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FUSARIC ACID FROM *FUSARIUM SOLANI*

NORMAN CLAYDON, JOHN FREDERICK GROVE and MICHAEL POPLE

Agricultural Research Council, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Falmer, Brighton BN1 9QJ England

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First isolated from *Gibberella fujikuroi* (conidial state: *F. moniliforme*) [1], the phytotoxin fusaric acid (5-*n*-butylpyridine-2-carboxylic acid) is also produced by a number of plant pathogens belonging to *Fusarium* section *Elegans*, particularly by certain *formae speciales* of *F. oxysporum*, formerly [2] described under the names *F. lycopersici*, *F. orthoceras* and *F. vasinfectum*. From these sources it usually occurs as a solid solution with the 5-but-3′-enyl analogue dehydrofusaric acid [3].

The phytotoxicity of the plant pathogenic *Fusarium* species of section *Martiella*, particularly *formae speciales* of *F. solani*, is attributed to production of the red naphthazarin pigments marticin, fusarubin and javanicin and their relatives, and no other phytotoxins have hitherto been found in cultures of these fungi [4].

As part of an investigation into the formation of insecticidal low molecular weight secondary metabolic products by *Fusarium* sp. pathogenic to invertebrates, we have examined, through the courtesy of Dr. D. V. Lightner, the strain isolated by him from *H. americanus* and identified as *F. solani* by Dr. C. Booth at the Commonwealth Mycological Institute, Kew.

The fungus was grown in surface culture on several media and, at intervals during the fermentations, solvent extracts of the culture filtrates were tested for insecticidal activity by injection, using the blowfly *Calliphora erythrocephala*. On Raulin–Thom medium (N source: NH_4^+) the insecticidal activity of the extracts was wholly accounted for by the presence of the napthazarin pigments anhydrofusarubin, fusarubin and javanicin [5], produced in yields comparable with those obtained from plant pathogenic strains of *F. solani* [6]. On Czapek-Dox medium (N source: NO_3^-), although pigment formation was almost completely inhibited, extracts were insecticidal. The active material was concentrated in the acid fraction and was isolated and identified as fusaric acid. It was completely free (MS) from dehydrofusaric acid. The yield of fusaric acid, estimated spectrophotometrically, was comparable with the best yields reported from plant pathogenic strains of Fusaria of the Elegans section.

EXPERIMENTAL

Plant material. Fusarium solani (Mart.) Sacc., CMI 197459; D. V. Lightner C166; culture 81 in our collection of entomopathogens. Isolated from an infected specimen of the cultivated lobster *Homarus americanus* at an experimental farm in New York [7].

Conical flasks (1 l.) containing the medium (250 ml) were inoculated with a spore/mycelium suspension (1 ml) of the fungus prepared from a 5 day shake culture on the same medium. The vessels were incubated at 25° in artificial light. At intervals, aliquots of the culture fluid were removed under sterile conditions from beneath the mycelial felts in selected vessels for determination of the pH and optical rotation and for spectrophotometric and TLC assays for secondary metabolites. In the photometric estimation of fusaric acid (ε_{269} 5700) the residue obtained by extracting culture fluid (5 ml) at pH 2.5 with EtOAc (2 × 2 ml) was dissolved in MeOH (10 ml) and the absorbance (1 = 1 cm) at 269 nm was recorded. After harvesting, the culture fluid was extracted with EtOAc, first at the natural pH and then, after the addition of HCl, at pH 2.5–3.0; neutral and acidic extracts were recovered in the usual way.

On Czapek-Dox medium 28 days after inoculation, culture fluid (690 ml; 380 mg/l fusaric acid) yielded neutral (44 mg) and acidic (278 mg) fractions. Recrystallization of the acidic fraction from EtOAc furnished prisms, mp 101–102°, of fusaric acid (Found: *M* 179.0944. Calc. for $C_{10}H_{13}NO_2$, 179.0946) identified by comparison of the infrared spectrum (chloroform · ν_{max} 3280, 1765, 1705, 1595, 1575 cm^{-1}) with that of an authentic specimen [3].

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REFERENCES

1. Yabuta, T., Kambe, K. and Hayashi, T. (1934) *J. Agr. Chem. Soc. Japan* **10**, 1059.
2. Gaumann, E. (1957) *Phytopathol. Z.* **29**, 1.
3. Grove, J. F., Jeffs, P. W. and Mulholland, T. P. C. (1958) *J. Chem. Soc.* 1236.
4. Kern, H. (1972) in *Phytotoxins in Plant Diseases* (Wood, R. K. S. ed.) pp 35. Academic Press, London.
5. Claydon, N., Grove, J. F. and Pople, M. (1977) *J. Invertebr. Pathol.* in press.
6. Kern, H., Naef-Roth, S. and Ruffner, F. (1972) *Phytopathol. Z.* **74**, 272.
7. Lightner, D. V. and Fontaine, C. T. (1975) *J. Invertebr. Pathol.* **25**, 239.