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TOXIC BUTENOLIDE PRODUCED BY FUSARIUM NIVALE (FRIES) CESATI ISOLATED FROM TALL FESCUE (FESTUCA ARUNDINACEA SCHREB.)

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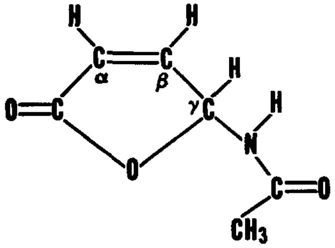
A toxic metabolite has been isolated from a culture of _Fusarium nivale_ and characterized as 4-acetamido-4-hydroxy-2-butenoic acid-γ-lactone (I). This mold was found on tall fescue hay from a pasture where fescue toxicity had been reported (1). At present we are investigating the pharmacological properties of the toxic metabolite to determine whether it is directly or indirectly involved in fescue toxicity. Here we offer evidence for the assigned $\Delta^{\alpha,\beta}$-butenolide structure.



(I)

The butenolide was isolated from a methylene chloride extract of a Sabouraud's agar culture of _F. nivale_ grown at 15°. The crude butenolide was recrystallized from ethyl acetate-cyclohexane, m.p. 116.5-118.5; Found: C, 51.2; H, 4.8; N, 10.1%; molecular weight 138 (vapor pressure osmometry). Calculated for $C_6H_7NO_3$: C, 51.1; H, 5.0; N, 9.9%; molecular weight 141.

The presence of a monosubstituted amide is indicated by IR bands at 3440 cm^{-1}, 3340 cm^{-1}, and 1705 cm^{-1} (FIG. 1). The amide structure was confirmed by gas-liquid chromatography (GLC)

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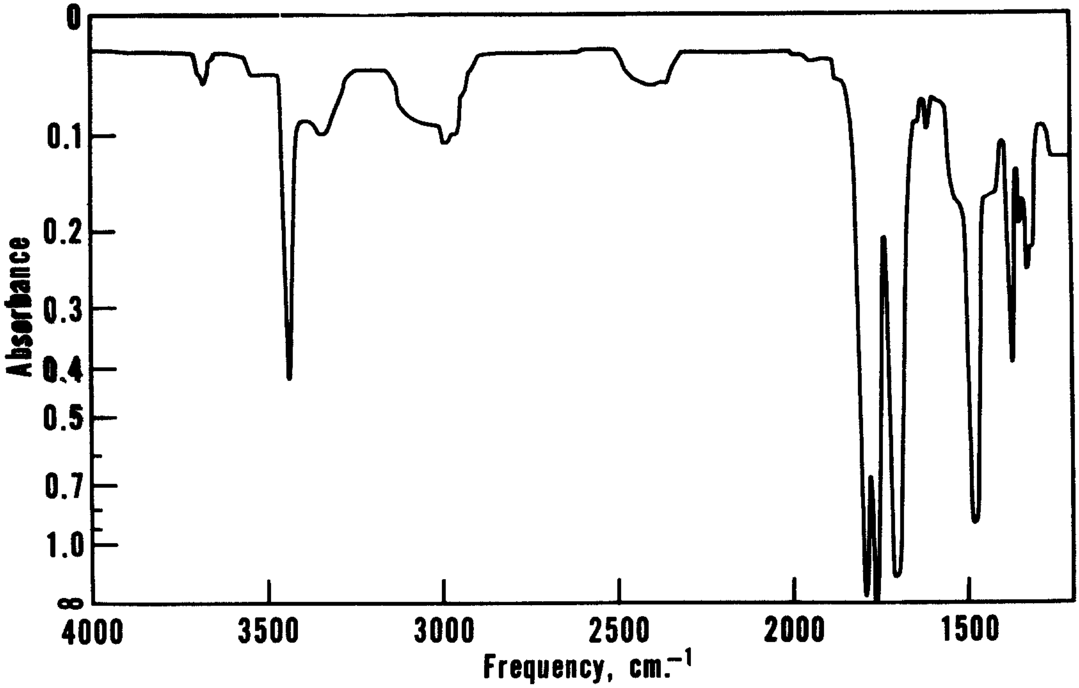

FIG. 1. Infrared spectrum of 4-acetamido-4-hydroxy-2-butenoic acid-γ-lactone in chloroform.

and NMR. Alkaline hydrolysis of I was followed by extraction of the products into ether. GLC of the ether-extracted products on a polar and a nonpolar column showed the presence of acetamide. The NMR spectrum of I (FIG. 2, CH_3-CO-peak at 118 cps) and the UV spectrum of the dihydro derivative of I (CH_3OH, λ max 201, ε = 2700) are consistent with an amide. The coupling pattern of the protons in the NMR spectrum (FIG. 2) and the positive Tollens test shown by the dihydro derivative of I prove that the amide is on the γ-carbon. 2-Acetamido-4-hydroxy-butanoic acid-γ-lactone (2), which does not have a potential aldehyde group at this position, gave a negative Tollens test.

The presence of the $\Delta^{\alpha,\beta}$-butenolide ring is indicated by the characteristic doublet in the IR spectrum (FIG. 1) at 1760 and 1790 cm^{-1}(3). In the dihydro derivative of the butenolide the doublet at 1760 and 1790 cm^{-1} disappears and one band is seen at 1775 cm^{-1}. The UV spectrum of I (CH_3OH, λ max 202, ε = 11,300) is consistent with the structure and is similar to that of β-angelica lactone (CH_3OH, λ max 203, ε = 15,200).

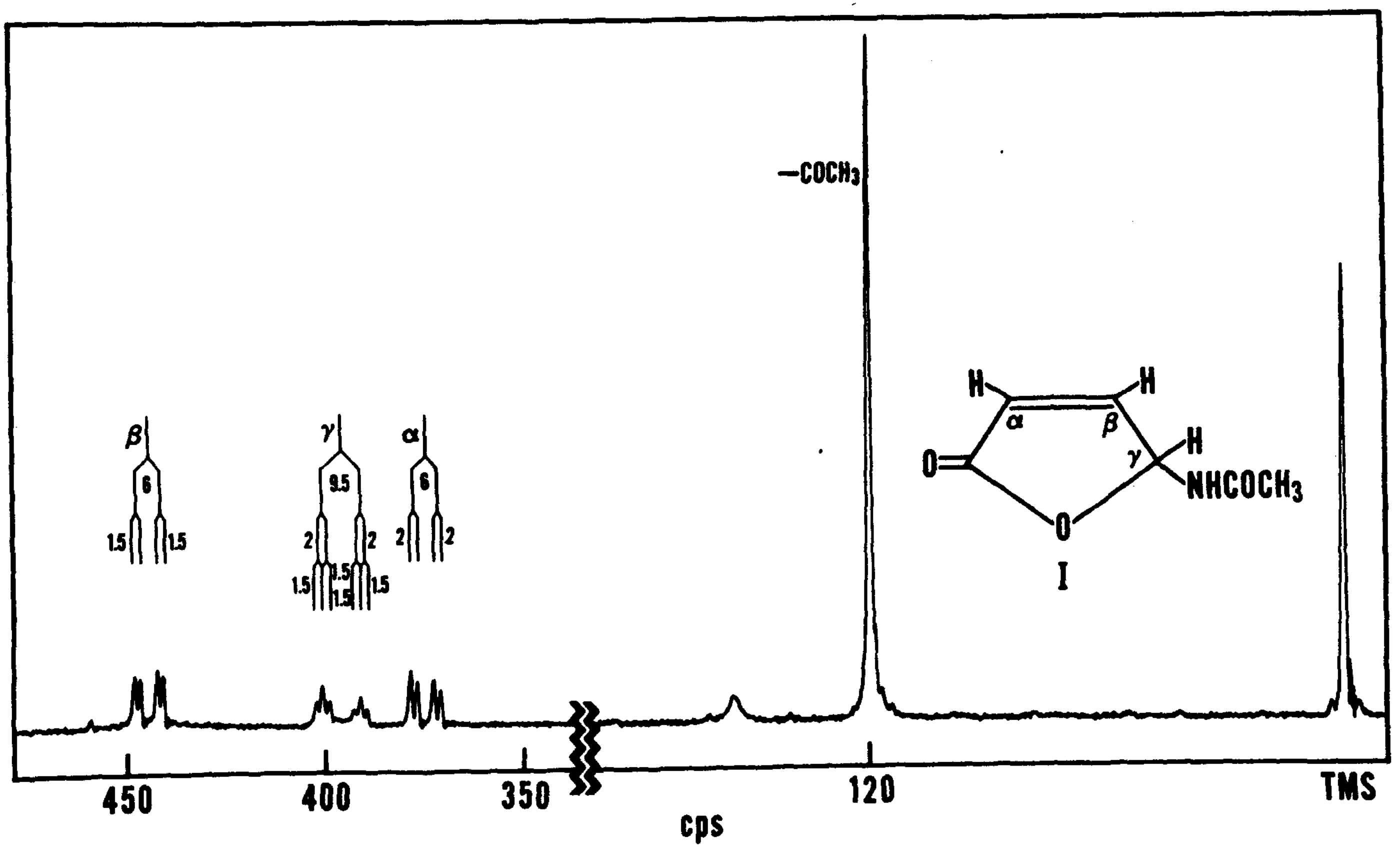

FIG. 2 NMR Spectrum of 4-acetamido-4-hydroxy-2-butenoic acid-γ-lactone (I) at 60 Mcps in CD_3CN

The NMR spectrum (FIG. 2) is also consistent with the assigned structure. The pair of triplets centered at 397 cps is due to the γ-proton and forms an unresolved triplet following D_2O exchange of the amide proton (FIG. 3).

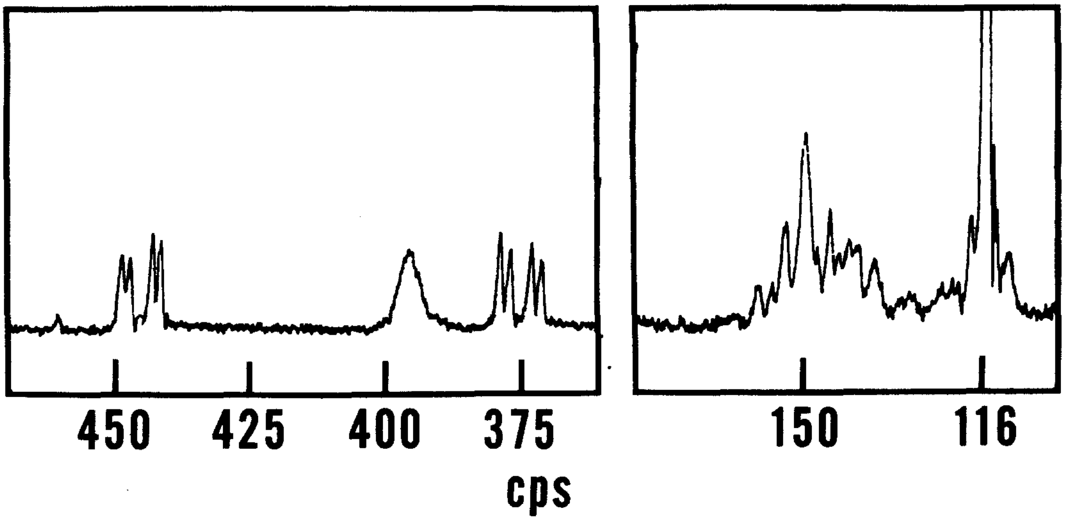

FIG. 3. Portions of 60 Mcps NMR spectra of I following D_2O exchange (left) and of the dihydro derivative of I (right); cps from TMS.

The NMR spectrum of the dihydro derivative of the butenolide (FIG. 3) shows a series of peaks at 150 cps for the methylene protons; this pattern is strikingly similar to the published spectrum of γ-valerolactone (4). A highly purified sample of the butenolide had no optical rotation between 600 and 290 mμ in spite of the chiral center at the γ-carbon. A once-crystallized sample shows optical rotation, but as yet the optical rotation has not been definitely assigned to the butenolide. Samples of ancepsenolide, another $\Delta^{\alpha,\beta}$-butenolide, also had variable optical rotations (5).

Details of isolation, structure determination, and the confirmation of the structure by synthesis by one of us (H. J. B.) will be published elsewhere.

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