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Note

Analysis of Cortinarius toxins by reversed-phase high-performance liquid chromatography

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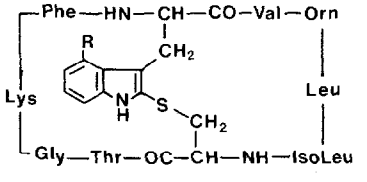
Several species within the Cortinarius genus of mushrooms have been responsible for a number of poisonings both in Britain and on the Continent[1–4]. The isolation and part characterisation of a polypeptide kidney toxin from *Cortinarius speciosissimus* was carried out in our laboratory[5]. This compound was called by us cortinarin A. Subsequent screening of some 60 different examples of Cortinarius by thin-layer chromatography (TLC) showed this compound or its analogues to be present in all of the species examined[6]. A normal adsorption phase high-performance liquid chromatographic (HPLC) system was developed which allowed the quantification of cortinarin A together with another major component of *C. speciosissimus*, cortinarin C[7]. A third compound, cortinarin B, which also shows nephrotoxicity in laboratory animals, has now been isolated from *C. speciosissimus* and the structures of all three cortinarins fully elucidated[8] (Fig. 1). This paper reports a method for the determination of these compounds in Cortinarius mushrooms using reversed-phase HPLC.



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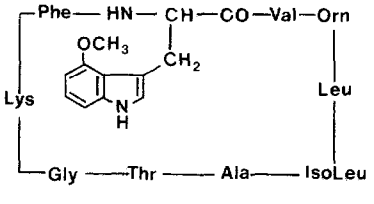

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Fig. 1. The structures of cortinarins A, B and C. (a) R = $-OCH_3$, cortinarin A; R = $-OH$, cortinarin B. (b) Cortinarin C.

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MATERIALS AND METHODS

Apparatus

A Pye Unicam LC3 pump was used to deliver solvent at 1.5 ml/min. The eluent was monitored at 220 nm with a Cecil Instruments CE212 variable-wavelength UV detector. The column was a 25 cm × 4.5 mm I.D. 5-μm ODS (Jones Chromatography) fitted with a Negretti and Zambra injection system incorporating a 20-μl loop. Separation was achieved with a mobile phase of acetonitrile–water (25:75). All solvents used were HPLC grade (Rathburn Chemicals).

Extraction procedure

Mushrooms collected in August 1982 were air dried at 40°C and stored over anhydrous silica gel until used. Dried powdered material was accurately weighed (100–500 mg) and Soxhlet extracted with petroleum ether (40–60) for 1 h prior to a similar 3-h methanol extraction. The methanol extract was evaporated to dryness under reduced pressure and the residue redissolved in 2 ml of methanol. Pure extracts of cortinarins A and C were obtained by preparative TLC using silica gel G plates (Merck) (20 cm × 20 cm × 0.5 mm) developed with cyclohexane–ethyl acetate (3:1). Cortinarin A was located as a fluorescent streak under UV light at 254 nm (R_F 0.50) and Cortinarin C, which is non-flourescent, by spraying one edge of the plate with acidified *p*-dimethylaminobenzaldehyde (R_F 0.30). Cortinarin B was similarly obtained by TLC using silica gel plates but with butanol–acetic acid–water (4:1:1) as the mobile phase (R_F 0.15), cortinarin B also showing a blue fluorescence when irradiated with UV light. All three samples were removed from the plate and immediately eluted with methanol. Straight-line calibration graphs were obtained for the cortinarins based on peak area measurements for concentrations of 25, 100, 200, 500, 750 and 1000 μg/ml. Each point was taken as the average of two determinations.

RESULTS AND DISCUSSION

A chromatogram typical of those obtained from extracts of Cortinarius mushrooms is shown in Fig. 2. The calibration lines correspond to least square regression equations of $y = 0.2417x - 5.7253$, $y = 0.1971x + 0.8008$ and $y = 0.5123x + 3.0913$ for cortinarins A, B and C respectively, with correlation coefficients of 0.9991, 0.9996 and 0.9991. Minimum detectable values showing a signal-to-noise ratio >2 were *ca.* 100 ng for cortinarins A and C and 200 ng for cortinarin B.

The procedure described above has been used in a study of the cortinarin content of a number of different species of Cortinarius mushrooms. A good separation of all three major components was obtained. Concentrations of cortinarins A and C determined by this method were in close agreement with results previously obtained by normal-phase HPLC[7] (Table I). Only three species have so far been found to contain cortinarin B. These are *C. orellanus*, *C. orellanoides* and *C. speciosissimus* which also contain relatively high concentrations of cortinarin A and are reported to be the three most toxic species within the genus Cortinarius. It would appear therefore that toxicity is proportional to the sum of concentrations of cortinarins A and B. Cortinarin C is now considered to be non-toxic and is probably an intermediary in the biosynthesis of the two toxins, cortinarins A and B.

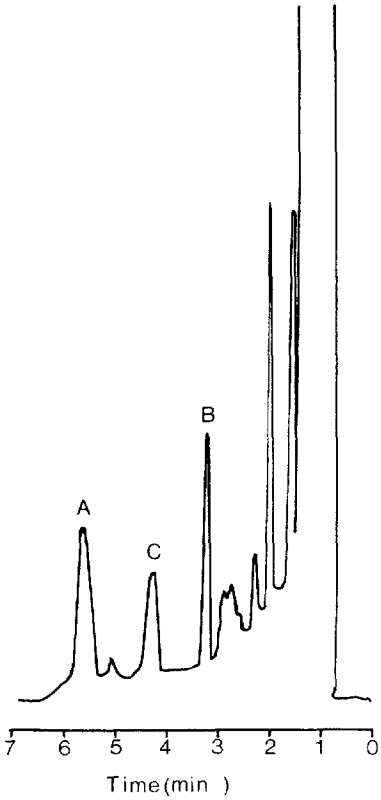

Fig. 2. HPLC chromatogram of a methanolic extract of *C. speciosissimus* showing Cortinarins A, B and C.

TABLE I

ANALYSIS OF CORTINARIUS SPECIES FOR CORTINARINS A, B AND C

Concentrations expressed as % dry weight of mushroom. Values in parentheses were obtained by adsorption-phase HPLC[7].

	Cortinarin A *(% w/v)*	*Cortinarin B* *(% w/v)*	*Cortinarin C* *(% w/v)*
C. speciosissimus	0.47 (0.47)	0.60	0.20 (0.20)
C. orellanus	0.42 (0.43)	0.52	0.24 (0.12)
C. orellanoides	0.45 (0.45)	0.47	0.20 (0.19)
C. pinicola	0.19 (0.20)	–	0.028 (0.03)
C. callisteus	0.20 (0.18)	–	0.19 (0.19)
C. turmalis	0.32 (0.33)	–	0.05 (0.043)
C. mucifluus	0.05 (0.06)	–	0.07 (0.076)
C. betuletorum	0.28 (0.28)	–	0.05 (0.04)
C. trivialis	0.10 (0.12)	–	0.06 (0.06)
C. torvus	0.01 (0.012)	–	0.12 (0.15)

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