lactones analogous to those isolated from other lettuce species growing in the countries of Central Europe [1, 2].

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3,7,8,15-TETRAHYDROXY-12,13-EPOXYTRICHOTHEC-9-EN IN A CULTURE OF Fusarium graminearum

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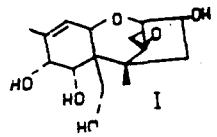
In an investigation of the metabolites formed by an isolate of Fusarium graminearum Schw. (479a VNIIZ [All-Union Scientific-Research Institute of Grain and the Products of its Processing]) on grain, together with the previously known 3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one (4-deoxynivalenol) and its 3-acetyl and 15-acetyl derivatives [1], we have found a new substance, 3,7,8,15-tetrahydroxy-12,13-epoxytrichothec-9-en (I).



I

The production of the biomass, extraction, and absorption purification of the extract were carried out as described previously [1]. The extract, purified in this way that had been obtained from 0.7 kg of dry biomass was then subjected to LC on a column of silica gel L (3.5 × 100 cm; 100-400 μm; 250 g) in a gradient mobile phase (1 liter each of mixtures of benzene and acetone in the form of successive 10% additions, with the collection of 200-ml fractions). The eluate obtained from the use of benzene–acetone (60:40) was evaporated to a dry residue, which was dissolved in 200 ml of chloroform and extracted with water (3 × 200 ml). The aqueous extract was evaporated to an oily residue (4.71 g). This residue was subjected to LC on a column of silica gel L (1.5 × 25 cm; 40-100 μm; 15 g) in the isocratic regime with the mobile phase chloroform–methanol (9:1), 10-ml fractions being collected.

The compositions of the fractions were monitored by TLC on Silufol in the mobile phases chloroform–methanol (7:1) (A) and benzene–acetone (2:3) (B). The substances were detected by means of a qualitative reaction for an epoxide group after the treatment of the Silufol plates with a 3% solution of 4-nitrobenzylpyridine (150°C, 30 min) and a 10% solution of tetraethylenepentamine in chloroform–carbon tetrachloride (2:3) [2].

The eluate fractions from the column that contained as their main component a substance with R_f 0.24 (A) were combined and evaporated to a dry residue (1.2 g), and then LC was conducted on a column of silica gel L (1.5 × 25 cm; 40-100 μm; 15 g) in a gradient mobile phase (100 ml each of mixtures of benzene and acetone in the form of 5% additions). The eluates from the column at a benzene:acetone ratio of 70:30, which contained a substance with R_f 0.22

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(B) were combined and evaporated to dryness in vacuum. When a mixture of benzene and acetone was added to the residue, a crystalline precipitate deposited.

After recrystallization from the same mixture, 700 mg of colorless crystals of substance (I) (yield 0.1% on the dry biomass) were obtained, with mp 185-187°C $[\alpha]_D^{25}$ −60° (c 1.02, methanol); IR spectrum (KBr, ν_{max} cm^{-1}): 3360 (OH), 2947, 2889, 1435, 1373, 1173, 833, 760 (C–H in CH_3, CH_2, CH), 1032-1065, 950-960 (C–OH). UV spectrum: $\lambda_{max}^{CH_3OH}$ 206 nm (log ε 3.2230). EI mass spectrum (70 eV) (m/z, %): 298 (4), 280 (42), 262 (42), 249 (46), 232 (90), 82 (100). Mass spectrum with bombardment by fast xenon atoms (6-8 keV): 299 (MH^+, 65), 281 (55), 263 (100), 251 (30), 233 (43). CI mass spectrum with ammonia: 316 $(MH + NH_3)^+$ (100%); calculated for $C_{15}H_{22}O_6$: C 60.40, H 7.38; found: C 60.32, H 7.37. Tetraacetate (acetic anhydride, pyridine, 22°C, one day): M 466 (mass spectrometry), R_f 0.61 (B); tetra-TMS ether (TBT/Tri–Sil, 60°C, 20 min); M 586 (mass spectrometry); ^1H NMR spectrum (δ, ppm, J, Hz): 3.52 (1H, J = 4.36, H-2), 4.53 (1H, J = 4.50, 9.38, H-3), 2.06 (2H; H-4), 4.05 (1H, H-7), 2.80 (7-OH), 4.49 (1H, J = 9.39, 5.28, H-5), 3.39 (1H, J = 9.61, 8-OH), 5.64 (1H, J = 5.69, H-11), 3.08, 3.24 (2H, J_{AB} = 4.30, H-13), 1.16 (3H, H-14), 3.72 (J = 12.05, 4.11), 3.88 (J = 12.42) (2H, H-15), 1.90 (3H, H-16).

A comparison of the NMR spectra of (I) and of 4-deoxynivalenol [3] showed that the substances were identical in structure and in substitution at C_3, C_7, and C_{15} and could differ only by the substitution in positions C_4 and C_8. According to its IR and UV spectra, polyol (I) contained no carbinol group, while, judging from the molecular masses of the complete acetyl and trimethylsilyl derivatives of this substance, it had one OH group more than 4-deoxynivalenol and differed from it in molecular mass by two units. This means that the metabolite isolated contained an OH group at C_8 or C_4. The presence of an OH group at C_4 was excluded by a consideration of the mass spectrum – in particular, from the absence in the EI spectrum of the peak of an ion corresponding to the ejection of 59 a.m.u. from M^+, which is characteristic for all 3,4-dihydroxy-substituted trichothecenes [4]. It followed from this that compound (I) was 3,7,8,15-tetrahydroxy-12,13-epoxytrichothec-9-ene.

Structure (I) was confirmed by a comparison of its NMR spectrum with that of 3,15-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene (7,8-dihydroxycalonectrin), recently isolated from culture fluids of Fusarium roseum (ATCC 28114) [5] and F. culmorum (CMI 14764, HIX 1503 [6, 7]. In actual fact, in the spectrum of (I) downfield shifts were observed of the signals of H-2 (3.52 ppm), H-3 (4.43 ppm), and H-15 (AB system, 3.72, 3.88 ppm) as compared with 7,8-dihydroxycalonectrin (3.80, 5.19, and 4.17, 4.48 ppm, respectively), which is obviously the result of the presence in (I) of OH groups at C_3 and C_{15}.

3,7,8,15-Tetrahydroxy-12,13-epoxytrichothec-9-en (I) has not been described previously. In view of terminological analogies in the trichothecene group, the new metabolite (I) may be called 7,8-dihydroxycalonectrintetraol.

Thus, from the biomass of an isolate of Fusarium graminearum Schw. (579a VNIIZ) has been obtained a substance for which, from a combination of physicochemical characteristics, the structure of 3,7,8,15-tetrahydroxy-12,13-epoxytrichothec-9-ene (7,8-dihydroxycalonectrintetraol) has been established.

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