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Isolation of Ergosterol Peroxide from *Alternaria dianthicola*

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Abstract

A second steroidal constituent of the *Alternaria* genus, ergosterol peroxide, isolated from *A. dianthicola*, may be an artefact.

Exhaustive extraction of the mycelium of *Alternaria dianthicola* revealed the presence of ergosterol peroxide.

Although ergosterol peroxide has been isolated from a number of micro-organisms,[1–7] this is the first report of the isolation of this compound from the genus *Alternaria*. The only other steroid isolated from this genus was a C_{19} steroid by Sugiyama *et al.*[8] However, this steroid has not been completely characterized.

Identification of ergosterol peroxide was accomplished by means of spectroscopic techniques and confirmed by comparison with an authentic sample. The infrared spectra of the natural product and the synthetic sample were superimposable.

It is not known, however, whether ergosterol peroxide is the actual metabolite or whether it is produced from ergosterol by photooxidation during the workup procedure. Ergosterol has previously been isolated from fungi[1,5] and Adam *et al.*[7] have shown that some fungal extracts contain coloured materials which are able to act as photosensitizers for the oxidation of ergosterol to ergosterol peroxide.

Experimental

Melting points were determined on a Kofler block and are uncorrected. The infrared, nuclear magnetic resonance and mass spectra were recorded on Perkin–Elmer 457, Jeol 100 and AEI MS 902 spectrometers respectively. The culture was obtained from the Department of Microbiology, University of Queensland. Kieselgel G (Woelm) was used for column chromatography.

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Growing Conditions

The medium used consisted of malt extract 30 g, mycological peptone 5 g and distilled water to 1 l. Approximately 1-cm square pieces of the fungus growing on mycological agar were added to 50 culture flasks, containing 200 ml of sterile liquid medium each. Incubation was at 37° for 14 days without shaking.

Extraction and Purification Techniques

The mycelium was separated from the liquid medium by filtration through gauze, air-dried and ground to a fine powdery material (80 g). This was extracted in a Soxhlet apparatus for 20 h with light petroleum (b.p. 40–60°), then with chloroform and then with acetone. Ergosterol peroxide was obtained from the light petroleum extract and was purified by column chromatography using the gradient elution technique. Yield 25 mg, recrystallized three times from ethanol, m.p. 176° (lit. 181·5–183°) [mol. wt (mass spectrum), 428·3289. Calc. for $C_{28}H_{44}O_8$: mol. wt, 428·3290]. Infrared spectrum (KBr disk): ν_{max} 3530m, 3415s, 2955s, 2870s, 1458s, 1376s, 1326w, 1290w, 1240w, 1162w, 1150w, 1104w, 1072w, 1040m, 1015m, 962s, 933w, 911w, 859w, 830w, 775w, 720w, 695w, 650w cm^{-1}. Ultraviolet spectrum: no absorption above 220 nm.

Synthetic ergosterol peroxide was prepared from ergosterol by the method of Windaus and Brunken,[9] m.p. 178–179°.

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