostated stainless steel fermentor containing the same potato broth under vortex conditions (130 mm ∅ propeller, 400 rpm at atm. pressure with 50 l/m air flow). Incubation was allowed for 4 days; after that time the glucose amount fell to ca. a half and the pH value to 5. At this stage 40 g of methionine dissolved in acid aqueous solution, pH 3, were added under sterile conditions and fermentation was continued for 6 days further. The micelium (dry weight 3 g/l) and the medium were then repeatedly extracted with chloroform.

The crude combined extracts were then fractionated on a Silica Gel column by extensive elution with chloroform and then with chloroform containing increasing amounts of ethyl acetate. Fractions were tested by TLC (Chloroform:Ethyl acetate 70:30) and the eluates were appropriately recombined. After elution of several red pigments, the structure of which has not been yet investigated, compound I was obtained which after crystallization from n-hexane-ethyl acetate afforded white needles, m.p. 178-80°.

The physical and spectral data of compound I are as follows:
$[\alpha]_D^{20}$ = + 224 (c 0.76 $CHCl_3$); m/e 400 (M^+); λ_{max} (MeOH) 227 nm (ε = 10500); ν_{max} ($CHCl_3$) 3650, 3485, 1750, 1685 cm^{-1}; δ ($CDCl_3$, 100 Mc) 0.88 (3H, s), 1.09 (3H, d, J = 7 Hz), 1.26 (3H, s), 1.70 and 1.74 (3H each, both bs), 3.54 (1H, m), 4.42 (1H, m, DR 1.72 d, 5.15 t), 4.93 (1H, t, J = 8 Hz), 5.15 (1H, bd, J = 7 Hz, DR 1.72 d, 4.45 bs), 6.95 (1H, bt).

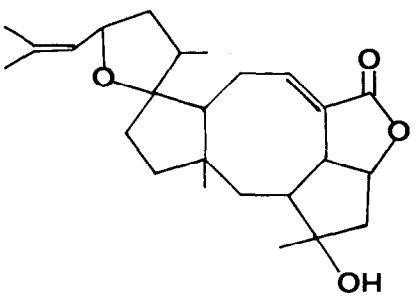
On the basis of the spectral evidences, structure I is suggested for the compound under investigation.

The same structure was reported for a compound obtained from Ophiobolin A by $LiAlH_4$ reduction followed by CrO_3 oxidation (3): comparison of an authentic specimen of the latter compound with I showed no appreciable difference in their physicochemical properties (UV, IR, NMR, and mixed m.p.).

Interestingly such a compound, so far obtained as a transformation product of Ophiobolin A, can constitute a true metabolite of Cochliobolus Miyabeanus although produced under modified fermentation conditions.

Acknowledgment

We thank Professor C.G.Casinovi for his helpful advices, and Mr A.Baragliu for technical assistance.



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