

Verruculogen Produced by Soil Fungi in England and Wales

D. S. P. PATTERSON,* B. J. SHREEVE, B. A. ROBERTS, AND S. M. MACDONALD

Central Veterinary Laboratory, Weybridge, Surrey KT15 3NB United Kingdom

Received 13 March 1981/Accepted 20 July 1981

Soil fungi, including *Aspergillus fischeri*, *Penicillium piceum*, *Penicillium nigricans*, and *Penicillium raistrickii*, produced a tremorgenic toxin previously described as toxin X. Chemical analysis showed that this toxin was predominantly verruculogen.

Penitrem A and an unknown tremorgenic toxin X, were produced by isolates of soil fungi obtained from pasture associated with an outbreak of disease resembling migran and ryegrass staggers in cattle and sheep (8, 10). Subsequently, it was reported (9; B. J. Shreeve, D. S. P. Patterson, B. A. Roberts, and S. M. MacDonald, Proc. Int. Conf. Vet. Pharmacol. Toxicol. Ther., 1980, in press) that toxigenic fungi could be isolated from the soil at 22 of 24 sites in England and Wales. Of 415 such isolates (mainly *Aspergillus* and *Penicillium* spp.), 83 produced toxin X and 31 produced penitrem A, demonstrating the wide distribution of soilborne toxigenic fungi, confirming the potential for tremorgenic ingestion by cattle and sheep grazing close to the soil, and stressing the predominance among tremorgenic species of fungi capable of producing toxin X.

Penitrem A and verruculogen have already been implicated in outbreaks of staggers syndromes (5-7), and it was therefore decided to test the hypothesis that toxin X and verruculogen were identical. The present communication records a comparison of the physical, chemical, and toxicological properties of the two toxins and establishes their probable identity.

One of our isolates (*Penicillium raistrickii*) was transferred to slopes of 2% malt extract agar and incubated at 25°C for 14 days. Spore suspensions in phosphate-buffered saline (1 ml, pH 7.0) were then used to inoculate a liquid medium (150 ml) containing 3% dehydrated mashed potatoes, 2% skimmed milk solids, and 2% sucrose (11), which was incubated in Roux flasks at 25°C for 14 days.

The presence of toxin X in the culture was confirmed in an ethyl acetate extract (10) and by a quantity of the toxin being purified by repeated preparative thin-layer chromatography (200- μ m layers of silica gel GF₂₅₄; developing solvents were chloroform-acetone, 96:4 (vol/vol) and diethylether-cyclohexane, 75:25 (vol/vol). The toxin was eventually recovered as a micro-

crystalline solid (ca. 12 mg); Table 1 shows a comparison of its properties with those of an authentic specimen of verruculogen.

That toxin X produced a tremorgenic response in cattle and sheep similar to that elicited by verruculogen (B. J. Shreeve, et al., Proc. Int. Conf. Vet. Pharmacol. Toxicol. Ther., 1980) and that much of the physicochemical data supported the conclusion that the two toxins were identical, but mass spectrometry perhaps not unexpectedly revealed that our preparation of toxin X also contained some fumitremorgen B.

It is known that verruculogen is produced by *Aspergillus fumigatus* (7), *Penicillium verruculosum* (2), *Penicillium piscarium* (4), and *Penicillium janthinellum* (6), but supporting our previous observations on the toxigenicity of certain soilborne fungi, the present data suggest that the following additional species should be considered potential producers of verruculogen: *Aspergillus fischeri*, *Penicillium piceum*, *Penicillium nigricans*, and *P. raistrickii*.

LITERATURE CITED

1. Cole, R. J., J. W. Kirksey, R. H. Cox, and J. Clardy. 1975. Structure of tremor-producing indole, TR-2. *J. Agric. Food Chem.* **23**:1015-1018.
2. Cole, R. J., J. W. Kirksey, J. H. Moore, B. R. Blankenship, U. L. Diener, and N. B. Davis. 1972. Tremorgenic toxins from *Penicillium verruculosum*. *Appl. Microbiol.* **24**:248-250.
3. Fayos, J., D. Lakensgard, J. Clardy, R. J. Cole, and J. W. Kirksey. 1974. Structure of verruculogen, a tremor-producing peroxide from *Penicillium verruculosum*. *J. Am. Chem. Soc.* **96**:6785-6787.
4. Gallagher, R. T., and G. C. M. Latch. 1977. Production of the tremorgenic mycotoxins verruculogen and fumitremorgen B by *Penicillium piscarium* Westling. *Appl. Environ. Microbiol.* **33**:730-731.
5. Gallagher, R. T., R. G. Keogh, G. C. M. Latch, and C. S. W. Reid. 1977. The role of fungal tremorgens in ryegrass staggers. *N. Z. J. Agric. Res.* **20**:431-440.
6. Lanigan, G. W., A. L. Payne, and P. A. Cockrum. 1979. Production of tremorgenic toxins by *Penicillium janthinellum* Biourge: a possible aetiological factor in ryegrass staggers. *Aust. J. Exp. Biol. Med.* **57**:31-37.
7. Mantle, P. G., J. B. Day, C. R. Haigh, and R. H. C. Penny. 1978. Tremorgenic mycotoxins and incoordi-

TABLE 1. Comparison of the properties of verruculogen and toxin X

Type of comparison and parameter	Toxin X ^a	Verruculogen ^a	Reference
Thin-layer chromatography			
R_f^b	0.18	0.2	6
R_f^c	0.09	0.1	
Absorbing spot ^d	Yes	Yes	
Light-brown spot ^e	Yes	Yes	
Photodecomposes ^f	Yes	Yes	
Cochromatography ^g	Indistinguishable	Indistinguishable	
Ultraviolet absorption spectrum (ethanol)			
Peak 1 (nm) (E ^h)	223 (4.80×10^4)	226 (4.75×10^4)	3
Peak 2 (nm) (E)	275 (8.52×10^3)	277 (11.0×10^3)	
Peak 3 (nm) (E)	293 (7.83×10^3)	295 (9.75×10^3)	
NMR spectrum ^a			
Proton NMR	Identical	Identical	1
Tremorgenic response in mice			
ED ₅₀ ⁱ	0.40	0.39	3

^a We are indebted to P. G. Mantle, Imperial College of Science and Technology, London, for the authentic sample of verruculogen and for the mass-spectral analysis, which indicated, by electron-impact mass spectrometry, that toxin X contained verruculogen with a smaller amount of fumitremorgen B (3, 6). We are also indebted to N. F. Janes, Rothamsted Experimental Station, Harpenden, for the nuclear magnetic resonance (NMR) spectrum.

^b Silica gel; chloroform-acetone, 93:7 (vol/vol).

^c Silica gel; diethyl ether-petroleum ether, 75:25 (vol/vol).

^d On chromatoplate with fluorescent additive.

^e With FeCl₃ spray.

^f Ultraviolet, 254 nm.

^g Both toxins were mixed and chromatographed in seven different solvent systems.

^h Molar absorbance.

ⁱ ED₅₀, 50% Effective dose. Values (approximate) are expressed in milligrams per kilogram.

- nation syndromes. *Vet. Rec.* **103**:403.
8. Patterson, D. S. P., B. A. Roberts, B. J. Shreeve, S. M. MacDonald, and A. W. Hayes. 1979. Tremorgenic toxins produced by soil fungi. *Appl. Environ. Microbiol.* **37**:172-173.
 9. Shreeve, B. J., D. S. P. Patterson, B. A. Roberts, and S. M. MacDonald. 1979. The occurrence of soil-borne tremorgenic fungi in England and Wales. *Vet. Rec.* **104**: 509.
 10. Shreeve, B. J., D. S. P. Patterson, B. A. Roberts, S. M. MacDonald, and E. N. Wood. 1978. Isolation of potentially tremorgenic fungi from pasture associated with a condition resembling ryegrass staggers. *Vet. Rec.* **103**:209-210.
 11. Wilson, B. J. 1971. Miscellaneous *Penicillium* toxins, p. 460-482. In A. Ciegler, S. Kadis, and S. J. Ajl (ed.), *Microbial toxins*, vol. 6. Academic Press, Inc., New York.