Production, Isolation, and Preliminary Toxicity Studies of Brevianamide A from Cultures of *Penicillium viridicatum*

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Brevianamide A, one of a novel group of alkaloids first obtained from *Penicillium brevis-compactum*, has also been isolated as a principal metabolite from two key isolates of *P. viridicatum*. Procedures for bioproduction and purification are described. This compound was not toxic when fed or injected into mice and had no antibiotic properties against several common bacterial and fungal microorganisms. Brevianamides A and B are among at least four yellow metabolites that may be formed by isolates of *P. viridicatum*.

Birch and Wright (2) in 1969 reported the isolation and structural characterization of a new class of alkaloids named brevianamides from *Penicillium brevis-compactum*. Figure 1 shows formulas for A through F of the series.

A major component of the group, brevianamide A, is a yellow crystalline compound which also fluoresces with an intense yellow color under long-wave ultraviolet illumination. Brevianamide B is stereoisomeric with A (about the spiro-center), whereas the C and D compounds represent colored photolysis products of A (1). Brevianamides E and F (cyclo-L-tryptophanyl-L-proline), on the other hand, are colorless crystalline substances.

We report that two out of three key isolates of *P. viridicatum* also produce brevianamide A (and possibly other brevianamides) when grown on several different substrates, and mention some previously unreported properties of the compound. The first of the two positive organisms was strain 66-68-2 obtained from John Tuite of Purdue University. This organism has been implicated as a toxin-producing agent when grown on popcorn and rice. Focal necrotic lesions in the livers, as well as damage to other organs, were noted in rats, mice, and guinea pigs fed experimentally contaminated diets (3–5, 7, 8). In miniature swine, similarly contaminated popcorn caused a disease syndrome characterized by severe visceral edema and nephropathy (6).

In addition to brevianamide A, another compound forming lustrous crystals of molecular weight 266 was obtained from chloroform extracts of contaminated corn. Further studies on the 266 metabolite were not possible since the product was obtained on only one occasion and in very low yield. Danish workers (9, 11) detected both oxalic acid and citrinin in cultures of *P. viridicatum* involved in the nephropathy syndrome of swine. Citrinin, long recognized to be nephrotoxic, is also a yellow crystalline material which fluoresces with an intense yellow color under ultraviolet illumination.

The second brevianamide A-positive strain of *P. viridicatum* was NRRL 963 provided by J. J. Ellis. Carlton et al. failed to demonstrate pathological effects for this organism when it was tested along with strains 66-68-2 in mouse feeding experiments (7). A third organism, designated 69-23 C, and reported to be a source of ochratoxins (12), was kindly provided by Mina Van Walbeek. This organism did not form the alkaloid under any culture conditions tested.

Brevianamide A was obtained as a major metabolite from chloroform extracts of experimentally inoculated corn, shredded wheat, and several chemically defined media in which fructose, galactose, sorbitol, sucrose, maltose, and soluble starch, respectively, served as the sole carbon source. The best yields from strain 66-68-2, amounting to approximately 25 mg per
100 g (dry weight) of substrate, were obtained from moistened shredded wheat on which the organism had grown for approximately 2 weeks at 25 C. Liquid medium shake cultures were usually devoid of alkaloid when tested at various periods up to 2 weeks at the same temperature.

The brightly fluorescing spot representing brevianamide A was readily noted on silica gel plates or prepared silica gel strips at about Rf 0.5 when these were developed in 10% acetone in chloroform. The same developer was used to separate preparative quantities of alkaloid from columns by using silica gel or florisil as adsorbents. Progress of elution was followed by intermittent illumination of the column with long-wave ultraviolet light. Orange to red colored bands, which probably represent brevianamides C and D (1), were also noted on the columns.

Pooled eluates enriched with brevianamide A were concentrated somewhat by evaporation and carefully treated with hexane until cloudy. The treated solutions were then held at 5 C for several days with small additions of hexane to permit formation of brevianamide A crystals. Pooled precipitates of crystalline material were collected, redissolved in acetone, and recrystallized in the cold to obtain light yellow rectangular plates. These were identified as brevianamide A by comparison of spectral and chromatographic data from authentic product kindly furnished by A. J. Birch. Dried crystals were stable to storage at room temperature for an indefinite period.

Both intragastric and intraperitoneal injections of brevianamide A at doses up to 40 mg in white female mice failed to give significant evidence of acute toxicity. The intraperitoneal injections, however, caused a marked yellow discoloration of subcutaneous tissues which persisted for several days. Acetone solutions of brevianamide A were used to saturate feed pellets which were then dried and fed to 20-g male mice ad libitum for a period of 2 weeks. The estimated dose consumed was approximately 100 mg per animal. All of the animals gained weight and had no gross or histological lesions in any of the visceral organs when sacrificed at the termination of the experiment. Although it is tentatively concluded that this alkaloid is not related to the animal disease syndromes mentioned above, longer term feeding studies may be required to confirm this hypothesis.

Brevianamide A was without antibiotic effect for Escherichia coli, Alkaligines fecalis, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa when tested by the disk method. It also had no inhibitory action against Aspergillus niger, A. flavus, Penicillium crustosum, Fusarium graminearum, F. moniliforme, Alternaria sp., and Cladosporium sp.

It is now evident that isolates of P. viridicatum are capable of producing several noteworthy metabolites. Perhaps the most recently discovered toxic compound is "viridicatumxin" obtained from isolates in South Africa (10). This compound is also a bright yellow pigment (C29H31NO9) whose structure has not yet been elucidated. Thus, the finding of an unknown yellow metabolite from P. viridicatum would necessitate one's differentiating at least among citrinin, brevianamides A and B, and viridicatumxin in identifying the compound.

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