APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1983, p. 1937–1938
0099-2240/83/061937-02$02.00/0

Vol. 45, No. 6

Isolation of Xanthomegnin from *Penicillium viridicatum* by Preparative High-Pressure Liquid Chromatography

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Received 13 January 1983/Accepted 14 March 1983

A method was developed for the production and purification of xanthomegnin from *Penicillium viridicatum* (NRRL 6430) cultured on rice at 15°C for 29 days. Liquid-liquid extraction followed by high-pressure liquid chromatography afforded 440 mg of crystalline xanthomegnin per kg of rice.

Xanthomegnin is a member of a family of 1,4-naphthoquinone pigments produced by several species in each of the genera *Penicillium*, *Aspergillus*, and *Trichophyton* (6, 9, 10). When xanthomegnin is fed to mice, hepatic lesions are produced that are identical to those observed after crude cultures of *Penicillium viridicatum* are fed (1). Extensive toxicopathological studies of the effects of *P. viridicatum* cultures in mice, rats, and swine have been carried out by Carlton and Tuite (3). Notable among these is the report of perirenal edema and nephropathy in miniature swine that received corn cultures of *P. viridicatum* as 50% of their diet (2). The strain of *P. viridicatum* used for these studies is not known to produce the nephrotoxins ochratoxin A and citrinin. Because acute and chronic toxicological studies of xanthomegnin in farm animals will require multigram amounts of compound, the objective of our study was to develop methods to produce and purify needed quantities of toxin.

Although high-pressure liquid chromatography has been used for quantitative determination of xanthomegnin in corn extracts (7), production schemes have utilized either a thin-layer (4, 6) or a gravity-flow column (8) chromatographic method. We report here a rapid procedure for the isolation of gram quantities of xanthomegnin from *P. viridicatum* on rice cultures, based on a selective solvent-solvent partitioning step followed by high-pressure liquid chromatography.

P. viridicatum NRRL 6430 (Purdue University strain no. 66-68-2) is maintained by the Agricultural Research Service Culture Collection (Northern Regional Research Laboratory). Cultures were maintained on Blakeslee malt agar slants. To obtain inoculum for production, 5 ml of 1:10,000 sterile aqueous Triton X-100 was added to a 7-day-old slant of *P. viridicatum* and swirled vigorously. A 1-ml portion of this spore suspension was added to a 300-ml Erlenmeyer flask containing 30 g of converted rice. The rice had been tempered with 13.5 ml of water overnight and autoclaved for 20 min at 121°C. The flasks were incubated overnight on a 200 rpm rotary shaker at 28°C. Spore production was then allowed to proceed statically for 6 days, with daily manual agitation to disperse clumps. A spore suspension for inoculation of production flasks was prepared by the addition of 200 ml of 1:10,000 sterile aqueous Triton X-100 with vigorous agitation. The medium was prepared by the addition of 500 g of converted rice and 500 ml of distilled water to 2.8-liter Fernbach flasks. The contents were mixed thoroughly and tempered overnight. The flasks were autoclaved for 20 min at 121°C and inoculated with 10 ml of spore suspension. The flasks were incubated at 15°C for 29 days, with daily manual shaking to prevent clumping. If the cultures were shaken on a rotary shaker, the rice grain structure deteriorated and resulted in reduced yields.

The contents of six flasks were individually blended with 2 liters of methylene chloride for 30 s in a Waring blender, pooled, stirred for 2 h with an air-driven stirrer, and filtered through filter paper on a Büchner funnel. The residual solids were re-extracted with 8 liters of methylene chloride by use of the air-driven stirrer and filtered. The combined organic phase (20 liters) was separated from the aqueous layer and concentrated under reduced pressure. The residual oil (ca. 100 ml) was partitioned between 300 ml of hexane and 500 ml of methanol-water (9:1) by shaking vigorously for 1 min. The aqueous methanol layer was separated and washed with a second portion of hexane. This resulted in deposition of a dark-brown, xanthomegnin-rich precipitate at the interface, which was removed by filtration and washed with hexane to give 3.3 g of material. Brevianamide A, a major metabolite of this strain of *P. viridicatum* (8), was partitioned into the methanolic phase.

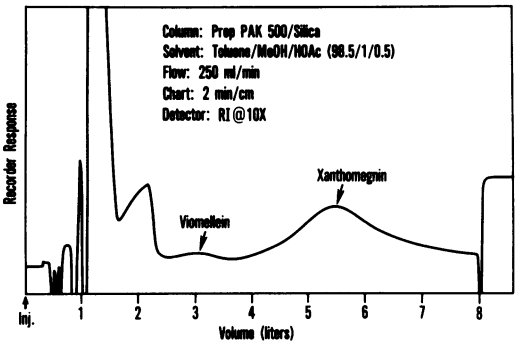

FIG. 1. Preparative high-pressure liquid chromatogram of xanthomegnin purification.

Chromatography of the precipitate dissolved in 80 ml of methylene chloride was performed with a Waters Associates Prep 500 chromatograph fitted with a Prep Pak 500 silica column and eluted with benzene-methanol-acetic acid (98.5:1:0.5, by volume) at 250 ml/min. Spontaneous crystallization occurred overnight at room temperature without evaporation of solvent in the fractions collected at between 5.0 and 6.5 liters of elution solvent. The resulting crystals were removed by filtration and dried to yield 1.33 g of xanthomegnin from 3 kg of rice. The xanthomegnin exhibited a single spot on thin-layer chromatography with R_f identical to an authentic sample in benzene-methanol-acetic acid (85:5:10, by volume) and produced nuclear magnetic resonance and UV spectral data consistent with reported values (5). For subsequent production runs, less toxic toluene was substituted for benzene in the same proportion. The elution pattern was essentially identical (Fig. 1), with xanthomegnin crystallizing in those fractions eluting between 4.5 to 6.5 liters. Small amounts of the related pigment viomellein were eluted between 2.5 and 3.5 liters and identified on the basis of mass spectral and nuclear magnetic resonance data (5).

We thank M. E. Stack, Food and Drug Administration, Washington, D.C., for an authentic sample of xanthomegnin.

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