Phytochemistry, 1972, Vol. 11, pp. 627 to 630. Pergamon Press. Printed in England.

SHORT COMMUNICATION

PRODUCTION OF CITREOVIRIDIN BY *PENICILLIUM PULVILLORUM*

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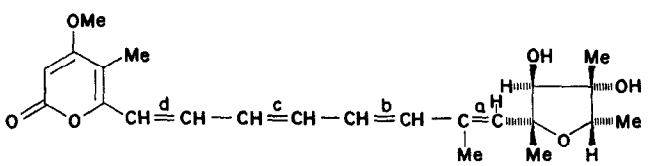
(*Received* 6 *July* 1971)

Abstract—The ability of some related *Penicillium* species to produce toxic metabolites on a natural substrate was investigated. It was found that maize meal was rendered toxic by inoculation with strains provisionally assigned to *Penicillium pulvillorum* Turfitt. The toxic component proved to be identical with citreoviridin, a yellow fluorescent, neurotoxic polyene. The isomerization of citreoviridin to isocitreoviridin was studied.

INTRODUCTION

DURING INVESTIGATIONS on the occurrence of toxigenic fungi on South African cereals, we examined two sclerotigenic *Penicillium* strains which were isolated from maize. Each strain was grown on sterilized, moistened maize meal[1] and the mouldy meals screened for toxicity by incorporating them into the feed of day-old ducklings. These two strains (CSIR 1405 and CSIR 1406) caused acute poisoning of the test animals. Extraction and systematic fractionation of the fungal cultures yielded a toxic yellow pigment in large quantities. This pigment was found to be identical with citreoviridin (I).[2,3] The procedure for the isolation of citreoviridin is shown in Fig. 1.



Citreoviridin (I)

Japanese scientists established that extracts of rice infected with *Penicillium toxicarum* Miyake[4] contained a toxic substance which affected the central nervous system, causing an ascending paralysis to experimental animals. According to Uraguchi[5] the same symptoms of poisoning were observed in cases of acute cardiac beriberi (Shoskin-kakke), which was reported to have occurred previously among the inhabitants of the major rice-producing

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[1] D. B. SCOTT, *Mycopath. Mycol. Appl.* **25**, 213 (1965).
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[5] K. URAGUCHI, *J. Stored Prod. Res.* **5**, 227 (1969).

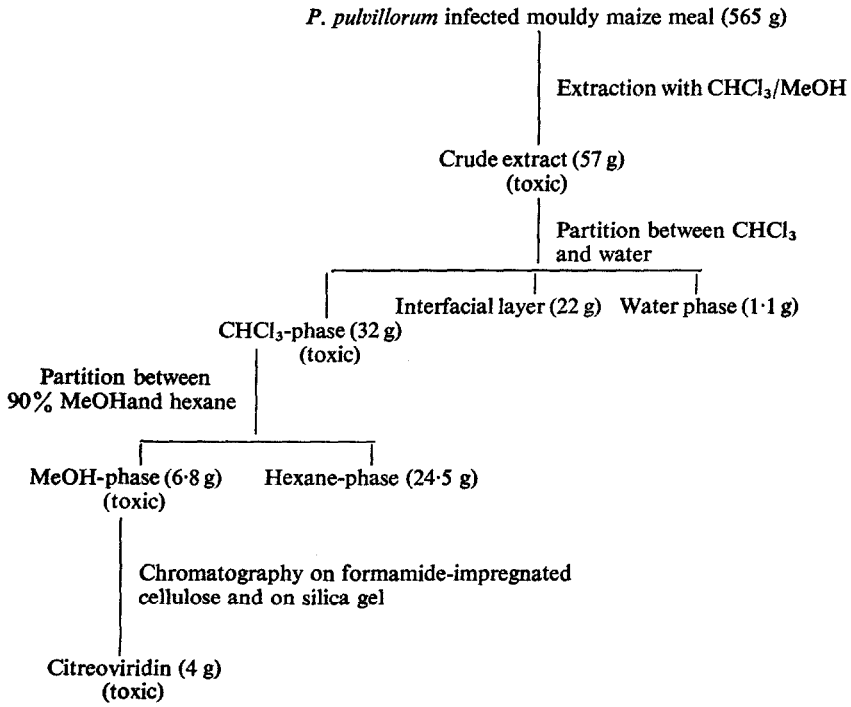

FIG. 1. FLOW SHEET FOR THE ISOLATION AND PURIFICATION OF CITREOVIRIDIN.

districts in Japan. The fungus associated with the toxic yellowed rice was later correctly identified as *P. citreoviride* Biourge.[6] Ueno[6] gave an extensive account of the production of citreoviridin by *P. citreoviride* and furthermore found that this pigment was also produced by *P. ochrosalmoneum* Udagawa.

The morphological characteristics of the two local strains (CSIR 1405 and CSIR 1406) were compared with those of the sclerotigenic *Penicillia* maintained in the Culture Collection of the Commonwealth Mycological Institute. This survey revealed that the characteristics of the two local strains correspond with those of five strains designated as IMI 59,911, IMI 89,983, IMI 99,085, IMI 96,225 and IMI 96,290. The latter strains were assigned to *P. lapidosum* Raper et Fennell, by Smith[7] when he amended the diagnosis of this species. However, Scott and Stolk,[8] who discovered the perfect state of *P. lapidosum* reported that these strains do not belong to *P. lapidosum* but should rather be classified as *P. pulvillorum* Turfitt.

All strains provisionally assigned to *P. pulvillorum* and some additional strains including type cultures of the latter species and related species such as *P. lapidosum* and *P. ochrosalmoneum* were therefore tested for the ability to produce citreoviridin. The survey was extended to include a number of *Eupenicillium* strains, e.g. *Eup. ehrlichii*, *Eup. anatolicum*, *Eup. parvum*, *Eup. terrenum* and *Eup. abidjanum*. The strains were grown in pure culture on maize meal and the pigment obtained as shown in Fig. 1. The presence of citreoviridin in the 90% MeOH-layer was established on silica gel plates (R_f 0·74 in $CHCl_3$–MeOH–

[6] Y. UENO, *Symposium on Mycotoxins in Human Health*, Pretoria, South Africa (September, 1970).
[7] G. SMITH, *Trans. Br. Mycol. Soc.* **44**, 42 (1961).
[8] D. B. SCOTT and A. STOLK, *Antonie van Leeuwenhoek* **33**, 297 (1967).

TABLE 1

Name of the *Penicillium* strain	Strain number	Yield of citreoviridin in mg/100 g maize meal
P. pulvillorum	CSIR 1406	1000
P. pulvillorum	CSIR 1405	850
P. pulvillorum	IMI 96,225	715
P. pulvillorum	IMI 59,911	640
P. pulvillorum	IMI 99,085	550
P. pulvillorum	IMI 96,290	535
P. ochrosalmoneum	CBS 231·60	457
P. pulvillorum	IMI 89,983	230
P. ochrosalmoneum	CSIR 1094	62
P. ochrosalmoneum	CBS 489·66	32

acetone, 45:3:2). The citreoviridin content was quantitatively estimated by measuring the absorbance at λ_{max} 383 nm (ϵ 44,000). A direct isolation of citreoviridin by silica gel chromatography gave the same results.

All seven strains provisionally named *P. pulvillorum* produced citreoviridin in high yield; fair amounts of this metabolite were also produced by three strains of *P. ochrosalmoneum*, as shown in Table 1. None of the other strains investigated, including type cultures of *P. pulvillorum* (CSIR 1407, CSIR 1408 and CSIR 1409) and *P. lapidosum* (CSIR 865) as well as the above mentioned *Eupenicillium* strains produced any citreoviridin.

During the isolation of citreoviridin from mouldy maize meal a minor yellow compound (yield approximately 0·5% of that of citreoviridin) was detected on silica chromatoplates and formamide-impregnated paper. In these systems the minor product, called isocitreoviridin, was less polar than citreoviridin.

Citreoviridin was completely stable towards heating in $CHCl_3$–MeOH for 24 hr. Treatment of a dilute solution of citreoviridin in diffuse light with a catalytic amount of iodine at room temperature gave a mixture of citreoviridin and isocitreoviridin in a ratio of 7:3. Similar treatment of isocitreoviridin gave the same isomeric mixture. The IR and mass spectra of citreoviridin and isocitreoviridin were very nearly identical and the main difference in their UV spectra, measured in methanol, was a shift of the bands at 294 and 285 nm for citreoviridin to 297 and 287 nm for isocitreoviridin. This bathochromic shift may be due to a change in the geometrical isomerism of citreoviridin and all other evidence suggests that the two compounds are geometrical isomers. Using molecular models, it appears from steric considerations that a change in geometrical isomerism from *trans* to *cis* has occurred at double bond c.[9,10] Isocitreoviridin is an artefact since when very pure citreoviridin was submitted to simulated cultural and extraction procedures, it gave a mixture of citreoviridin and isocitreoviridin.

It is of importance to note that citreoviridin caused the mortality of all the experimental animals within two hours on subcutaneous injection of a single dose of 100 mg/kg. At this dosage level, isocitreoviridin had no effect on the rats.

EXPERIMENTAL

Isolation of citreoviridin (I) from infected maize meal. Infected maize (565 g) was extracted and purified as shown in Fig. 1. Recrystallization from MeOH gave citreoviridin (I) m.p. 105–107°. $[\alpha]_D^{18·6°}$ −107·8 (c = 1 in $CHCl_3$). λ_{max}^{MeOH} 383, 294, 285, 238 and 206 nm (ϵ 44,000, 24,800, 22,000, 10,500 and 14,600, respectively).

[9] L. ZECHMEISTER, *Fortschr. Chem. Org. Naturstoffe* **18**, 234 (1960).
[10] L. PAULING, *Fortschr. Chem. Org. Naturstoffe*, **3**, 203 (1939).

$\nu_{max}^{CHCl_3}$ 3450, 3010, 2950, 1700, 1635, 1620, 1605, 1580, 1550, 1465, 1415, 1257, 1100, 1003 cm^{-1}. It was identical to an authentic sample, by IR (KBr disc), MS, NMR and TLC co-chromatography.

Isocitreoviridin. I (500 mg) in C_6H_6 (1 l.) was treated with I_2 (2·5 mg) and kept for 10 min in diffuse light at room temp. Removal of the solvent at low temp. gave a residue which was separated on formamide-impregnated cellulose powder. Elution with C_6H_6–hexane (7:3) gave isocitreoviridin (100 mg, 20%). R_f 0·79 on SiO_2 TLC in $CHCl_3$–MeOH–Me_2CO (45:3:2). λ_{max}^{MeOH} 382, 297, 287, 232 and 206 nm (ϵ 43,200, 28,800, 27,800, 18,200 and 24,900, respectively). Accurate mass M^+ 402·2080. Calc. for $C_{23}H_{30}O_6$ 402·2042. The NMR spectrum was essentially identical to that of (I).

Further elution with C_6H_6–hexane (7:3) gave I, R_f 0·74 on SiO_2 TLC in $CHCl_3$–MeOH–Me_2CO (45:3:2).

Acknowledgements—Thanks are due to Dr. S. J. van Rensburg, South African Medical Research Council, Pretoria, for bio-assay, and to Mrs. Joy Datel of the Microbiology Research Group, for preparing the bulk cultures on maize meal.

Key Word Index—Penicillium pulvillorum; Fungi; neurotoxic polyenes; citreoviridin.