IDENTIFICATION OF ENNIATINS FORMED BY Fusarium gibbosum ON GRAIN

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Enniatins – cyclic hexadepsipeptides consisting of residues of α-hydroxyisovaleric acid, of N-methylvaline, and (or) of N-methylisoleucine – are known as specific secondary metabolites of individual strains of fungi of the genus Fusarium [1] that exhibit a toxic action on mycobacteria, fungi, and plants in vitro [2]. Particular interest is presented by their capacity for forming complex compounds with inorganic cations, and the possibility of the transport of these complexes through biological membranes and the applied aspects of complex-formation are being studied [3]. The present paper reports the identification of enniatins B, B_1, and A_1 in the biomass obtained after the growth of Fusarium gibbosum Appl. et Wr. emend. Bilai, var. accuminatum (El. et Ev.) Bilai comb. nova (isolate 1/3 VNIIVS) and F. gibbosum Appl. et Wr. emend. Bilai (isolate 2/2 VNIIVS) on grain. The total yield of enniatins was 1.5-2.0%. This was the first time that a capacity of fusaria for forming enniatins on grain has been established. Their accumulation in fusarial grain under natural conditions is possible.

The initial biomass was obtained by inoculating sterile rice with spore suspensions of isolates and growth in a thermostat at 29°C for 40 days. In each case the biomass was homogenized with a mixture of acetonitrile and water (5:1), the homogenate was filtered, and the filtrate obtained was passed through a column containing active carbon and neutral alumina. The eluate was concentrated to an aqueous phase and was left for crystallization. The precipitate was recrystallized from acetonitrile. When subjected to TLC on Silufol in the mobile phase toluene–ethyl acetate–formic acid (5:4:1), the colorless crystalline products obtained from samples of biomass had R_f 0.3 and were revealed in the form of a yellow spot on a violet background after the plates had been treated with iodine vapor.

The results of elementary analysis [C 61.99, H 8.71, N 6.60%, mp 161-163°, $[\alpha]_D^{20}$ −96° (c 0.8; chloroform); the IR spectrum [(ν_{max}, cm^{-1}): 1653 (O=C–N), 1732 (O=C–O)]; the UV spectrum [$\lambda_{max}^{CH_3OH}$ (log ε): 209 nm (4.17)]; and the ^1H NMR spectrum [($CDCl_3$, δ, ppm, J, Hz): 5.13 d (J = 8.6; α-CH–O), 4.56 d (J = 9.5; α-CH–N), 3.15 s (N–CH_3), 2.29 m (β-C$\underline{H}$(CH_3)$_2$), 1.0 m (($C\underline{H}_3$)$_2$CH–)] coincided with those described for cyclo(N-methyl-l-valyl-D-α-hydroxyisovaleryl-)$_3$, which is known as enniatin B [4]. The assignment of the signals in the ^{13}C NMR spectra in the light of their multiplicities corresponded to the structure of enniatin B (δ, ppm): 170.076 s (C=O, N–MeVal); 169.203 s (C=O, α-hydroxyisovaleryl); 75.692 d (α-CH–O); 62.977 d (α-CH–N); 33.129 q (N–CH_3); 30.030 d (β-CH, α-hydroxyisovaleryl); 28.034 d (β-CH, N–MeVal); 20.451 q, 19.577 q (γ-CH_3, N–MeVal); 18.794 q, 18.590 q (γ-CH_3, α-hydroxyisovaleryl) [5].

The electron-impact mass spectrum (70 eV) consisted of the sequence of the fragmentation of enniatin B, m/z (%): 639(9), 624(4), 596(9), 556(10), 540(7), 538(10), 510(6), 496(7), 456(5), 427(6), 409(11), 396(7), 296(33), 282(27), 214(14), 196(100), 182(29), 169(58), 154(20), 141(23), 86(100) [6], the sequence of fragmentation established in [7] for enniatin B_1 differing by the fact that one N-methylvaline in the molecule has been replaced by a N-methylisoleucine residue [7]: 653(6), 610(4), 570(5), 423(5), 310(10), 210(17), 100(17), and also a peak with m/z 667 corresponding to the molecular ion of enniatin A_1, in the molecule of which two N-methylvaline residues have been replaced by N-methylisoleucine residues [7]. N-Methylisoleucine fragments were also detected in the NMR spectra from a weak doublet at 4.80 ppm, J = 9.5 Hz, corresponding to the α-CH proton in the N-methylisoleucine molecule [8].

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The mass spectrum obtained by bombardment with accelerated xenon atoms (6-8 keV) contained three peaks of MH^+ ions with m/z 640, 654, and 668, corresponding to enniatins B, B_1, and A_1. The ratio of the intensities of the peaks was 69:29:2 for the isolate 1/3 VNIIVS and 58:32:10 for the isolate 2/2 VNIIVS. Enniatin A, cyclo(N-methyl-L-isoleucyl-D-hydroxyvaleryl-)$_3$, which has been described in natural materials, was not detected in the samples of biomass studied.

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MICROELEMENT COMPOSITION OF POLLEN OF SOME POLLEN-YIELDING PLANTS OF THE FLORA OF THE LITHUANIAN SSR

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It is known that pollen (the pollen load of bees) is rich in macro- and microelements [1-4]. We give the results of an investigation of the microelement compositions of the monofloral pollens (pollen loads) of eight species of pollen-yielding plants of the Lithuanian flora: Malus domestica Borkh. (cultivated apple), Trifolium pratense L. (red clover), Pyrus communis L. (common pear), Sinapis arvensis L. (charlock), Salix caprea L. (goat willow), Taraxacum officinale Wigg. (common dandelion), Pisum sativum L. (garden pea), and Ranunculus acer (tall buttercup).

The microelements were determined by instrumental neutron-activation analysis in a nuclear reactor. The principle of the method is the measurement of the gamma spectra obtained on the irradiation of a pollen sample with neutron fluxes of different densities. Sodium, magnesium, aluminum, chlorine, titanium, and copper were determined from the radionuclides formed on the brief (30-second) irradiation of samples weighing 0.2 g with a neutron flux having a density of $1.6 \cdot 10^{13}$ neutrons/cm^2, with subsequent measurement of the gamma spectra after 2.5 min and 2.5 h. To determine bromine, potassium, cobalt, iron, and zinc, samples of pollen weighing 0.1 g were irradiated with a neutron flux of $1.4 \cdot 10^{13}$ neutrons/cm^2 for 17 h. The gamma spectra of the samples were measured after 2 and 24 days on a semiconductor spectrometer.

Twenty different elements were determined in the samples of pollen investigated. Of them the macroelements potassium, calcium, and magnesium were found in the largest amounts, and the microelements cobalt and vanadium in the smallest amounts. The pollen also contained considerable amounts of aluminum and chlorine (Table 1). These were followed with

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