idite was added. The reaction was monitored in TLC in the chloroform–ethanol (9:1) system with the addition of 2% of triethylamine. After 1 h, the reaction mixture was poured into a saturated solution of $NaHCO_3$ and was washed with a saturated solution of NaCl. The organic layer was dried with Na_2So_4 and was evaporated in a rotary evaporator. Yield 90%.

Hydrolysis of ACGGAU$^{2'F}$ and ACGGAU^{ara} with a Mixture PME and PDE from Snake Venom. A solution of 0.7 OU (10 mmole) of one of the oligonucleotides in 30 µl of water was treated with 15 µl of buffer 6 and then with 15 ul each of solutions of PME and PDE (the concentration of each enzyme in the reaction mixture was 0.1 mg/ml). The mixture was kept at 37°C for 3 h, and then the enzymes were extracted with chloroform–isoamyl alcohol (24:1). The hydrolysate was analyzed by HPLC on a Tracor chromatograph (Netherlands) with a 0.46 × 25 cm column containing the support Ultrasphere octyl. Standard conditions: linear gradient (3-35%) of methanol in 0.1 M ammonium acetate; rate of elution 1 ml/min; temperature 40°C. The enzymatic hydrolysate was compared with a control mixture containing nucleosides in a ratio corresponding to the nucleoside composition of the oligomer under investigation.

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FUSARENON X AND 7-DEOXYNIVALENOL IN CULTURES OF *Fusarium graminearum* ISOLATES

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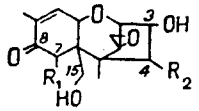
UDC 547.811.02:543.644

It has been established that the biomass of isolates of *Fusarium graminearum* Schw. that form nivalenol on grain contain two of its structural analogs – 7-deoxynivalenol and 4-acetylnivalenol (fusarenon X). The substances were identified by a combination of chromatographic characteristics and the chemical-ionization mass spectra of their complete trimethylsilyl ethers. 7-Deoxynivalenol is a new, not previously described, metabolite of fusariogenic nature.

We have previously reported the presence of nivalenol (3,4,7,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one) (I) in the biomass of an isolate of *Fusarium graminearum* Schw. 15/2 VNIIVS [1]. We have now identified nivalenol (I) in the biomasses of five other isolates of the same species of fungus from feed grain attacked by fusarial wilt.



I. $R_1=R_2=OH$
II. $R_1=OH$, $R_2=OCOCH_3$
III. $R_1=H$, $R_2=OH$

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The use of a combination of chromatographic and spectroscopic methods has enabled us to establish the presence in samples of the biomasses of these isolates of (together with (I)) 4-acetylnivalenol (fusarenon X) (II) and 7-deoxynivalenol (III). The investigation of the eluates obtained as a result of the adsorption purification of aqueous acetonitrile extracts of the biomass of the isolates was made by TLC and combined GC-mass spectrometry in the regime of CI by ammonia.

Component (II) had R_f 0.47 in mobile phase (MP) (A) and was well separated from nivalenol (I) with R_f 0.18. In addition to a positive reaction with the Takitani reagent, (II) gave a complex with $AlCl_3$ fluorescing at 366 nm, which showed the presence of a $-CO-C(OH)-$ fragment in the molecule. The chromatographic mobilities of (II) coincided with those given in the literture for 4-acetylnivalenol [2, 3].

In contrast to (II), component (III) could not be separated from nivalenol (I) on TLC in MP (A) nor in the other MPs used in the chromatography of trichothecenes on silica gel [3]. Some separation of these substances was observed only in MP (B), when nivalenol (I) has R_f 0.42, and component (III) R_f 0.34. This chromatographic behavior of (III) indicated that the substance had either the same number of OH groups as nivalenol or in it the OH groups that could participate in an intramolecular hydrogen bond were substituted or absent and therefore had little effect on the mobility of the substance on silica gel. In the molecules of 8-oxotrichothecenes, such a group is the hydroxyl at C_7.

Component (III) did not form a fluorescing complex with aluminum chloride, but when it was treated with a 20% alcoholic solution of sulfuric acid and heated at 100°C for 1-2 min, a product having a greenish yellow fluorescence at 366 nm was formed. The appearance of fluorescence on such treatment has been described for a large group of natural trichothecenes having a methylene unit in position 7 [3]. The chromatographic mobilities on silica gel in nonaqueous MPs coinciding with those of nivalenol (I), the formation of a fluorescent product with sulfuric acid, and the incapability of the substance of forming a complex with aluminum chloride permitted the assumption that component (III) was the 7-deoxy analog of nivalenol.

On an investigation of the products of the trimethylsilylation of purified extracts of the biomass of the isolates by GC-mass spectrometry, it was found that they contained, together with the TMS ether of nivalenol (TMS-I), another two TMS ethers (TMS-II and TMS-III) with shorter retention times (Table 1). The NI mass spectrum of TMS-I coincided completely with that which we have described previously [1].

Analysis of the mass spectra of the component TMS-II showed that it was the TMS ether of 4-acetylnivalenol. Thus, in the PI spectrum the values of m/z of two diagnostic ions $(NH)^{+\cdot}$ and $(MNH_4)^{+\cdot}$ were 571 and 588. The NI spectrum contained, as was to be expected, a weak M^- ion with m/z 570 (5%) and an ion with the maximum intensity having m/z 297, and also the peak of an ion with m/z 273 (30) (Fig. 1a).

It is known that on the chemical ionization of 8-oxotrichothecenes, in particular, by the reactant ion OH^-, the molecular ion-radical breaks down as a result of the cleavage of the $C_{11}-O$ and C_5-C_6 bonds into two anion-radicals consisting of the fragments of left- and right-hand parts of the molecule with the substituents present in them [4]. The same nature of fragmentation is retained for the TMS ethers on chemical ionization by methane [5] and by ammonia, the peak of the ion formed from the left-hand part of the molecule predominating in the spectrum. The presence of the peak of an ion at m/z 297 with the maximum intensity in the NI spectrum of the component TMS-II showed that the left-hand part of the molecule contained two O—TMS groups, i.e., at C_7 and C_{15}, while the peak at m/z 297 confirmed the presence of OAc and O—TMS groups in the right-hand part. Thus, component (II) was 4-acetylnivalenol (fusarenon X).

An investigation of the product of the trimethylsilylation of an authentic sample of fusarenon X from Serva under the same conditions of GC-mass spectrometry showed that the main component did in fact coincide in relation to its retention time (23.00 min) and mass spectrum with the component TMS-II on the chromatograms obtained for samples of the biomass. At the same time, the commercial sample also contained small amounts of nivalenol (I), as was confirmed by comparing the retention time and mass spectrum of the TMS ether, and also an unknown component the TMS ether of which had a retention time of 22.80 min – TMS-IV.

It follows from a comparison of the NI spectra of TMS-III present in the products of the trimethylsilylation of extracts of the biomass of fungi and of TMS-IV contained in the product of the trimethylsilylation of the authentic sample of fusarenon X that they had clear

TABLE 1. Results of the GC of the Products of the Trimethylsilylation of Samples Obtained from the Biomass of F. graminearum Isolates and a Commercial Sample of Fusarenon X

TMS ether	Retention time	Heights of the peaks, % of the maximum		
		(1)*	(2)	(3)
TMS -I	23,54	100	100	10
TMS -II	23,03	20	0	100
TMS -III	23,23	53	48	0
TMS -IV	22,80	0	0	7

*1) Purified extract of the biomass of an 82/3 VNIIVS isolates; 2) product obtained as the result of the chromatographic purification of an extract of the biomass of a 15/2 VNIIVS isolate; 3) commercial sample of fusarenon X.

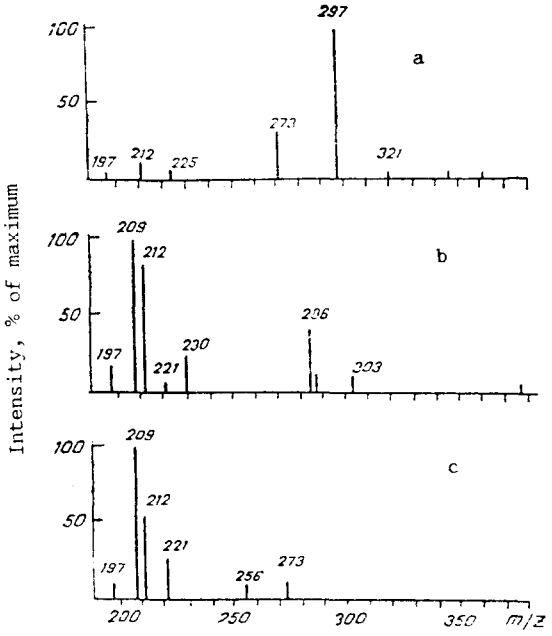

Fig. 1. Fragments of the CINI mass spectra of the TMS ethers of: a) 4-acetylnivalenol, TMS-II; b) 7-deoxynivalenol, TMS-III; and c) 4-acetyl-7-deoxynivalenol, TMS-IV, obtained by the GC-mass-spectrometry of the products of the trimethylsilylation of the samples listed in Table 1.

analogies (Fig. 1b and c). In both spectra, the strongest peak was that of an ion with m/z 209 and there were common peaks with m/z 107, 212, 221, and 230. At the same time, the spectra differed by the values of two peaks at a distance of 17 a.m.u.. For TMS-III the peaks of these ions were present at m/z 303 and 386, and for TMS-IV at m/z 273 and 256. Judging from these facts, the two ethers had the same structure of the left-hand part of each of their molecules and contained one O-TMS (m/z 209) in it, but differed in the nature of the substitution in the right-hand part.

The m/z values of two peaks for TMS-III of 303 and 286 permitted the assumption of the presence in the right-hand part of its molecule of two O-TMS groups. The displacement of the m/z values of the analogous ion peaks by 30 a.m.u. into the region of lower masses for TMS-IV indicated that it contained OAc in place of one O-TMS group. Thus, according to the results of NI mass spectra, (III) and (IV) can be identified as the 7-deoxy analogs of nivalenol and fusarenon X, respectively. This conclusion was confirmed by the results of PI mass spectroscopy. Intense peaks of the ions $(NH)^{+\cdot}$ and $(MNH_4)^+$ were present in the spectrum of TMS-I at

m/z 601 (100%) and 618 (60%), and in the spectrum of TMS-III at m/z 513 (42%) and 530 (90%), i.e., they were shifted by 88 a.m.u. (difference of one O—TMS group).

For the additional identification of component (III), we carried out the preparative isolation of a fraction containing nivalenol and (III). Judging from IR and UV spectra, the product obtained was free from contamination by substances with a different chemical nature. However, in the mass spectra obtained on bombardment with accelerated xenon atoms (6-8 keV), and also on CI by ammonia, two peaks of $(MH)^{+\cdot}$ were observed, at m/z 297 and 313, which obviously corresponded to component (III) ($M^{+\cdot}$ 296) and nivalenol ($MM^{+\cdot}$ 312), differing by one hydroxy group.

Under the conditions of HPLC on a Zorbax ODS, 5 μm, column, 4.6 mm × 25.0 cm, in the MP 5% THF in water at a rate of flow of 1.0 ml/min with UV detection at 224 nm, again the presence in the product of two components with close retention times was revealed. The results of a GC-mass-spectrometric study of the product of the trimethylsilylation of the mixture (see Table 1) again confirmed that its components were nivalenol and 7-deoxynivalenol (III).

This is the first description of the identification of 7-deoxynivalenol as a component of fungal metabolites. In an authentic commercial samples of 4-acetylnivalenol most probably having fusariogenic nature, judging from the NI mass spectrum of the trimethylsilylation product, the main substance was accompanied by its 7-deoxy analog. The presence of small amounts of 4,15-diacetyl-7-deoxynivalenol in the culture of the fungus F. crookwellense sp. nov. DAOM 193611 has recently been shown [6]. 4,7-Dideoxynivalenol is known as a metabolite of two strains of F. sp., accompanying 4-deoxynivalenol [7]. In a culture liquid of F. roseum (ATCC 28114), together with 3-acetyl-4-deoxynivalenol, 3-acetyl-4,7-dideoxynivalenol has been identified [8]. The formation of 7-deoxy analogs is apparently typical for the biosynthesis of 8-oxotrichothecenes by Fusarium fungi.

EXPERIMENTAL

Samples of the biomass of isolates of Fusarium graminearum Schw. 15/2, 82/3, and 83/4 isolated and identified by L. S. Malinovskii in the microbiology laboratory of the All-Union Scientific-Research Institute of Veterinary Medicine (Moscow) were investigated. The production of the biomass, extraction, and the adsorption purification of the extracts were carried out as described previously [1]. For TLC we used Silufol and the mobile phases (MPs), chloroform—methanol (6:1) (A) and hexane—isopropanol—water (10:10:1) (B). The substances were detected on the plates by heating after spraying with a 10% methanolic solution of $AlCl_3$ and by means of the Takitani reagent [2].

The GLC-mass spectrometric investigation of the samples after their exhaustive trimethylsilylation (Pierce TRI-SIL/TBT, USA; 60°C, 20 min) was performed in a SPB-1 capillary column (0.32 mm × 30 m) in a Finnigan MAT 4615 quadrupole mass spectrometer at 60°C (1 min) and then with programming of the temperature from 60 to 290°C at the rate of 10°C/min and under isothermal conditions at 290°C for 10 min. The mass spectra were recorded in the regime of electronic impact (EI) and chemical ionization with the simultaneous recording of positive and negative ions (DIPI, CINI), the reagent gas being ammonia at 0.7 mm Hg, and the ionizing voltage 70 eV.

For the preparative isolation of fractions containing nivalenol (I) and component (III), the biomass of a 15/2 VNIIVS isolate (dry weight 1.2 kg) was homogenized with a mixture of acetonitrile and water in a ratio of 5:1 and the resulting extract was filtered through a column containing a bottom layer of activated carbon and an upper layer of neutral alumina. The eluate from the column was concentrated in vacuum at 50°C. The residue obtained was dissolved in 600 ml of chloroform—methanol (9:1), and the solution was filtered through a column containing silica gel L 40/100; then elution was continued with the same solvent, 500-ml fractions being collected.

The compositions of the fractions were monitored by TLC in MP (B) with detection by the Takitani reagent [2]. The eluates containing (I) and (III) were evaporated to dryness, the residues (12.27 g) consisting of a viscous oily brown product. It was dissolved in 500 ml of chloroform and the solution was extracted with water (3 × 300 ml), after which the aqueous extract was freeze-dried. The dry residue (6.98 g) was subjected to LC under pressure in a column of LiChrosorb RP-8, 10 μm, dimensions 22.7 mm × 25.0 cm in the MP 5% of methanol in water at a rate of flow of 14 ml/min, with refractometric detection. The dry residues from the fractions with retention times of 4.0-6.0 min and 6.0-8.0 min consisted of mixtures containing nicotinic (pyridine-3-carboxylic) acid and a mixture of free pyrimidine bases of which the main one quantitatively was uracil (2,4-dioxypyrimidine).

A benzene solution of the dry residue from the fraction with a retention time of 6.0-8.0 min yielded 157.6 mg of uracil in crystalline form, Found, %: C 42.11, H 4.00; N 23.72; $C_4H_4N_2O_2$. EI mass spectrum, m/z (intensity, % of the maximum): 112 (100), 69 (65), 68 (23), 42 (67), 41 (34), 40 (37), 39 (9), 28 (68). UV spectrum, $\lambda_{max}^{CH_3OH}$, nm: 258 (log ε 4.12).

The dry residue (1.9 g) from the fraction with a retention time of 8.0-12.0 min, which contained (I) and (II) and accompanying substances, was chromatographed on column of silica gel L 40/100 in a gradient MP of benzene–acetone. The eluate obtained with the MP benzene–acetone (55:45) was evaporated to dryness, giving 370 mg of a product in the form of a colorless film. IR spectrum, ν, cm^{-1}: 3379 (O–H), 2918 (C–H), 1676 (C=C–C=O), 1448, 1377, 1169, 850, 754. UV spectrum: $\lambda_{max}^{CH_3OH}$, nm: 221 (log ε 3.67).

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PHEROMONES OF INSECTS AND THEIR ANALOGS.

XXVII. SYNTHESIS OF 10-HYDROXY-4,8-DIMETHYLDECA-4E,8E-DIENOIC ACID AND OF RACEMIC 4,8-DIMETHYLDECANAL FROM GERANYL ACETATE

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UDC 542.91+547.473+632.936.2

10-Hydroxy-4,8-dimethyldeca-4E,8E-dienoic acid and racemic 4,8-dimethyldecanal (components of beetle pheromones) have been synthesized from geranyl acetate.

A number of schemes for the synthesis of 10-hydroxy-4,8-dimethyldeca-4E,8E-dienoic acid (I) [1-4] and of racemic 4,8-dimethyldecanal (II) [5-10] have been published. The first of these compounds is an acyclic precursor of the aggregation component of the pheromone of the grain beetle Cryptolests ferrugineus [1, 2], and the second exhibits a high activity [5] in relation to the flour beetles Tribolium castaneum and T. confusum, the aggregation pheromone of which is, according to the literature [11-13], a mixture of the optically active 4R,8R and 4R,8S diastereomers of the aldehyde (II). We have developed a scheme for the synthesis of compounds (I) and (II) that starts from geranyl acetate (III). The latter was converted into 8-acetoxy-2,6-dimethylocta-2E,6E-dien-1-ol (IV) and then into the corresponding chloride (V) according to [3, 14]. The interaction of the chloride (V), as of the corresponding bromide [3] with sodiomalonic ester led to the coupling product (VI) with a yield of 76%. We have

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