REACTION OF FERVENULIN AND ITS 3-HYDROXY DERIVATIVE WITH 1,3-DIMETHYL-6-HYDRAZINOURACIL

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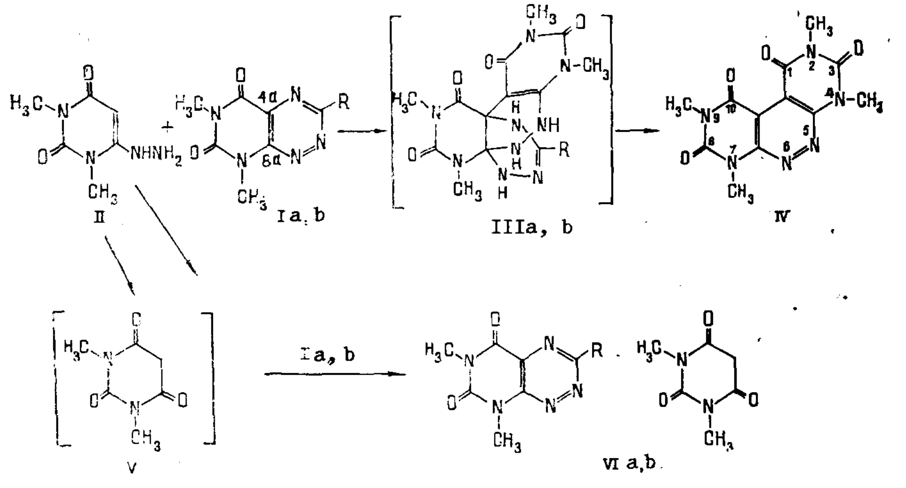
It has recently been shown that, under the conditions of acid catalysis, the antibiotic fervenulin (Ia) adds indole at the angular C-4a atom. At the same time, it is reported that the 3-substituted derivatives of the antibiotic (Ia) are transformed into the xanthine derivatives as the result of the nucleophilic attack by formamide on the C-8a atom in the pyrimidotriazine nucleus [2]. The transformation is also known of toxoflavin (1,6-dimethylpyrimido-[5,4-e][1,2,4]-triazin-5,7-dione) into 3-alloxazine [3], which is apparently the result of the addition of o-phenylenediamine to the C-4a and C-8a atoms of the pyrimidotriazine nucleus of this antibiotic and the subsequent splitting out of its triazine ring.

We have found that (Ia) and its 3-hydroxy derivative (Ib) interact with 1,3-dimethyl-6-hydrazinouracil (II) on heating in boiling ethanol in the presence of hydrochloric acid (30-40 min) with the formation of 2,4,7,9-tetramethyl-1,2,3,4,7,8,9,10-octahydropyrimido[4',5':3,4]pyridazino[3,4-d]pyrimidine-1,3,8,10-tetraone (IV) (mp above 300°C). The molecular weight of (IV) determined by mass spectrometry corresponds to the empirical formula $C_{12}H_{12}N_6O_4$. The PMR spectrum of a solution of (IV) in DMSO-d_6 had only two signals (3.33 and 3.74 ppm) of four methyl groups equivalent in pairs. The number of signals of ^{13}C atoms in the NMR spectrum of (IV) in trifluoroacetic acid solution (32.40, 33.09, 110,43, 151.03, and 152.01 (another signal in the 162-165 ppm region where the ^{13}C atoms of carbonyl groups at C-4 of uracils usually resonate [4] was apparently masked by the solvent) was only half the number that there should be according to the empirical composition. This also shows the symmetry of the molecule of (IV). In the IR spectrum of crystals of (IV) ($\nu_{C=O}$ 1675, 1720, 1741 cm^{-1}) there was a $\nu_{C=O}$ absorption band of higher frequency than in the initial (Ia) ($\nu_{C=O}$ 1675, 1680, 1696, 1706, 1728 cm^{-1}). This may be due to the presence of field effects between the closely located oxygen atoms of the carbonyl groups at C-1 and C-10. The conversion of compounds (Ia, b) into (IV) can be represented as the result of the splitting out of the triazine ring of intermediate (III) on the aromatization of its pyridazine fragment.



e-R=H, b-R=OH

When the mother solution after the separation of (IV) from the reaction mixture was concentrated, the adducts (VIa, b) of the initial compounds (Ia, b) respectively, with 1,3-dimethylbarbituric acid (V) were obtained, the (V) having been formed as the result of the

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hydrolysis of compound (II) under the reaction conditions. It is obvious that the low yield of (IV) on the interaction of equimolar amounts of (II) and (Ia) (~5%) and of (II) and (Ib) (~30%) is a consequence of the parallel hydrolysis of (II). At the same time, the considerable difference in the yields of (IV) show a higher reactivity of (Ib) than of (Ia).

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CHROMATO-MASS SPECTROMETRIC IDENTIFICATION OF FOUR 12,13-EPOXYTRICHOTHEC-9-EN-8-ONES IN A SAMPLE OF FUSARIUM-INFECTED GRAIN

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The first report on the identification of two 12,13-epoxytrichothec-9-en-8-ones – the 3,4,7,15-tetrahydroxy derivative (nivalenol) and 4-deoxynivalenol – in a sample of barley was made in Japan in 1977 [1]. The combined presence of 4-deoxynivalenol and its 15-acetyl derivative in samples of Fusarium-infected maize has been described recently [2]. In the present paper we report the identification of 4-deoxynivalenol and its 3-acetyl and 15-acetyl derivatives and of 4,7-dideoxynivalenol from the results of TLC and the chromato-mass spectrometry of the trimethylsilyl (TMS) derivatives under the conditions of chemical ionization by positive and negative ions (CIPI, CINI) in an extract of a sample of Fusarium-infected wheat.

A sample of flour (200 g) was extracted with 600 ml of acetonitrile–water (5:1) in a mixer for 10 min. The extract was filtered through a column containing 10 g of a mixture of activated carbon and Celite and 10 g of neutral alumina. The filtrate was evaporated to dryness, the residue was dissolved in water, and the solution was passed through a PRE SEP C18 column (Czechoslovakia). A methanol–water (1:1) eluate from the column was evaporated to dryness in vacuum, the residue was dissolved in 5 ml of ethyl acetate, the solution was filtered through a layer of anhydrous sodium sulfate, and the product was analyzed by TLC on Silufol in comparison with authentic samples of natural 12,13-epoxytrichotec-9-en-8-ones. The substances were detected from their blue fluorescence in UV light (366 nm) after separate treatment of the plates with 10% solutions of H_2SO_4 and $AlCl_3$ in ethanol and heated at 92°C for 1 and 10 min, respectively.

TLC with chloroform–methanol (7:1) as the mobile phase revealed the presence in the eluate, together with 4-deoxynivalenol (I), R_f 0.26, of 4,7-dideoxynivalenol (II) with the same mobility as (I) but giving a characteristic blue fluorescence after treatment with acid and heating. In ethyl acetate–hexane (3:1) as the mobile phase, the substances of the eluate were found to have the same R_f values as 4-deoxynivalenol 3-acetate (III) (R_f 0.26) and 4-deoxynivalenol 15-acetate (IV) (R_f 0.15).

The dry residue from the filtrate was treated with 50 µl of TBT silylation mixture (USA), and after 20 min at 60°C the reaction mixture was analyzed on a Finnigan MAT 4615 chromato-mass spectrometer (0.32 mm × 45 m capillary column, 25 µm of OV-351) in the isothermal regime at 220°C with the recording of mass spectra in the CIPI and CINI regimes, the reagent gas being ammonia (0.7 mm Hg) and the ionizing voltage 70 V.

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