Detection of Griseofulvin and Dechlorogriseofulvin by Thin-Layer Chromatography and Gas-Liquid Chromatography

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A rapid and accurate method is described for the determination of griseofulvin and dechlorogriseofulvin extracted from Penicillium urticae with chloroform. Thin-layer chromatography was used to tentatively identify griseofulvin or decholorogriseofulvin, or both. Two gas-liquid chromatographic systems provided additional qualitative information and simultaneous quantitation of the individual compounds.

Several methods have been described for rapid detection and quantitation of griseofulvin in body fluids and in fermentation media. Most of them are based on spectrophotometry (1, 3, 5–7, 10, 18, 22), spectrofluorometry (2, 4, 8, 13, 16), and colorimetry (19, 23). Some spectrophotometric methods minimized error due to irrelevant materials by measuring absorbancies of the extracts at several equally spaced wavelengths and calculating the concentration of griseofulvin mathematically (1, 3, 5, 6). Holbrook et al. (11) converted griseofulvin to isogriseofulvin with methanesulfonic acid in methanol and determined its amount by measuring the resulting shift in ultraviolet (UV) absorption. Fischer and Riegelman (9) quantitated griseofulvin and griseofulvin-4'-alcohol by measurement of fluorescence directly on thin-layer chromatograms. MacMillan (17) described a sensitive color test to detect decholorogriseofulvin in the presence of griseofulvin. He reported that dechlorogriseofulvin gave an intense blue-violet color with nitric acid, whereas griseofulvin gave a pale yellow color.

A method for griseofulvin determination in fermented broths of Penicillium griseofulvum and P. nigricans has been described in which the antibiotic was extracted from the fermentation broth with chloroform, and iodine was added in stoichiometric ratios (24). Compounds which are structurally related to griseofulvin, such as dechlorogriseofulvin, can interfere with the analysis. Rezabek (20) and Kleine-Natrop et al. (14) assayed for griseofulvin on the basis of colony growth of Trichophyton persicolor and Trichophyton rubrum, respectively. Other biological methods for griseofulvin determination were reported by Knoll et al. (15) and Stepanisscheva and Ziserman (21).

The present study describes a rapid and accurate method for determination of griseofulvin and dechlorogriseofulvin by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

MATERIALS AND METHODS

A griseofulvin-producing isolate of Penicillium urticae was used in this study. The fungus was cultured in 500-ml Erlenmeyer flasks containing 25 g of shredded wheat that was moistened with 50 ml of Mycological Broth (Difco) supplemented with 0.5% each of yeast and malt extract. After 10 to 14 days of growth at 28 C, fungal cultures from each flask were transferred into a Waring Blender and extracted with 100 ml of chloroform. The chloroform extracts were filtered through anhydrous sodium sulfate and a 10 μlter sample of the extract was spotted onto thin-layer chromatographic plates (0.25 mm) (MN-Kieselgel G-HR, Brinkman Instruments, Westbury, N.Y.), along with authentic griseofulvin. The plates were developed in chloroform-acetone (93:7, v/v) to a height of 10 cm. They were examined for the presence of griseofulvin or dechlorogriseofulvin, or both, first under long-wave UV light and then in normal light after being sprayed with 50% sulfuric acid and heated at 110 C for 30 min.

GLC analyses were made with a Barber Colman series 3000 gas chromatograph equipped with a hydrogen-flame ionization detector and disc integrator. The liquid phases used were 1% QF-1 and 1 to 2% SE-30 coated onto Anakrom ABS 80 mesh (Analab Corp., Hamden, Conn.) by the method of Horning et al. (12). The GLC supports were packed into silanized glass columns. Precautions similar to those taken for steroids (12) were rigorously observed when preparing the GLC columns, column...
Griseofulvin and dechlorogriseofulvin were isolated and purified from chloroform extracts of *P. urticae* by precipitation from chloroform solution with *n*-hexane, followed by silica gel column chromatography (0.05- to 0.20-mm mesh) (Brinkman Instruments, Westbury, New York) of the precipitate with chloroform as the eluting solvent. Fractions (25 ml) were collected automatically and subsequently monitored by GLC for the presence of griseofulvin and dechlorogriseofulvin. The fractions containing griseofulvin and dechlorogriseofulvin were combined, evaporated to dryness, and recrystallized from *n*-hexane-chloroform solution.

Analytical confirmation of the structures of the purified griseofulvin and dechlorogriseofulvin from *P. urticae* was based on melting points, TLC, GLC, and UV, infrared (IR), nuclear molecular resonance (NMR), and mass spectral analyses. Melting points were taken on a Fischer-Johns melting point apparatus; UV spectra were determined in methanol solution with a model DB-G spectrometer (Beckman Instruments, Inc., Fullerton, Calif.). Infrared spectra were measured with a Perkin-Elmer model 257 spectrometer as a thin film coated onto a KBr window. NMR spectra were performed with a Varian A-60A spectrometer in deuterated chloroform. Samples for mass spectra were introduced into the mass spectrometer by the direct-probe method.

**RESULTS AND DISCUSSION**

Griseofulvin and dechlorogriseofulvin were determined accurately in crude extracts from *P. urticae* by TLC and GLC analysis. These two compounds appeared together as a bright blue fluorescent spot at RF 0.65 on our TLC system. The difference in polarity between these two compounds was insufficient for their complete separation.

The limit of detection of griseofulvin by TLC was 0.05 µg. Thus, this chromatographic method provided a rapid and sensitive technique for tentative identification of both griseofulvin and dechlorogriseofulvin. Two GLC systems were then used to separate these compounds (Fig. 1, Table 1). This permitted accurate quantitation of the individual compounds without extensive purification. Spectrophotometric and spectrofluorometric methods would not make this distinction on a sample containing a mixture of these compounds. The colorimetric method described by MacMillan (17) would detect both substances in a purified sample mixture, but interfering substances in a crude extract might affect accuracy. No metabolites in the extracts from *P. urticae* interfered with either the GLC or TLC analyses.

The use of TLC is valuable for preliminary screening, since several extracts can be evaluated on one chromatographic plate in minimal time. Extracts that appear to contain griseofulvin or dechlorogriseofulvin, or both, can then be simultaneously analyzed qualitatively and quantitatively by GLC.

In addition to being an excellent qualitative and quantitative method for analysis of these compounds, GLC also served as a monitor during purification. Use of GLC showed that griseofulvin and dechlorogriseofulvin were not resolved from each other by silica gel column chromatography; however, they were separated by fractional recrystallization. This was consistent with previously reported data (17).

The melting point and UV, IR, NMR, and mass spectra of the metabolite identified as griseofulvin from *P. urticae* and an authentic griseofulvin standard were compared to confirm that the compound observed on TLC and GLC was griseo-
fulvin. No authentic standard for dechloro-
griseofulvin was available; however, purified
dechlorogriseofulvin from *P. urticae* was iden-
tified by comparison of its UV, IR, NMR, and mass
spectra with those of griseofulvin.

The melting point (220 °C), UV spectra [wave-
length (λ) maxima in methyl alcohol at 291, 236, and
325 nm], and IR spectra of griseofulvin from
*P. urticae* and authentic griseofulvin were identi-
cal. The molecular weights of these compounds,
as determined by mass spectroscopy [molecular extinction coefficient (m/e) 352], and of the
major fragments (m/e 214 and m/e 138) were
also identical. In addition, the mass spectra were
identical in their overall fragmentation patterns.

The mass spectrum of dechlorogriseofulvin showed parent peak at m/e 318 with major frag-
mentation at m/e 180 and m/e 138. This is con-
sistent with the spectrum of griseofulvin less
chlorine. The UV spectrum of dechlorogriseofulvin
was identical with griseofulvin and the IR
spectrum had only minor differences. The NMR
spectra for griseofulvin from *P. urticae* and
authentic griseofulvin were identical. The NMR
spectrum of dechlorogriseofulvin showed the
presence of an additional proton at 6.08 parts
per million (ppm) which was coupled with a
proton at 6.26 ppm (J = 2 Hz). This is consistent
with the expected coupling of protons that are
meta to each other such as the protons at car-
bon 5 coupling with the proton replacing the
chlorine group at carbon 7 in dechlorogriseofulvin.

The absorptions of the two aromatic methoxy
groups in griseofulvin (4.00 ppm and 4.05 ppm)
were shifted in dechlorogriseofulvin so as to be
superimposed at 3.92 ppm.

The above analytical data prove that the two
compounds, from crude extracts of *P. urticae*
which were analyzed via TLC and GLC, were
griseofulvin and dechlorogriseofulvin.

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**LITERATURE CITED**

1. Ashton, G. C., and A. P. Brown. 1956. Determination of

2. Beddor, C., K. J. Child, and E. G. Tomich. 1939. Spectro-

3. Daly, C. 1961. A simplified spectrophotometric assay of

4. Drydon, L. 1966. Analysis of therapeutic antifungal com-

5. Efimova, G. V., and A. V. Markovich. 1962. Spectropho-

6. Efimova, G. V., and A. V. Markovich. 1963. Spectrophot-
ometric determination of griseofulvin and 6-dimethyl-

7. Efimova, G. V., and A. V. Markovich. 1964. Spectrophoto-
metric determination of griseofulvin in mycelia. Anti-
biotiki 9:592-595.

8. Eisenbrandt, K. 1964. Photofluorometric determination of

mination of griseofulvin and griseofulvin-4'-alcohol in plasma
by fluorometry on thin-layer chromatograms. J.
Chromatogr. 21:268-274.

for the determination of griseofulvin in fermentor broth.

11. Holbrook, A., F. Bailey, and G. M. Bailey. 1963. Quan-
titative chromatographic method for the determination of purity

1963. Separation and determination of steroids by gas

13. Ivanov, W. M., A. A. Nevinskii, and N. S. Redl. 1965. Fluoro-
metric determination of griseofulvin. Lab. Delo. 9:564-566.

Comparative griseofulvin serum level determinations after
administration of griseofulvin AWD and Grinic using new

Plate assays for griseofulvin in pharmaceutical preparations

the spectrophotofluorometric determination of griseofulvin.
J. Pharm. Sci. 54:655-656.

1697-1702.


Venerol. 37:26-29.


23. Underman, H. W. 1965. Colorimetric determination of griseo-

griseofulvin determination in fermentation fluids. Anti-
biotiki 13:319-322.