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Tremorgenic Mycotoxin from *Penicillium paraherquei*

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A tremorgenic mycotoxin was isolated from *Penicillium paraherquei* Abe ex G. Smith and identified as verruculogen. It was produced at the rate of approximately 1 mg/g of the dried fungal mycelium cultured on peptone-enriched Czapek-Dox medium at 28°C.

One of the fungal isolates (culture strain no. 312) from the air in Kochi, a city located in the southwest area of Japan, caused tremorgenic activity in mice administered the chloroform extract of its fungal mycelium. The species clearly belonged in the Biverticillata-Asymmetrica section and was usually assigned to the *Penicillium herquei* series, as in the classification of Raper and Thom (9). The correct placement of the species, however, remained somewhat in doubt, since it frequently developed more divaricate penicillia than other species belonging to the *P. herquei* series. The fairly luxuriant growth on the usual media, the floccose texture in its colonies on Czapek agar, the rough walls of conidiophores, and the long ellipsoid conidia with fine warts arranged in spiral bands were characteristic. Its colony reverse was usually in dull yellow shades, rather than green colored as in *P. herquei*. From these observations, the species was identified as *Penicillium paraherquei* Abe ex G. Smith. Although it was isolated originally from Japanese soil (1), and later from agricultural soil in Kochi, it has rarely been found in the temperature zone. In our experience, it has been encountered frequently in soils and agricultural commodities in Papua, New Guinea, and seems to be unusually prevalent in tropical and subtropical areas in the Pan-Pacific (11).

The toxin production was performed on 0.5% peptone-enriched Czapek-Dox medium (pH 6.8) at 28°C for 10 to 12 days. The fungal mycelium was dried at room temperature and extracted with chloroform to yield 22 g of the extract from 250 g of the dried mycelium. It was chromatographed on a silica gel column (5 by 23 cm) and eluted subsequently with ethyl ether-*n*-hexane (15:85), vol/vol), chloroform-methanol (97:3, vol/vol), and methanol. The second eluate was further chromatographed on a silica gel column using chloroform-methanol (97:3, vol/vol). The toxic eluate was treated with ethanol, and soluble pigments were discarded. The resultant insoluble matter (1.54 g) was crystallized from benzene to yield fine needles with a melting point of 234 to 235°C (decomposed). From 100 g of the dried fungal mycelium, approximately 100 mg of tremorgen was isolated in pure form. The toxin gave a single spot with R_f values of 0.75 (chloroform-methanol, 97:3, vol/vol), 0.70 (toluene-ethyl acetate-formic acid, 5:4:1, vol/vol/vol), 0.56 (dichloromethane-acetone, 95:5, vol/vol), and 0.31 (ethyl ether) on thin-layer chromatography plates of Silica Gel G. Elemental analyses showed: C, 63.05%; H, 6.42%; N, 7.74%; and O, 22.79%; as calculated for $C_{27}H_{33}N_3O_7$: C, 63.39%; H, 6.50%; N, 8.21%; and O, 21.90%. The ultraviolet spectrum of the tremorgen showed λ_{max}^{EtOH} at 223, 274, and 293 nm. The infrared spectrum showed prominent absorptions at 3,430 (hydroxyl group) and at 1,690 and 1,670 cm^{-1} (diketopiperadine ring). The 90-MHz proton magnetic resonance spectrum taken in $CDCl_3$ showed the following absorptions: δ 1.01 (s, 3H), 1.74 (s, 6H), 2.01 (s, 3H), 1.8 to 2.6 (bd, 6H), 3.60 (t, 2H), 3.83 (s, 3H), 4.10 (s, 1H), 4.49 (m, 1H), 4.78 (d, J=3 Hz, 1H), 5.04 (d, J=8 Hz, 1H), 5.65 (d, J=3 Hz, 1H), 6.05 (d, J=10 Hz, 1H), 6.60 (d, J=2 Hz, 1H), 6.64 (d, J=8 Hz, 1H), 6.82 (dd, J=2, 9 Hz, 1H), and 7.88 (d, J=9 Hz, 1H). Adding D_2O to the $CDCl_3$ solution, a signal at δ 4.78 diminished, and a signal at δ 5.65 was changed from doublet to singlet. Reduction of the tremorgen with 5% palladium-on-carbon in chloroform at room temperature under atmospheric pressure afforded a decomposition product, the proton magnetic resonance spectrum of which was identical to that of TR-2 (4). The mean lethal dose of the pure toxin was 6.3 mg/kg when injected intraperitoneally into male mice of ddy strain, and typical trembling was observed at doses over 0.9 mg/kg. From these facts, the tremorgen produced by *P. paraherquei* was confirmed as verruculogen (6).

In addition to *Aspergillus* spp. (3, 10, 14, 15), several *Penicillium* spp. are well known to

produce toxic tremorgens such as tremortins and verruculogen (2, 5–8, 13). But it has never been reported that the tremorgenic mycotoxin is produced by *P. paraherquei*, from which penicillic acid was isolated as a toxic metabolite in the culture filtrate (12). It is noteworthy that this species is exceptionally different in its distribution from other *Penicillium* spp. (11).

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