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Production of Xanthomegnin and Viomellein by Isolates of *Aspergillus ochraceus, Penicillium cyclopium*, and *Penicillium viridicatum*

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Fungal isolates from legumes were cultured on rice and examined for production of the toxic mold metabolites xanthomegnin and viomellein. Six of 14 *Aspergillus ochraceus* isolates produced from 0.3 to 1.3 mg of xanthomegnin per g and 0.1 to 1.0 mg of viomellein per g. One of nine isolates of *Penicillium cyclopium* produced 0.1 mg of xanthomegnin per g and 0.06 mg of viomellein per g. Three of nine *P. viridicatum* isolates produced from 0.4 to 1.6 mg of xanthomegnin per g and 0.2 to 0.4 mg of viomellein per g. This is the first report of xanthomegnin and viomellein production by *A. ochraeus* and *P. cyclopium*.

The mycotoxin xanthomegnin is a metabolite of *Penicillium viridicatum* (17), *Aspergillus melleus* and *A. sulphureus* (7), *Trichophyton rubrum* (19), *T. megnini* (2), *T. violaceum* (12), and *Microsporum cookei* (8). The chemically related mycotoxin viomellein is a metabolite of *P. viridicatum* (17), *A. melleus*, and *A. sulphureus* (7). When these two mycotoxins are administered to laboratory animals, lesions are produced in the liver (3) and kidneys (4). Tests on isolated rat liver mitochondria show that xanthomegnin may be a strong uncoupler of oxidative phosphorylation (8). Most of the toxicological studies of these two compounds have involved xanthomegnin- and viomellein-producing isolates of *P. viridicatum* rather than the pure mycotoxin. The chemistry (18) and toxicology (5) of toxins from *P. viridicatum* have been reviewed.

Isolates of *A. ochraceus* produce hepatic lesions similar to those caused by xanthomegnin- and viomellein-producing strains of *P. viridicatum* (20), but there are no reports that *A. ochraceus* produces either one or both of these toxins. We examined 14 isolates of *A. ochraceus* to determine their capacity to elaborate these two toxins. We also examined nine isolates of *P. cyclopium*. These isolates were included because: (i) except for colony color, *P. cyclopium* and *P. viridicatum* share similar macroscopic and microscopic characteristics (11); (ii) these two species are perhaps the most frequently encountered *Penicillium* contaminants of foods/feeds (13); and (iii) no reports exist showing that *P. cyclopium* produces either of the two compounds. Nine isolates of *P. viridicatum* were also examined for production of these two metabolites.

MATERIALS AND METHODS

The 32 isolates investigated were freshly obtained from dried pinto beans and split peas. The seeds were plated on malt agar amended with 7.5% NaCl and 40 μg of chlortetracycline hydrochloride per ml. Emerging colonies were subcultured on Czapek-Dox and malt agar plates and identified according to Raper and Thom (15) and Raper and Fennell (14). The isolates were subsequently grown for 21 days at 23 to 26°C on sterile, polished rice (50 g of rice and 50 ml of water) and were then extracted with chloroform. Extracts were filtered through filter paper, concentrated on a rotary evaporator, and then examined for the presence of xanthomegnin and viomellein by thin-layer chromatography, using silica gel Adsorbosil 1 and a benzene-acetic acid-methanol (90:5:5, vol/vol/vol) solvent system. The mycotoxins were quantitated by high-pressure liquid chromatography, using a method developed for xanthomegnin but which also measures viomellein (16). The nine extracts from the *P. viridicatum* isolates were examined, in addition, for the presence of citrinin and ochratoxin A, using the above silica gel and solvent system.

RESULTS AND DISCUSSION

***A. ochraceus* extracts.** Six of the 14 extracts contained both xanthomegnin and viomellein in concentrations similar to those reported previously for *P. viridicatum* isolates (17; see Table 1). Since these compounds had not previously been reported as metabolites of *A. ochraceus*, mass spectrometric confirmation was obtained for one of the seven thin-layer chromatography-positive extracts.

TABLE 1. *Production of xanthomegnin and viomellein on rice*

Isolate[a]	Xanthomegnin (mg/g)	Viomellein (mg/g)
Ao 48	1.3	1.0
Ao 49	1.1	0.5
Ao 45	0.4	0.1
Ao 204	1.1	0.4
Ao 217	0.9	0.3
Ao 211	0.3	0.2
Pc 613	0.1	0.06
Pv 222	1.6	0.4
Pv 186	0.6	0.3
Pv 43	0.4	0.2

[a] Ao, *A. ochraceus*; Pc, *P. cyclopium*; Pv, *P. viridicatum*.

***P. cyclopium* extracts.** One of the nine extracts contained small amounts of both xanthomegnin and viomellein. Since *P. cyclopium* had not previously been reported to produce these compounds, the presence of xanthomegnin and viomellein in this thin-layer chromatography-positive extract was confirmed by mass spectrometry. In addition, since *P. cyclopium* and *P. viridicatum* are, except for colony color, macroscopically and microscopically very similar (11), we re-examined the one positive isolate by serial dilution to ascertain that the isolate was a pure culture of *P. cyclopium*. Serial dilution results showed this to be the case.

***P. viridicatum* extracts.** Of the nine *P. viridicatum* isolates examined, three produced a strong moldy, earthy odor on diagnostic agar media; the other six isolates did not. All three extracts from those isolates having an earthy odor contained both xanthomegnin and viomellein but neither citrinin nor ochratoxin A. None of the extracts of the odorless, or slightly fragrant, isolates contained either xanthomegnin or viomellein. However, five of the six extracts contained citrinin, and two of the six contained ochratoxin A plus citrinin. These citrinin-ochratoxin A findings in nonearthy-smelling versus earthy-smelling isolates of *P. viridicatum* agree with the findings of Ciegler et al. (6) regarding citrinin and/or ochratoxin A production by isolates of *P. viridicatum*.

Accumulating evidence indicates that xanthomegnin and viomellein are important mycotoxins. They are produced by a number of mold species, several of which are regularly encountered in foods/feeds. Carlton et al. (3) have described the hepatic and renal damage produced in laboratory animals by isolates of *P. viridicatum*. Hamilton et al. (P. B. Hamilton, W. E. Huff, J. R. Harris, and R. D. Wyatt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, O21, p. 248) have reported on livestock illnesses and deaths in North Carolina, possibly due to corn contaminated with ochratoxin A. The corn was not analyzed for xanthomegnin and/or viomellein. The presence of *A. ochraceus* and *P. viridicatum* in feeds has been associated with nephrotoxicity in poultry and swine in Northern Europe (9, 10), and a nephrotoxic strain of *P. cyclopium* has been isolated from maize in a geographical area of Europe in which endemic Balkan nephropathy is present (1). It is possible that xanthomegnin and/or viomellein may play an important role in liver and kidney diseases in animals.

LITERATURE CITED

1. **Barnes, J. M., P. K. C. Austwick, R. L. Carter, F. V. Flynn, G. C. Peristianis, and W. N. Aldridge.** 1977. Balkan (endemic) nephropathy and a toxin-producing strain of *Penicillium verrucosum* var. *cyclopium*: an experimental model in rats. Lancet i(8013):671-675.
2. **Blank, F., W. C. Day, and G. Just.** 1963. Metabolites of pathogenic fungi. I. The isolation of xanthomegnin from *Trichophyton megnini* Blanchard 1896. J. Invest. Dermatol. **40**:133-137.
3. **Carlton, W. W., M. E. Stack, and R. M. Eppley.** 1976. Hepatic alterations produced in mice by xanthomegnin and viomellein, metabolites of *Penicillium viridicatum*. Toxicol. Appl. Pharmacol. **38**:455-459.
4. **Carlton, W. W., and J. Tuite.** 1970. Nephropathy and edema syndrome induced in miniature swine by corn cultures of *Penicillium viridicatum*. Pathol. Vet. **7**:531-546.
5. **Carlton, W. W., and J. Tuite.** 1977. Metabolites of *P. viridicatum* toxicology, p. 525-541. *In* J. Rodricks, C. Hesseltine, and M. Mehlman (ed.), Mycotoxins in human and animal health. Pathotox Publishers, Inc., Park Forest South, Ill.
6. **Ciegler, A., D. I. Fennell, G. A. Sansing, R. W. Detroy, and G. A. Bennett.** 1973. Mycotoxin-producing strains of *Penicillium viridicatum*: classification into subgroups. Appl. Microbiol. **26**:271-278.
7. **Durley, R. C., J. MacMillan, T. J. Simpson, A. T. Glen, and W. B. Turner.** 1975. Fungal products. XIII. Xanthomegnin, viomellein, rubrosulphin, and viopurpurin, pigments from *Aspergillus sulphureus* and *Aspergillus melleus*. J. Chem. Soc. Perkin Trans. **1**:163-169.
8. **Ito, Y., K. Kawai, and Y. Nozawa.** 1973. Biochemical studies of pigments from the pathogenic fungus *Microsporum cookei*. J. Biochem. **74**:805-810.
9. **Krogh, P., F. Elling, B. Hald, B. Jylling, V. E. Petersen, E. Skadhuage, and C. K. Svendsen.** 1976. Experimental avian nephropathy. Acta Pathol. Microbiol. Scand. Sect. A **84**:215-221.
10. **Krogh, P., E. Hasselager, and P. Friis.** 1970. Studies on fungal nephrotoxicity. II. Isolation of two nephrotoxic compounds from *Penicillium viridicatum* Westling: citrinin and oxalic acid. Acta Pathol. Microbiol. Scand. Sect. B. **78**:401-413.
11. **Mislivec, P. B.** 1977. The genus *Penicillium*, p. 41-57. *In* T. Wyllie and A. Morehouse (ed.), Mycotoxic fungi, mycotoxins, and mycotoxicoses: an encyclopedic handbook. Marcell Dekker, New York.
12. **Ng, A. S., G. Just, and F. Blank.** 1969. Metabolites of pathogenic fungi. VII. On the structure and stereochemistry of xanthomegnin, vioxanthin, and viopurpurin, pigments from *Trichophyton violaceum*. Can. J. Chem.

47:1223-1227.
13. **Pohland, A. E., and P. Mislivec.** 1976. Metabolites of various *Penicillium* species encountered on foods. Adv. Chem. Ser. **149**:110-143.
14. **Raper, K. B., and D. I. Fennell.** 1965. The genus *Aspergillus*. The Williams & Wilkins Co., Baltimore.
15. **Raper, K. B., and C. Thom.** 1949. A manual of the Penicillia. The Williams & Wilkins Co., Baltimore.
16. **Stack, M. E., N. L. Brown, and R. M. Eppley.** 1978. High pressure liquid chromatographic determination of xanthomegnin in corn. J. Assoc. Off. Anal. Chem. **61**:590-592.
17. **Stack, M. E., R. M. Eppley, P. A. Dreifuss, and A. E. Pohland.** 1977. Isolation and identification of xanthomegnin, viomelein, rubrosulphin, and viopurpurin as metabolites of *Penicillium viridicatum*. Appl. Environ. Microbiol. **33**:351-356.
18. **Stack, M. E., R. M. Eppley, and A. E. Pohland.** 1977. Metabolites of *Penicillium viridicatum*, p. 543-555. *In* J. Rodricks, C. H. Hesseltine, and M. Mehlman (ed.), Mycotoxins in human and animal health. Pathotox Publishers, Inc., Part Forest South, Ill.
19. **Wirth, J. G., T. E. Beesley, and S. R. Anand.** 1965. The isolation of xanthomegnin from several strains of the dermatophyte *Trichophyton rubrum*. Phytochemistry **4**:505-509.
20. **Zimmermann, J. L., W. W. Carlton, J. Tuite, and D. I. Fennell.** 1977. Mycotoxic diseases produced in mice by species of the *Aspergillus ochraceus* group. Food Cosmet. Toxicol. **15**:411-418.