



Methods of immobilizing heparin and p-aminophenethylheparin on a collagen film

ene-4-sulfonate (CMEC) was used: by this method it was possible to add 86 mg of p-aminophenethylheparin to 1 g of collagen.

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#### INFLUENCE OF COUMARINS ON THE BIOSYNTHESIS OF MELANIN BY THE FUNGUS

##### *Verticillium dahliae*

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Up to the present time, in the fight against some diseases of agricultural crops, such as piriculariosis of rice, synthetic preparation (tricyclazole, pyroquilon, etc.) are used the mechanism of the action of which is based on the inhibition of the biosynthesis of melanin in the pathogen. Of natural ecologically harmless substances a capacity for blocking the pentaketide pathway for the biosynthesis of melanin has been established only for coumarin (the lactone of cis-ortho-hydroxycinnamic acid) [1]. In the present communication we give information on the influence of some coumarin derivatives on the melaninogenesis of the fungus *Verticillium dahliae* Kleb.

An isolate of *V. dahliae* KhL-1,3 from the collection of fungi of the Division of General Genetics of the Cotton Plant of the Tadzhikistan SSR Academy of Sciences was cultivated on an agarized Czapek-Dox medium in Petri dishes in the dark at 24-25°C for 7-10 days. All the compounds in this investigation were supplied by V. M. Malikov and E. Kh. Batirov of the coumarin and terpenoid chemistry laboratory in the Institute of the Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences. As a standard we used tricyclazole - a known inhibitor of the pentaketide pathway for the biosynthesis of melanin by fungi [1]. The substance was added to the cultivation medium as described previously [2].

The capacity of the substances investigated for blocking the melaninogenesis of the fungus *V. dahliae* was judged from the disappearance of the black coloration of the micro-

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sclerotia, the blue or dark orange coloration of the cultivation medium, and the appearance in the culture of the fungus of 2-hydroxyjuglone and flaviolin, i.e., pentaketides usually formed on blocking [3]. The treatment of the culture of the fungus *V. dahliae* and the identification and quantitative determination of the substances mentioned were performed by methods given in the literature [3, 4].

We found that of the 14 substances investigated — umbelliferone, scopoletin, daphnetin, haploperoside A, haploperoside E, conferol, fraxetin, capensin, obtusinin, obtusicin, osthole, gummosin, conferone, and samarkandin — the biosynthesis of the *V. dahliae* was inhibited by the first five of these substances in concentrations of from 1 to 500 µg/ml. They all acted in the same way as tricyclazole, i.e., they blocked the conversion of 1,3,8-trihydroxynaphthalene into vermelone and of 1,3,6,8-tetrahydroxynaphthalene into scytalone [1].

The results obtained form the basis for further investigations to establish the link between the structures of coumarins and their capacity for inhibiting the pentaketide pathway of melanin biosynthesis, and also for elucidating the role of these substances in protecting plates for certain phytopathogenic fungi.

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