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LAGOPODIN METABOLITES AND ARTEFACTS IN CULTURES OF *COPRINUS**

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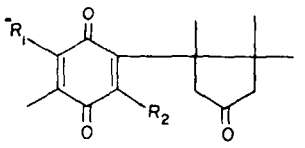
The lagopodins are sesquiterpenoid quinones which have been isolated from "*Coprinus lagopus* Fr." [1] and *C. macrorhizus* var. *microsporus* [2]. We found that shake-flask cultures of monokaryons and a dikaryon of *C. cinereus* produced a range of lagopodins. We isolated and fully characterised lagopodin A (**1**) and lagopodin B (**2**), for which fuller NMR data than are available in the accessible account for these compounds [1] are appended; in our view these data support structure (**2**) for lagopodin B, at least for the predominant form in $CDCl_3$ solution, rather than the alternative hemiketal tautomer [1,2]. We also encountered the dimeric quinone lagopodin C, confirming that as suggested by Thomson [1] this is an artefact formed during work-up, and identified hydroxylagopodin B (**3**) [2] as another component.



(1) $R_1 = R_2 = H$
(2) $R_1 = H$; $R_2 = OH$
(3) $R_1 = R_2 = OH$

However, during work-up by conventional procedures it became apparent that both (**1**) and (**2**) are relatively labile compounds in aqueous solution and especially at neutral or slightly alkaline pH. Under these conditions there was considerable conversion of (**1**) into other products, including (**2**).

In further trials, purified (**2**) was incubated in sterile non-inoculated media under the growth conditions used for the cultures, i.e. using 10 mg (**2**) per 100 ml in 500 ml flasks, stoppered with cotton wool and kept at 37° in darkness on a gyrotatory shaker, 7.5 cm throw, 150 rev. min^{-1}; culture media contained glucose, inorganic salts, and ammonium tartrate and/or urea, and the pH was adjusted with NaOH or HCl. In a convenient TLC system (Merck silica gel F254 with C_6H_6–EtOAc–MeOH, 2:2:1), (**2**) has R_f 0.85. After 6 h incubation at pH 4.4, recovered (**2**) was accompanied by at least 4 other products of R_f 0.45–0.80; from medium at pH 6.8 there were at least 7 such products together with more polar materials, and from a similar incubation at pH 7.6 at least 12 products, covering the whole R_f range, could be seen. This last chromatogram closely resembled the TLC picture obtained from worked-up culture filtrates.

The half-life of (**2**) in shaken culture media was approximately 12 h at pH 6.8–7.6. A considerable amount of red-brown water-soluble pigment, not extractable with Et_2O, was formed from (**2**) and appears similar to the non-extractable pigment which is conspicuous in culture filtrates.

We conclude that a major part of the apparent complexity of the pigment mixture in our cultures, and presumably in cultures of related *Coprinus* species, is due to pH-dependent non-enzymic reactions of the parent quinones in the aerobic aqueous culture media. The complexity of reactions possible under such conditions is increased by the marked tendency of these fungi to release free amino acids and (when urea is the N source) ammonia into the culture medium. This explains our finding that some of the 'natural' lagopodins from our cultures contain 1 or 2 N atoms (per C_{15} unit, by MS) and behave as typical aminoquinones.

NMR data (100 *MHz*, *CDCl*$_3$, δ). Lagopodin A (**1**): 0.97 (3H, *s*), 1.25 (3H, *s*), 1.40 (3H, *s*) (3 Me–Csat); 2.03 (3H, *d*, Me–Ar); 2.0–2.36 (4H, *m*, CH_2–C=O); 6.55 (1H, *q*, Ar–H); 6.66 (1H, *s*, Ar–H). Lagopodin B (**2**): 0.96 (3H, *s*), 1.08 (3H, *s*). 1.36 (3H, *s*) (3 Me–Csat.); 1.99 (3H, *d*, Me–Ar); 2.08–2.20 (4H, *m*, CH_2–C=O); 5.50 (1H, *s*, ArO–H); 6.39 (1H, *q*, Ar–H). The similarity of the methylene signals in the two spectra suggests the cyclopentanone structure for both compounds.

* *Plant*: *Coprinus cinereus* (Schaeff. ex. Fr.) S. F. Gray (= *C. lagopus sensu* Lewis), from Dr. D. Moore, Manchester University Department of Botany.

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