

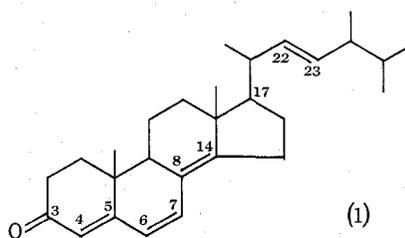
The Occurrence of Ergosta-4,6,8(14),22-tetraen-3-one in Several Fungi

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Abstract

Ergosta-4,6,8(14),22-tetraen-3-one has been identified in extracts of rice infected with *Aspergillus*, *Penicillium* and *Fusarium* species.



Ergosta-4,6,8(14),22-tetraen-3-one (1) has been isolated previously from *Penicillium rubrum*,¹ yeast (*Candida utilis*),² *Fomes officinalis*³ and the bioluminescent mushroom *Lampteromyces japonicus*.⁴ Metabolic studies^{1,5} indicate that it arises from ergosterol, ergosterol peroxide and ergosta-6,22-diene-3 β ,5 α ,8 α -triol by a set of multiple pathways. The widespread

natural occurrence of ergosterol and ergosterol peroxide in fungi⁶ suggests that the tetraenone may be more common than literature reports indicate.

Our interest stemmed from the investigation of samples of rice from various parts of Papua New Guinea. These samples were heavily infected with fungi, and extracts contained a strongly fluorescent compound. From the rice at least 25 infecting species were isolated,⁷ among them *Aspergillus flavus* Link ex Fr., *A. fumigatus* Fres., *A. ochraceus* Wilhelm, *Fusarium moniliforme* Sheld., *Penicillium islandicum* Sopp., *P. patulum* Bainier and *P. citrinum* Thom.

When uninfected rice was inoculated with each of the above organisms, the same blue-green fluorescent compound appeared between 18 and 30 days later. With defatted rice the metabolite was evident after less than 18 days. The compound was

¹ White, J. D., and Taylor, S. I., *J. Amer. Chem. Soc.*, 1970, **92**, 5811.

² Morimoto, H., Imada, I., Murata, T., and Matsumoto, N., *Justus Liebigs Ann. Chem.*, 1967, **708**, 230.

³ Schulte, K. E., Rucker, G., and Fachmann, H., *Tetrahedron Lett.*, 1968, 4763.

⁴ Endo, M., Kajiwara, M., and Nakanishi, K., *Chem. Commun.*, 1970, 309.

⁵ White, J. D., Perkins, D. W., and Taylor, S. I., *Bioorg. Chem.*, 1973, **2**, 163.

⁶ 'Dictionary of Organic Compounds' 5th Cumul. Supp. (Ed. R. Stevens) p. 368 (Eyre & Spottiswoode: London 1969).

⁷ Shaw, D. E., Brown, J. S., and Cunningham, A., *Papua New Guinea Med. J.*, 1972, **15**, 108.

isolated from a large-scale culture of *P. citrinum* on defatted rice and purified by repeated column and thick-layer chromatography.

The material was chromatographically homogeneous and exhibited R_F values identical to those of authentic tetraenone (1) in two solvent systems. The mass spectrum indicated a molecular weight of 392 with an intense peak at $M - 124$ corresponding to loss of the side chain from C17. An ultraviolet absorption maximum at 348 nm is in agreement with published figures for the tetraenone.^{3,5} The n.m.r. spectrum clearly showed the C6-C7 AB system as a pair of doublets (δ 6.6 and 6.03, J_{AB} 10 Hz) with the characteristic C22-C23 multiplet at δ 5.32.

A further vinylic proton resonance (H4) was evident as a singlet at δ 5.82. Comparison of the infrared spectra of the isolated and authentic compounds finally established the identity of the metabolite as (1).

The occurrence of ergostatetraenone in these additional seven organisms suggests that the biosynthetic pathways involving this compound are probably widespread within the fungi.

Experimental

The u.v. spectrum was recorded on a Beckman D.B. spectrophotometer. The mass spectrum was recorded on a Varian-MAT CH7 spectrometer at 70 eV. The n.m.r. spectrum was recorded on a Varian HA-60 spectrometer on a deuteriochloroform solution with tetramethylsilane as internal standard. I.r. spectra were recorded in carbon disulphide using a Perkin-Elmer Infracord 337 spectrophotometer and in Nujol with a Jasco IRA-1 grating spectrophotometer.

Rice Extraction

Samples (10 g) of whole grain rice (Australian brown rice, commercial) were inoculated with fungal culture and sealed. For the accumulation studies *A. fumigatus*, *P. patulum* and *P. citrinum* were used and samples taken as 1, 3, 10, 18 and 30 days after inoculation. Chloroform-methanol-hexane (8 : 2 : 1, 100 ml) was added to each flask, which was shaken for 2 h. After filtration the procedure was repeated and the combined filtrates concentrated to 2 ml. Each sample was chromatographed on silica gel G plates (0.25 mm) in chloroform-acetone (9 : 1) and hexane-acetone-acetic acid (18 : 2 : 1). Detection was by u.v. fluorescence.

The tetraenone exhibited R_F values of 0.68 and 0.3 respectively in the two systems.

Extraction of Ergostatetraenone

Australian brown rice (1.3 kg) was ground and defatted by Soxhlet extraction with chloroform in order to remove lipid material which otherwise interfered with later chromatography. The dried, defatted rice was divided into 12 portions and each was inoculated with *Penicillium citrinum*.

After 30 days the cultures were extracted as previously and the extracts combined and the solvent evaporated to yield 43 g of a light brown gum. This was chromatographed on silica gel (500 g) in chloroform-hexane and the progress of the fluorescent band monitored by u.v. light. Evaporation of the fluorescent fractions afforded 5.35 g. This was rechromatographed and then subjected to thick-layer chromatography on silica gel from which 940 mg were obtained. This was chromatographed once more on silica gel thick layer then finally purified by thin-layer chromatography. Mass spectrum: m/e 392 (37%), 349 (5), 268 (100), 267 (82), 253 (25), 242 (12), 240 (13), 227 (10), 226 (11), 225 (12), 214 (30), 173 (25), 155 (17), 109 (38). N.m.r.: δ 6.6 (1H, d, J 10 Hz), 6.03 (1H, d, J 10 Hz), 5.83 (1H, s), 5.32 (2H, m), 1.12, 0.96, 0.88 and 0.8 (6 \times Me). U.v.: (EtOH) 348 nm (ϵ 26000), 282 (6800), 237 (4600). I.r.: 1665, 1640, 1593, 1460, 1375, 1350, 1325, 1260, 1220, 1195, 1120, 1072, 1035, 1020, 970, 950, 925, 875, 805, 760, 735, 695, 665, 655, 615 cm^{-1} . Specific rotation: $[\alpha]_D^{25} + 565^\circ$ (c, 0.37 in CHCl_3). M.p. 113.5-114.5 $^\circ$ (crystallized from ethanol); no depression of the m.p. occurred when mixed with an authentic sample synthesized by the method of Elks.⁸

⁸ Elks, J., *J. Org. Chem.*, 1954, 468.

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