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Production of Verrucarol

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Verrucarol was obtained from a simple procedure that involved the hydrolysis of a crude extract of a culture of *Myrothecium verrucaria* ATCC 24571.

The trichothecene complex of mycotoxins (1, 3, 5, 8, 13, 16) has presented formidable problems for chemists working on the development of synthetic organic compounds. This challenge has been taken up in recent times by several distinguished chemists who have developed a variety of cleverly conceived syntheses for these compounds (9–11).

The most complex trichothecenes, the trichoverroids (7) and the macrocylic trichothecenes (5, 13, 14), present the greatest challenge. The total synthesis of these compounds, in a formal sense, has had to await the total synthesis of verrucarol (10), the simple trichothecene from which nearly all of the trichoverroids and macrocyclic trichothecenes are derived (Fig. 1).

Although verrucarol has been prepared by total synthesis (10), none of these synthetic pathways produces verrucarol economically. Verrucarol can be readily prepared from naturally occurring anguidine (15); however, the total synthesis of anguidine is also quite laborious (2). Herein, we report the preparation of verrucarol in significant quantities by a very simple technique of fermentation by *Myrothecium verrucaria* ATCC 24571, which produces a variety of macrocyclic (5, 13, 14) and trichoverroid (7) trichothecenes. Upon base hydrolysis, these complex trichothecenes produce verrucarol in good yields.

Lyophilized spores of *M. verrucaria* ATCC 24571 were grown on Sabouraud agar slants and then transferred to sterilized grains of oats. The fungus was allowed to grow out on the oats until the kernels were covered with a thick growth that was rich in spores. It is very important to inoculate with spores since no trichothecenes are produced in the absence of spores. We have observed this phenomenon for all of the many strains of *M. verrucaria* and *Myrothecium roridum* with which we have worked. In addition, *M. verrucaria* also sporulated well on bee pollen (obtained from a local health food store) and on rice with added peptone (0.5%). Several kernels were transferred to a sterile solution of 9.5 g of glucose and 6 ml of corn steep liquor (obtained from Illinois Cereal, Paris, Ill.) in 600 ml of water contained in a 4-liter Erlenmeyer flask. This seed medium was placed on a rotary shaker (100 rpm) at 28°C. After 2 days, 100-ml portions of this seed medium were added to six 4-liter Erlenmeyer flasks each with 1 liter of production medium containing $NH_4H_2PO_4$ (1.0 g), K_2HPO_4 (3.0 g), NaCl (5.0 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), sucrose (40.0 g), and glycerol (10 ml) in 1 liter of water. The flasks were then shaken on a rotary shaker (100 rpm) for 7 days at 28°C. Each flask was treated with 1 g of sodium azide (to inhibit growth and further metabolism) and 200 ml of XAD-7 resin (Rohm and Haas, Philadelphia, Pa.), and shaking was continued for another 5 h. The purpose of the resin was to adsorb the trichothecenes from the aqueous medium. XAD-7 resin adsorbs these trichothecenes more efficiently than does either XAD-4 or XAD-2 resin. Of the trichothecenes, only trichoverrols A and B (7) are not efficiently removed from the solution by this technique. These more hydrophilic compounds can be extracted from the aqueous phase with ethyl acetate. However, the amount of trichoverrols (less than 100 mg/6 liters) would not seem to justify a laborious extraction of 6 liters of an aqueous solution.

The medium containing the mycelium-resin mixture was filtered through a Büchner funnel. To facilitate filtration, a layer of sand (2-cm thick) was used on the top of the filter paper, and the sand layer was covered with another piece of filter paper. The mixture of mycelium and resin was washed with acetone (4 to 5 liters). The initial washings were bright yellow, whereas the later washings were pale yellow. The mycelium-resin mixture was soaked in fresh acetone (1 liter) overnight and filtered. This filtrate was combined with the filtrate obtained in the previous step. The mycelium-resin mixture that was recovered by filtration was soaked in 5 liters of water, stirred vigorously, and decanted quickly. The heavy resin settled faster than did the mycelium, facilitating the removal of the mycelium, which was discarded. The process was repeated twice to remove most of the mycelium. The recovered resin was soaked in 3 liters of 1% sodium azide overnight, filtered, and stored for further use.

The acetone extract (ca. 6 liters) was concentrated in vacuo, and the aqueous portion thus obtained was extracted three times with ethyl acetate (200 ml per extraction). The ethyl acetate extract was dried (Na_2SO_4) and concentrated in vacuo to give 14 g of an oil which was dissolved in 150 ml of methanol. To this mixture was added 6 g of NaOH dissolved in 35 ml of water, and the mixture stood at room temperature for 15 h. After the solution was washed three times with 100 ml of hexane, it was neutralized with 5% HCl and concentrated by rotary evaporation. The aqueous mixture was diluted with 150 ml of saturated sodium carbonate and extracted four times with 100 ml of ethyl acetate. The ethyl acetate extract was dried ($MgSO_4$) and concentrated to give 4.5 g of oil. Flash chromatography (12) over 200 g of silica gel (40 to 60 μm, 70% ethyl acetate-hexane) gave 0.6 g of verrucarol (R_f = 0.3, 70% ethyl acetate-hexane, silica gel thin-layer chromatography) with a melting point of 157 to 158°C from ether-hexane (literature value [4], 155 to 156°C). A slightly more polar fraction (R_f = 0.25, 70% ethyl acetate-hexane, silica gel thin-layer chromatography) than that of verrucarol yielded 50 mg of 8α-hydroxyverrucarol with a melting point of 176 to 178°C (literature value [6], 177 to 179°C). ^1H nuclear magnetic resonance spectral data agreed

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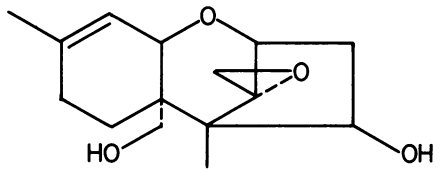

FIG. 1. Structure of verrucarol.

with those of authentic samples of verrucarol and 8α-hydroxyverrucarol. Caution should be exercised in handling the crude extracts, chromatography fractions, and pure compounds. The trichothecenes are acutely toxic and cause severe rashes upon contact with the skin.

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