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Note

Analysis of Cortinarius mushrooms by high-performance liquid chromatography

I. R. TEBBETT and B. CADDY*
Forensic Science Unit, University of Strathclyde, Glasgow (U.K.)
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The toxicity associated with the Cortinarius genus of mushrooms is highlighted by reports of deaths following ingestion of *Cortinarius orellanus* in Poland[1], renal failure caused by consumption of *C. speciosissimus* and *C. gentilis* in Scotland[2] and Finland[3] and a recent case of poisoning by *C. splendens* in France[4]. Cortinarius is the largest genus of fungi in Europe and the majority of the two hundred and fifty or so British species are suspected of being toxic. It seems likely therefore that further examples of accidental Cortinarius poisoning will be encountered in the future. The isolation and part characterisation of a polypeptide kidney toxin (cortinarin A) obtained from *C. speciosissimus* has been carried out in our laboratory and is described elsewhere[5]. Subsequently, methanolic extracts of some 60 species of Cortinarius were screened by thin-layer chromatography (TLC) to determine the presence of this toxin or its analogues[6]. Extracts from all 60 species showed the presence of a single blue fluorescent spot, when examined under ultraviolet light (254 nm), at the same R_F as that of the toxin isolated from *C. speciosissimus*. The intensity of the fluorescent compound in each species was recorded on an arbitrary concentration scale expressed by: (+) weak, (+ +) fairly strong and (+ + +) very strong. All of the samples examined were also found to contain a non-fluorescent compound which reacted with *p*-dimethylaminobenzaldehyde (pDAB) and which showed properties consistent with it being of similar structure to cortinarin A. This compound was called by us cortinarin C. High-performance liquid chromatography (HPLC) offered a rapid technique for the determination of both cortinarins A and C and gave a more satisfactory method of assessing the toxicity of individual mushrooms. Since preliminary structural elucidation of compounds isolated from *C. speciosissimus* suggested that very closely related structures were present, it was considered that a liquid–solid chromatographic method would provide a good separation. Such a method is now described.

MATERIALS AND METHODS

Apparatus

The liquid chromatography system incorporated a Pye Unicam LC3 pump which delivered solvent at 1 ml/min. A Cecil Instruments CE212 variable-wavelength UV detector was used to monitor the eluent at 270 nm, this wavelength being near the λ_{max} for both cortinarin A and C (Fig. 1). The column was a 25 cm × 4.5 mm I.D. Spherisorb 5 μm (Jones Chromatography) fitted with a Negretti and Zambra

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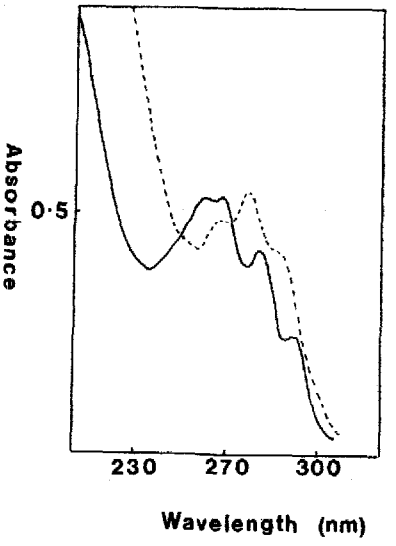

Fig. 1. UV spectra in methanol of cortinarin A (– – –) and cortinarin C (———).

injection system incorporating a 20-μl loop. Separation was achieved with a mobile phase of hexane–ethanol (90:10). All solvents used were either HPLC grade (Rathburn Chemicals) or glass redistilled.

Extraction procedure

Mushrooms were air dried at 40°C for several hours and stored over anhydrous silica gel until used. Powdered samples were accurately weighed (100–500 mg) and

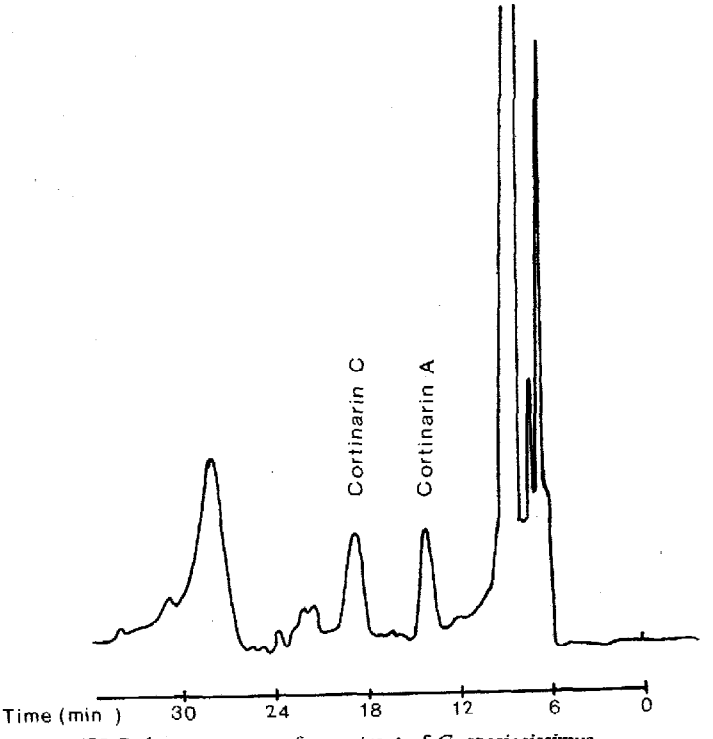

Fig. 2. HPLC chromatogram of an extract of *C. speciosissimus*.

TABLE I

HPLC ANALYSES OF CORTINARIUS SPECIES FOR CORTINARINS A AND C

Concentrations expressed as % dry weight of mushroom.

	Cortinarin A % (w/w)	*Cortinarin C % (w/w)*
Myxacium*		
C. pseudosalor	0.26	0.03
C. mucosus	0.19	0.196
C. elatior	0.05	0.024
C. pinicola	0.20	0.03
C. trivialis	0.12	0.06
C. mucifluus	0.06	0.076
Phlegmacium*		
C. rapaceus	0.04	0.27
C. crocolitus	0.02	0.11
C. subpurpurascens	0.06	0.01
C. caesiocyaneus	0.01	0.36
C. cyanopus	0.04	0.005
C. largus	0.07	0.06
C. melliolens	0.01	0.23
C. porphyropus	0.04	0.03
C. purpurascens	0.125	0.03
C. turmalis	0.33	0.043
C. varicolor	0.20	0.06
C. decolorens	0.08	0.17
C. subtriumphans	0.05	0.03
C. splendens	0.35	0.032
Sericeocybe*		
C. subargentatus	0.05	0.10
C. anomalus	0.01	0.048
C. lepidopus	0.05	0.038
C. tabularis	0.004	0.026
C. myrtillinus	0.018	0.24
C. spilomeus	0.01	0.05
Cortinarius*		
C. violaceus	—	0.08
Leprocybe*		
C. gentilis	0.36	0.03
C. betuletorum	0.28	0.04
C. callisteus	0.18	0.19
C. orellanus	0.43	0.12
C. speciosissimus	0.47	0.20
C. orellanoides	0.45	0.19
Dermocybe*		
C. cinnamomeus	0.064	0.11
C. semisanguineus	0.11	0.09
C. croceofolius	0.004	0.02
C. cinnamomeobadius	0.004	0.03
Hydrocybe/telamonia*		
C. decipiens	0.07	0.20
C. hinnuleus	0.01	0.20
C. torvus	0.012	0.15
C. armillatus	0.01	0.03
C. evernius	0.03	0.01
C. saturninus	0.07	0.09

* These are the seven subgenera of Cortinarius.

Soxhlet extracted with light petroleum (b.p. 40–60°C) for 1 h. This was followed by a similar 3-h methanolic extraction. The methanol fraction was evaporated to dryness under reduced pressure and the residue redissolved in 2 ml of ethanol. Of this extract 20 μl was then injected onto the column. Standard solutions of cortinarins A and C were obtained by preparative TLC using silica gel G plates (20 cm × 20 cm × 0.5 mm) with cyclohexane–ethyl acetate (3:1) as the mobile phase[5]. Cortinarin A was located as a fluorescent streak under UV light at 254 nm (R_F 0.50) and cortinarin C by spraying one edge of the plate with pDAB (R_F 0.31). Both samples were removed from the plate and eluted with ethanol. Straight-line calibration graphs were obtained for both cortinarins A and C based on peak area measurements for concentrations of 25, 100, 250, 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 squares regression equations of $y = 0.3044x + 0.8948$ and $y = 0.4028x - 1.071$ for cortinarin A and cortinarin C, respectively, where y is the peak area and x the corresponding concentration. Correlation coefficients for both analyses had a value of 1. Minimum detectable values showing a signal-to-noise-ratio > 2 were about 200 ng.

The analytical procedure described above has been used for the analysis of 42 different species of cortinarius mushrooms, including examples of each of the seven subgenera of the genus (Table I). The reported toxicity of each species appears to be directly proportional to the concentration of cortinarin A. *C. orellanus* (0.43% w/w), *C. speciosissimus* (0.47% w/w), *C. gentilis* (0.36% w/w) and *C. splendens* (0.35% w/w) have all been responsible for poisonings. Whereas *C. orellanoides* (0.45% w/w)[7], *C. turmalis* (0.35% w/w)[8], *C. betuletorum* (0.28% w/w) and *C. callisteus* (0.18% w/w) are all considered as highly suspect. Only *C. violaceus*, which is listed as being edible, was found to be devoid of cortinarin A. Similar results were obtained on analysis of mushrooms of the same species but collected from different areas and at different times. No correlation could be made between cortinarin C content and toxicity and it seems doubtful whether this compound is indeed toxic. Detailed results of toxicity tests involving both of these compounds together with other cortinarins will be published at a later data.

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