A Method for the Isolation of Luteoskyrin from *Penicillium Islandicum* Sopp

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Investigations over the past decade have suggested that environmental toxicants play a major role in the causation of liver cancer among the people of rice-eating regions of the world. In particular, mycotoxins have been pinpointed as being a major causative factor in the disease.

One of the most prominent of the molds producing hepatotoxin is Penicillium islandicum Sopp (HOWARD and RAISTRICK 1949). Cirrhosis and carcinoma of the liver have been found in animals fed a diet containing Penicillium islandicum contaminated rice or the fungal mat.

Eight major pigments have been isolated from the mycelium of Penicillium islandicum. Toxicity studies have shown the most hepatotoxic of these to be luteoskyrin (TATSUNO et al. 1955). Luteoskyrin is a dimer of an anthraquinone derivative and occurs as orange, needle-shaped crystals. Because of its similarity to the other pigments present in the mycelium of Penicillium islandicum luteoskyrin has been isolated by chromatographic procedures only.

Since luteoskyrin was the pigment we were primarily interested in, the purpose of this investigation was to develop a chromatographic system in which the luteoskyrin was eluted first. In addition to isolating luteoskyrin, a thin layer chromatography system was developed as a method for determining the purity of the isolated samples and establishing their identities.

Procedures

Penicillium islandicum[a] was grown in surface culture on a Raulin-Thom 5% glucose broth (ASHLEY et al. 1937) at a controlled temperature (25-27°C). The culture flasks were harvested after 20-25 days by filtering the contents of each flask through a Buchner funnel. After discarding the liquid filtrate, the mycelial mats were combined, dried at 70°C for 24 hours and ground in a mortar and pestle.

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In order to reduce the amount of extraneous anthraquinone type pigments to a minimum, the preliminary extraction procedure described by Morooka (MOROOKA 1956) was utilized. The precipitate obtained from this extraction procedure was then dissolved in acetone and placed on a 60-200 mesh size Silica Gel[b] 5 x 48 cm column and eluted with acetone (USP). A yellow band moved off first from the column source followed by an orange-red band and then by a brown color which trailed back to the column source. After the yellow band was eluted from the column it was dried under vacuum and then 2N HCl was added to remove impurities. Ether was added and the mixture shaken in a separatory funnel. The colorless aqueous layer was discarded and the yellow ethereal layer transferred to a beaker and allowed to air dry. Clean needle-shaped crystals of luteoskyrin appeared on drying.

Ultraviolet spectra were determined for both the column eluant and a luteoskyrin standard on the Bausch and Lomb 505 Automatic Recording Ultraviolet Spectrophotometer. Ethanol solutions of both compounds gave identical peaks at 270, 350, and 440 mu. The melting point of the crystals was determined as 278-279° C (with decomposition).

In order to verify the purity of isolated samples of luteoskyrin and further confirm their identity, an adequate thin layer chromatography system was needed. The system adopted utilized $CaHPO_4$ (USP Powder) as stationary phase. Plates were prepared from a slurry of one part $CaHPO_4$ to two parts water. A solvent system of Chloroform-Toluene-Methanol (10:10:1) moved the isolated luteoskyrin and the reference standard with an Rf of 0.8. The column source was also separated into three distinctly colored fractions (Table 1).

TABLE 1

$CaHPO_4$ Thin Layer Chromatography System

Material	Rf Values 0.3	0.8	Solvent Front
Isolated Luteoskyrin	-	yellow	-
Column Source	orange	yellow	red
Reference Standard	-	yellow	-

b. Grace-Davison Chemical Co., Baltimore, Md.
c. Mallinckrodt Chemical Works, St. Louis, Mo.

Summary

Luteoskyrin, a toxic metabolite of Penicillium islandicum can be isolated by an acetone-Silica Gel chromatography column. A preliminary procedure separates the luteoskyrin and 2 other similar metabolites from the mycelial mat. The column used has the advantage of eluting the luteoskyrin as the first band from the source. A thin layer system utilizing $CaHPO_4$ as stationary phase is also available to separate the components of the column and to verify isolation of luteoskyrin.

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