Cortinarins in *Cortinarius speciosissimus*? A critical revision

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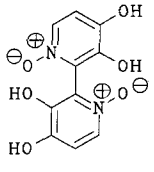
Summary. Fluorescent cyclic decapeptides, the so-called cortinarins, have been claimed to cause the toxicity of the deadly poisonous mushroom *Cortinarius speciosissimus*. As the structure elucidation described in the literature is doubtful in decisive parts, the proposed structures must be disputed. The present study demonstrates that the fluorescence is mainly due to ergosta-4,6,8(14),22-tetraen-3-one (**8**) and to photodecomposition products of the nephrotoxin orellanine (**1**). Moreover, we were not able to reproduce the isolation of fluorescent or any other cyclic peptides related to cortinarins from *C. speciosissimus*, by various methods.
Key words. Cortinarin; *Cortinarius speciosissimus*; *Cortinarius rubellus*; fluorescence; mushroom; steroid; toxin.

Several species within the genus *Cortinarius* are extremely toxic (e.g. *C. orellanus* or *C. speciosissimus*), as several reports relating to lethal poisonings indicate[1]. Characteristic symptoms[2] of the so-called orellanus syndrome include an extremely long latent period (2–12 days) and renal failure, often extending to severe renal damage. Hepatobiliar affection, which is typical of the phalloides-syndrome, is rare[3], and the latency for *Amanita phalloides* ('deadly agaric') intoxication is 8–12 h.

A slow-acting orellanus toxin was first isolated in an impure state by Grzymala[4] in 1962; further purification was achieved by Antkowiak and Gessner[5]. These authors, in cooperation with Steglich[6], identified the toxin as 3,3′,4,4′-tetrahydroxy-2,2′-bipyridyl-bis-N-oxide (**1**). Orellanine, which was also isolated from *C. speciosissimus* (recently renamed in *C. rubellus*), decomposes in light to **2** and **3**, which show blue fluorescence on chromatograms exposed to UV light[7]. The structures of **1**–**3** were verified by synthesis[8,9]. Toxicological studies confirmed the nephrotoxic activity of orellanine[10]. Furthermore, deposition of a substance exhibiting blue fluorescence in tubules of kidneys damaged by the toxin has been demonstrated[11]. Isolation as well as analytical data concerning orellanine were reproduced by different authors[12–14].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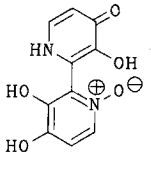
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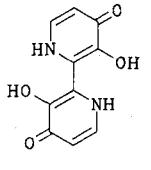
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In 1984 Tebbett and Caddy claimed to have isolated three novel cyclopeptides from *C. speciosissimus* and assigned to them the structures **4b** (cortinarin A), **4a** (cortinarin B), and **5b** (cortinarin C) (fig. 1). The nephrotoxic effect of the mushroom was ascribed to two of these compounds[15]. The investigations were based on results published by Testa[16], which had stated that methanolic extracts of *Cortinarius orellanus* contained fluorescent components of presumed peptide structure.

The cortinarins (**4a, 4b, 5b**) display a structural similarity to the amanitins but contain a 4-hydroxyindole moiety. Until now, the occurrence of 4-hydroxyindoles in nature has only been demonstrated in the case of fungus alkaloids related to psilocin/psilocybin and heteroyohimbines like mitragynine from *Mitragynia speciosa*[17].

Tebbett and Caddy performed their structure elucidation by partial degradation and spectroscopic investigation of the products thereof. Desulfurization of **4b** and TFA-hydrolysis of **5b** resulted solely in cleavage of the peptide bond between Ile and Ala. This strongly resembles the partial hydrolysis of phalloidin but does not take into account the intramolecular assistance of the hydroxy leucine (forming a lactone), which is not present in **5b**. Consequently other authors also questioned the identity of these compounds[6,18].

Therefore, the aim of our studies was to check the structures of the so-called cortinarins by synthesis followed by comparison with isolated material. However, in spite of several attempts using mushroom samples collected in different places over a period of 9 years, the peptide isolation could not be reproduced. Instead, our results indicate that the fluorescence must be attributed to decomposition products of orellanine (**1**), namely orellinine (**2**) or orelline (**3**) – and to a great extent to steroids.

Materials and methods

General: *^{1}H-NMR spectra*: Varian FT-80A, XL 100, 200, 500, VXR 200 spectrometer (tetramethylsilane as internal standard). *Mass spectra*: low resolution: Varian MAT 311 A (70 eV), high resolution: Varian MAT 731 (peak-matching, perfluoro kerosene, resolution 10000). *UV spectra*: Cary 219 (Varian). *Gas chromatography* (GC): Varian 3700 (column: CP Sil-5, 20 m × 0.32 mm; Chrompack). *Thin layer chromatography* (TLC): TLC-sheets 40 × 80 mm and 80 × 80 mm (Polygram SIL G/UV$_{254}$; Macherey-Nagel & Co); TLC-sheets 20 × 20 cm (aluminum oxide 60 F 254, neutral Typ E; Merck);

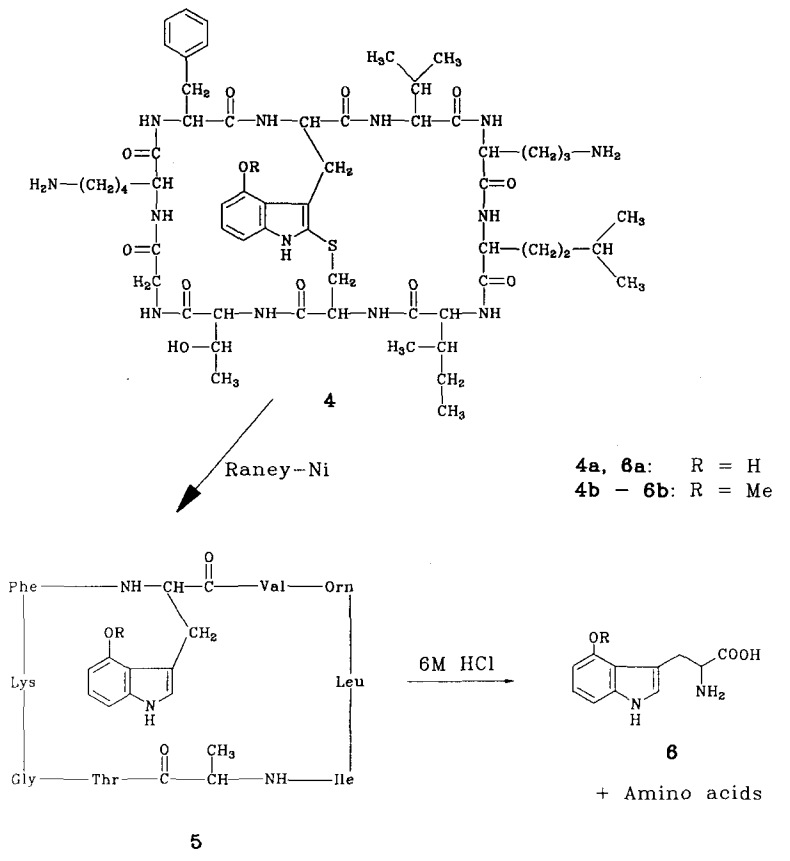


Figure 1. Structure and chemical interconversions of the cyclic decapeptides **4b** (cortinarin A), **4a** (cortinarin B), and **5b** (cortinarin C) according to Tebbett and Caddy[15].

TLC-glass plates 20 × 20 cm (cellulose CEL 300-25; Macherey-Nagel & Co). *Preparative thin layer chromatography* (PTLC): A slurry of 55 g silica gel (silica gel P/UV; Macherey-Nagel & Co) in 120 ml water was poured on glass plates (20 × 40 cm), air-dried and subsequently activated by heating 3 h at 130 °C. *Column chromatography* (CC): aluminum oxide (neutral, Brockmann activity I; Woelm). Columns were packed dry. *HPLC*-apparatus (Knauer; F.R.G.): Two pumps type 364.00 were used to deliver solvent at 0.7 ml/min (3.3 MPa). The eluent was monitored at 266 nm with a variable-wavelength monitor (type 731.87). The column (8 × 250 mm) was packed with 7 µm Lichrosorb RP 18 and was fitted with an injection system (type Reodyne) incorporating a 20 µl loop. Knauer software 1.42C was used. Filtration of samples: S & S disposable filter holders (Spartan 13/30; Schleicher and Schüll Dassel, Germany). All solvents used were HPLC grade. N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was purchased from Fluka Chemical Co.

Compounds for comparison: *4-Hydroxytryptophane* (**6a**) and *4-methoxytryptophane* (**6b**) were synthesized following a sequence of Pasini[19]. – **6a**: ^{1}H-NMR (d-DMSO, 100 MHz): δ = 10.84 (s; 1 H, NH), 7.07 (d, $J_{1,2}$ = 2 Hz; 1 H, 2-H); 6.86 (m; 2 H, 5-, 7-H), 6.38 (dd, J = 5, J' = 4 Hz; 1 H, 6-H), 4.78 (s, br; NH_2, OH), 3.68 (m; 1 H, α-H), 3.5–3.00 (m; 2 H, β-H_2). – UV [(0.1% trifluoroacetic acid (TFA) in acetonitrile/water-azeotrope (16.3% H_2O)]: λ_{max} (lg ε) = 260.8 nm (sh; 3.69), 267.1 (3.96), 280.2 (3.68), 290.1 (3.63).

6b: ^{1}H-NMR (d-DMSO, 80 MHz): δ = 10.95 (s; 1 H, NH), 6.99 (d, $J_{1,2}$ = 2 Hz; 1 H, 2-H); 6.87 (m; 2 H, 5-, 7-H), 6.35 (dd, J = 4.5, J' = 3.75 Hz; 1 H, 6-H), 4.95 (s, br; NH_2, OH), 3.8 (s, 3 H, CH_3), 3.55 (m; 1 H, α-H), 3.5–2.65 (m; 2 H, β-H_2). – UV [(0.1% TFA in acetonitrile/water-azeotrope (16.3% H_2O)]: λ_{max} (lg ε) = 260.1 nm (sh; 3.76), 266.4 (3,80), 282.6 (3.58), 292.1 (3.51).

4-Methoxyindole was prepared according to Repke[17]: ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.96 (s, 3 H, CH_3); 6.53 (dd, $J_{5,6}$ = 7.7 Hz, $J_{5,7}$ = 1.0 Hz; 1 H, 5-H), 6.66 (ddd, $J_{3,2}$ = 3.5 Hz, $J_{3,1}$ = 2.75 Hz, $J_{3,7}$ = 0.9 Hz; 1 H, 3-H), 7.02 (ddd, $J_{7,6}$ = 8.0 Hz, $J_{7,5}$ = 1.0 Hz, $J_{7,3}$ = 0.9 Hz; 1 H, 7-H), 7.11 (ddd, $J_{2,3}$ = 3.5 Hz, $J_{2,1}$ = 2.5 Hz; 1 H, 2-H), 7.12 (dd, $J_{6,7}$ = 8.0 Hz, $J_{6,5}$ = 7.7 Hz; 1 H, 6-H), 8.20 (s, br; 1 H, NH).

^{1}H-NMR (CD_3OD, 500 MHz); δ = 3.90 (s, 3 H, CH_3); 6.47 (dd, $J_{5,6}$ = 7.8 Hz, $J_{5,7}$ = 1.5 Hz; 1 H, 5-H), 6.47 (dd, $J_{3,2}$ = 3.2 Hz, $J_{3,7}$ = 1 Hz; 1 H, 3-H), 6.98 (ddd, $J_{7,6}$ = 8 Hz, $J_{7,5}$ = 1.5 Hz, $J_{7,3}$ = 1 Hz; 1 H, 7-H), 7.00

(dd, $J_{6,5} = 7.8$ Hz, $J_{6,7} = 8$ Hz; 1 H, 6-H), 7.07 (d, $J_{2,3} = 3.2$ Hz; 1 H, 2-H). – ^{1}H-NMR (CD_3OD, 250 MHz)[20]: $\delta = 3.8$ (s, 3 H, CH_3), 7.4 (m, aromatic H), 7.7 (s, 1 H, NH).

MS (70 eV): $m/z = 147$ (100%; M^+), 133 (7), 132 (80), 116 (11), 104 (43), 89 (9), 76 (8), 77 (12), 51 (10).

R_f value (BuOH/HOAc/H_2O 4:1:1) found 0.96, reported[20] 0.35.

Steroids in fluorescent fractions

Extraction and separation: Dried *Cortinarius speciosissimus* (160 g) was powdered and extracted in a Soxhlet apparatus with petroleum ether (40–60 °C; 1 h). After evaporation of the solvent, the residue (3.6 g) was redissolved in a minimum volume of dichloromethane and separated by PTLC (cyclohexane/25% ethyl acetate). An intense blue-fluorescent band ($R_f = 0.5$) was located in UV_{366} and immediately eluted with dichloromethane/2% methanol. From this concentrate, fatty acids were separated by filtration over aluminum oxide using dichloromethane/2% methanol as the eluent.

Identification: A dichloromethane solution (1 µg/µl) of the sterol mixture obtained as described was evaporated in a capillary tube. Sodium acetate (1 crystal), 3 µl of MSTFA and 3 µl of pyridine, were then added, and the tube sealed and heated (12 h, 60 °C).

GC/MS (70 eV, 200 °C, 5 °C/min): *Ergosta-4,6,8 (14),22-tetraen-3-one* (**8**), $t_R = 16.2$ min (key fragments of the ring system in italics): $m/z = 392.3081$ (30%; M^+, cal. 392.30790 for $C_{28}H_{40}O$), *269* (18), *268.1827* (100; cal. 268.18243 for $C_{19}H_{24}O$), *267.1748* (62; cal. 267.17497 for $C_{19}H_{23}O$), 253 (14), 240 (4), 214 (20), 207 (6), *173* (12), 155 (6), *109* (15), 95 (7), 81 (20), 79 (20).

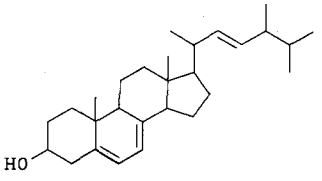
22,23-Dihydroxyergosta-4,6,8 (14)-trien-3-one, mono-TMS-ether (**9**), $t_R = 16.3$ min: $m/z = 498$ (53%; M^+), 483 (24; $M^+ - CH_3$), 394 (15), 393 (67), 392 (8), 281 (4), *269* (4), *268* (45), *267* (37), 227 (4), 207 (19), *173* (10), 148 (8), 135 (19), 121 (12), *109* (34), *95* (25), *79* (100), 73 [34; $Si(CH_3)_3^+$].

(3β)-24-Methylenlanost-8-en-3-ol, TMS-ether (**10**), $t_R = 17$ min): $m/z = 512$ (100%; M^+), 497 (32; $M^+ - CH_3$), 408 (30), 407 (88), 323 (8), 283 (8), 281 (9), 255 (8), 241 (18), 227 (12), 229 (10), 215 (9), 207 (14), 289 (12), 287 (14), 175 (9), 173 (10), 163 (12), 161 (13), 149 (9), 147 (18), 145 (8), 135 (29), 133 (16), 109 (46), 107 (19), 97 (27), 95 (39), 92 (19), 82 (40), 81 (43), 73 (80).

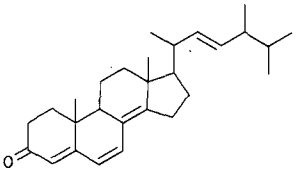
For further purification of **8**, the steroid mixture was applied to a 1 × 12 cm column of aluminum oxide. The column was eluted with dichloromethane and the strongly fluorescent fractions collected. **8** was obtained as a viscous, yellowish oil. – ^{1}H-NMR ($CDCl_3$, 200 MHz): $\delta = 6.63$, 6.05 (2 d, $J_{6,7} = J_{7,6} = 10$ Hz, each 1 H, 6-, 7-H), 5.75 (s, 1 H, 4-H), 5.24 (m, 2 H, 22-, 23-H), 1.29–0.80 (m, 18 H, 6 CH_3), 1.30–2.5 (m, 17 H, steroid skeleton-H).

Indirect essay of cortinarins via 4-substituted tryptophanes

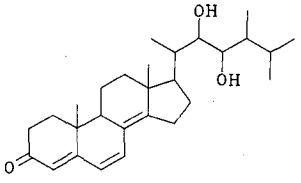
Peptide enrichment: Dried mushroom powder (11.8 g), previously defatted with petroleum ether was exhaustively extracted (2 × 3 h) with methanol in a Soxhlet apparatus. The extracts were evaporated to dryness under reduced pressure, the residue (total amount 5.7 g) redissolved in distilled water (100 ml) and acidified with 2 N HCl to pH 2.2. After separation of insoluble substances by centrifugation, the solution was treated with several portions of Amberlite IR 118/20–50 mesh (H^+ form) until the supernatant was free of substances reacting with cinnamaldehyde/HCl or ninhydrin. The resin was applied to a 25 × 3 cm column and washed twice with water. The absorbed substances were subsequently eluted with a) 200 ml 0.15 N NaOH, b) 200 ml 0.3 N NaOH and c) 200 ml 2 N NaOH in 50 ml-fractions. Fractions that were positive in spot tests with cinnamaldehyde/HCl or ninhydrin (1–8, pH = 4–14, 400 ml) were pooled and then neutralized with 2 N HCl. After evaporation under reduced pressure, the residue was redissolved in methanol and filtered from precipitated NaCl. In this way 3.7 g of a fraction A enriched with amino acids and peptides was obtained.



7 (Ergosterin)



8 (Ergosta-4,6,8(14),22-tetraen-3-on)



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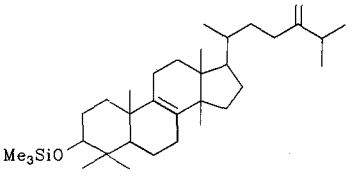


10 (Eburicol-TMS)

Hydrogenation and hydrolysis of the peptide fraction: A part of fraction A (3.0 g) was treated with Raney nickel (12 g) in boiling methanol (120 ml) for 2 h. The catalyst and undissolved organic remainder were filtered off; the filtrate was divided into two portions and evaporated (40 °C, 15 Torr; A_1 = 1200 mg, A_2 = 560 mg), followed by addition of thioglycollic acid (0.5 ml) and 6 N HCl (3 ml). To A_2 were added 0.84 mg 4-OH-Trp (**6a**) and 1.09 mg 4-OMe-Trp (**6b**). A_1 (unchanged amino acid profile) and A_2 were heated in sealed tubes under nitrogen atmosphere (110 °C, 6 h), subsequently concentrated under nitrogen (70 °C) and finally dried in vacuo (10^{-3} Torr, 40 °C).

Chromatography of hydrolysates: Each of the dried residues of hydrolysates A_1 and A_2 was redissolved in 10 ml of acetonitrile/water azeotrope (16.3% H_2O) with ultrasonic vibration and filtered. A 1-ml aliquot of each solution was subjected to PTLC using cellulose plates and dioxane/20% water. Synthetic **6a** and **6b** were co-chromatographed at the edge of the plates for comparison. Zones with the chromatographic properties of these references (R_f = 0.35, 0.31) were scraped off and eluted with 5% formic acid. After evaporation of the solvent, the residue was redissolved in acetonitrile/water azeotrope and prepared for HPLC analysis by filtration.

HPLC-analysis: Separation of **6a** and **6b** was achieved with a 50/50 mixture of acetonitrile/water-azeotrope (16.3% H_2O) and 0.2% TFA in H_2O, the column was pre-equilibrated with 0.1% TFA in H_2O (flow rate: 0.7 ml/min (3.3 MPa); detection at 266 nm). Retention times: **6a** 3.4 min, **6b** 3.85 min, tryptophane: 3.58 min. Absorption of phenylalanine (3.08 min), tyrosine (3.03 min) and histidine (3.28 min) under the given conditions was negligible.

The compounds **6a** and **6b** resisted treatment with HCl (pH 2.2) or 5% formic acid at least for 120 min (room temperature). **6b** proved to be stable in 25% NH_4OH for 90 min, whereas ca 70% of **6a** changed into three main decomposition products. Hydrolysis of pure **6a** and **6b** in 6 N HCl under nitrogen (without thioglycollic acid) led to >70% decomposition.

Results and discussion

Isolation and identification of fluorescent compounds: According to the original method[15], the dried mushroom was freed of apolar compounds by extraction with petroleum ether prior to extraction of the active substance with methanol, because Tebbett and Caddy attributed the highest biological activity to the fluorescent zones of the methanolic extract.

On reinvestigation, using cyclohexane/25% ethyl acetate for TLC we found three compounds showing the R_f-values and color reaction with acidic ninhydrin described by Testa[16] for his grzymalin [zone I, presumably identical to Tebbett's[15] cortinarin A (**4b**)], benzonin [zone II, presumably identical to cortinarin C[15] (**5b**)] and cortinarin [zone III, unidentified, but not a peptide]. These compounds were concentrated mainly in the petroleum ether extract and exhibited an intense, blue fluorescence; this result is not consistent with the presence of peptides. In addition, on direct chromatographic comparison, the structurally related peptides α-amanitin and phalloidin remained at the starting point whereas for **4b** and **5b** R_f-values of 0.5 and 0.3 respectively were reported[20]. The methanolic extract, however, contained only traces of fluorescent compounds with the R_f-values of zone I–III. In the petroleum ether and in the methanolic extract, the compounds in zone I were identical, as confirmed by mass spectroscopy.

In UV_{366}, zone I and III showed blue fluorescence. Zone II, however, first exhibited quenching of fluorescence (254 nm); the intrinsic fluorescence described by Testa became evident after about one hour. The same result was found by Gamper[21]. 2D-TLC showed that the fluorescent compound from zone II was identical to I, which therefore appears to be reformed during the isolation procedure (fig. 2).

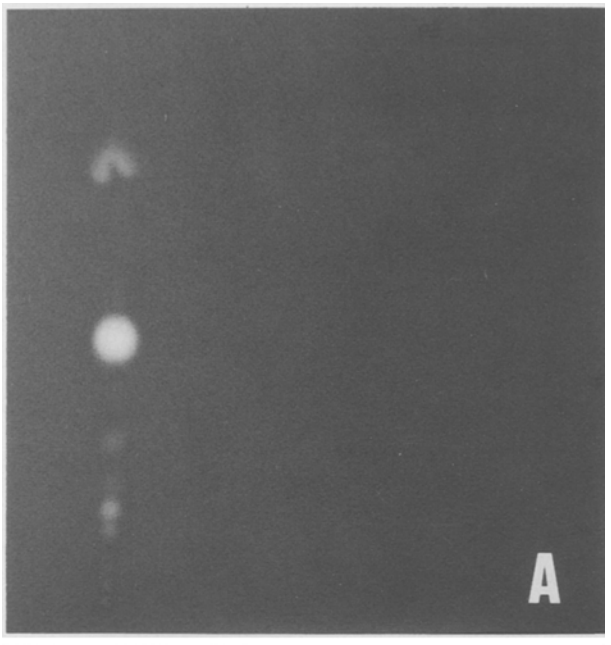
After PTLC in the absence of light, component II crystallized from ethanol/water as colorless platelets. It was identified as ergosterol (**7**) by IR and ^{1}H-NMR spectroscopy and comparison with an authentic sample. In chromatograms, **7** produces a blue color after spraying with 2 N or 6 N HCl and subsequent heating. Ergosterol (**7**) gives a red to brown color with acidified ninhydrin solution. Obviously this property has led to its erroneous assignment as a peptide[16].

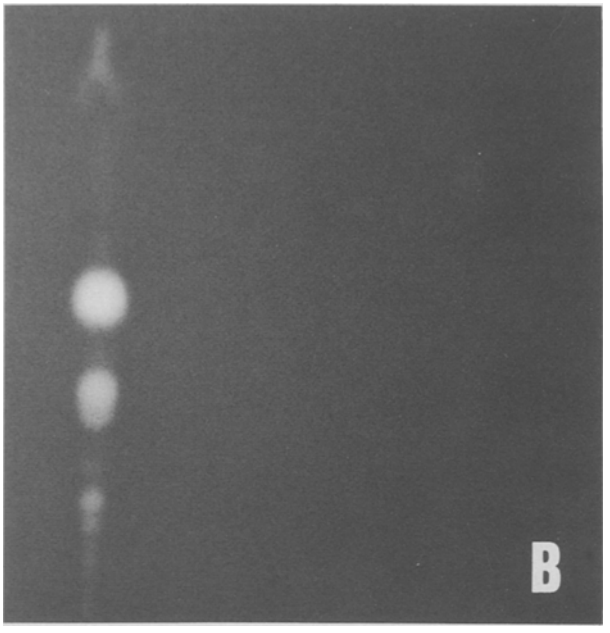
Component I was purified by chromatography on silica gel and aluminum oxide. GC/MS of a silylated sample revealed a mixture of three substances, namely: ergosta-4,6,8(14),22-tetraen-3-one (**8**), the mono-TMS-ether of 22,23-dihydroxyergosta-4,6,8(14)-trien-3-one (**9**) and (3β)-24-methylenlanost-8-en-3-ol as TMS-ether (eburicol-TMS, **10**).

Compound **9** seems to be new[22]. It gave the same key fragment (cleavage between the steroid ringsystem and side chain) as **8**. As several other fragments are also identical, we postulate the same ring system for both compounds. Possibly **9** results from **8** via the corresponding epoxide after hydrolytic cleavage. As the substances appear together, isomeric skeletons are not likely.

Pure **8** was prepared by column chromatography on aluminum oxide. Its ^{1}H-NMR-spectrum proved to be identical with a sample isolated from *Laricifomes officinalis* ('*Fungus laricis*') according to Fachmann et al.[23]. Compound **8** is in fact responsible for the intense blue UV-fluorescence of extracts of both fungi.

The component of zone III gave a grey-blue color with Ehrlich reagent, but acidic hydrolysis did not yield amino acids; it was therefore not examined further. The weakly fluorescent photo-products **2** and **3** of orellanine (**1**) are – as expected – located in the methanolic extract, and remained on TLC (cyclohexane/25% ethyl acetate) at the starting point.





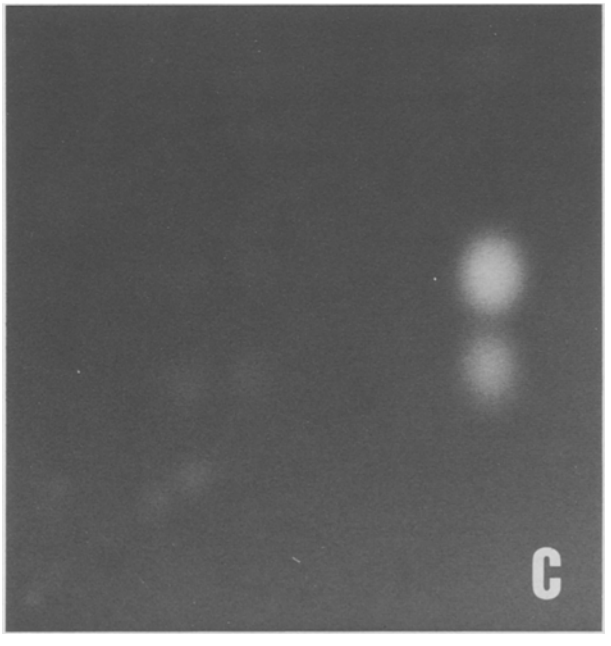


Figure 2. Chromatograms (cyclohexane/25% ethyl acetate) of *C. speciosissimus* petroleum ether extract in UV_{366}. Immediately after development (*A*): Strongly fluorescent spot = ergosta-4,6,8(14),22-tetraen-3-one (**8**, $R_f = 0.5$). Ergosterol (**7**, $R_f = 0.3$) exhibits quenching of fluorescence in UV_{254} and is therefore not visible in this chromatogram. After 1 h exposure to UV light (*B*): A new fluorescent spot appears at the R_f-value of **7**. 2nd dimension (*C*): Both fluorescent spots show identical chromatographic behavior; fluorescent **8** is reformed from non-fluorescent ergosterol (**7**).

Analysis to find cortinarins in methanolic extracts: As described above, if cortinarins are present, they should only be located in the weaker fluorescent methanolic extracts. Since authentic samples were not available, in spite of substantial efforts[24,18], we tried to trace cortinarins indirectly by identifying products of degradation, namely 4-OH-Trp (**6a**) and 4-OMe-Trp (**6b**).

Tebbett and Caddy[15] performed the cleavage of the sulphur bridge in cortinarin A by hydrogenation, yielding **5b**, which was subsequently hydrolyzed, generating 4-OMe-Trp (**6b**). After purification by Sephadex column chromatography followed by incubation with tryptophanase, 4-methoxyindole was liberated and identified by ^{1}H-NMR and UV.

It is, however, not clear from the report whether the hydrolysis of **5b** (20 mg) 'by refluxing with hydrochloric acid' was performed in the presence of antioxidants in an inert atmosphere. The instability of Trp and 6-OH-Trp in strong acids has been extensively documented[25,26]. Nevertheless, in this way 5 mg of **6b** and, after enzymatic cleavage, 3 mg of 4-methoxyindole were obtained, which exceeds the theoretical yield for each step.

Authentic 4-methoxyindole, on the other hand, showed completely different chromatographic behavior in the solvent system reported. The ^{1}H-NMR data for 4-methoxyindole, presented in the original literature[20] are also inconsistent with our measurements. Signals for 3-H and 5-H are missing completely and for 1-H (NH) – according to the illustration[20] – a sharp singlet is indicated ($\delta = 7.7$). This signal is unexpected, or should be very broad at least, because of the rapid H/D exchange in CD_3OD. Indeed, we could detect it only by measuring in $CDCl_3$ as the solvent. The chemical shift and splitting pattern of the aromatic signals exhibit other differences (for details see materials and methods), indicating that the structure assignment is incorrect.

According to our method, corresponding to standard procedures, the methanolic extract was processed in order to isolate amino acids and peptides by cation-exchange chromatography. On this occasion, conditions

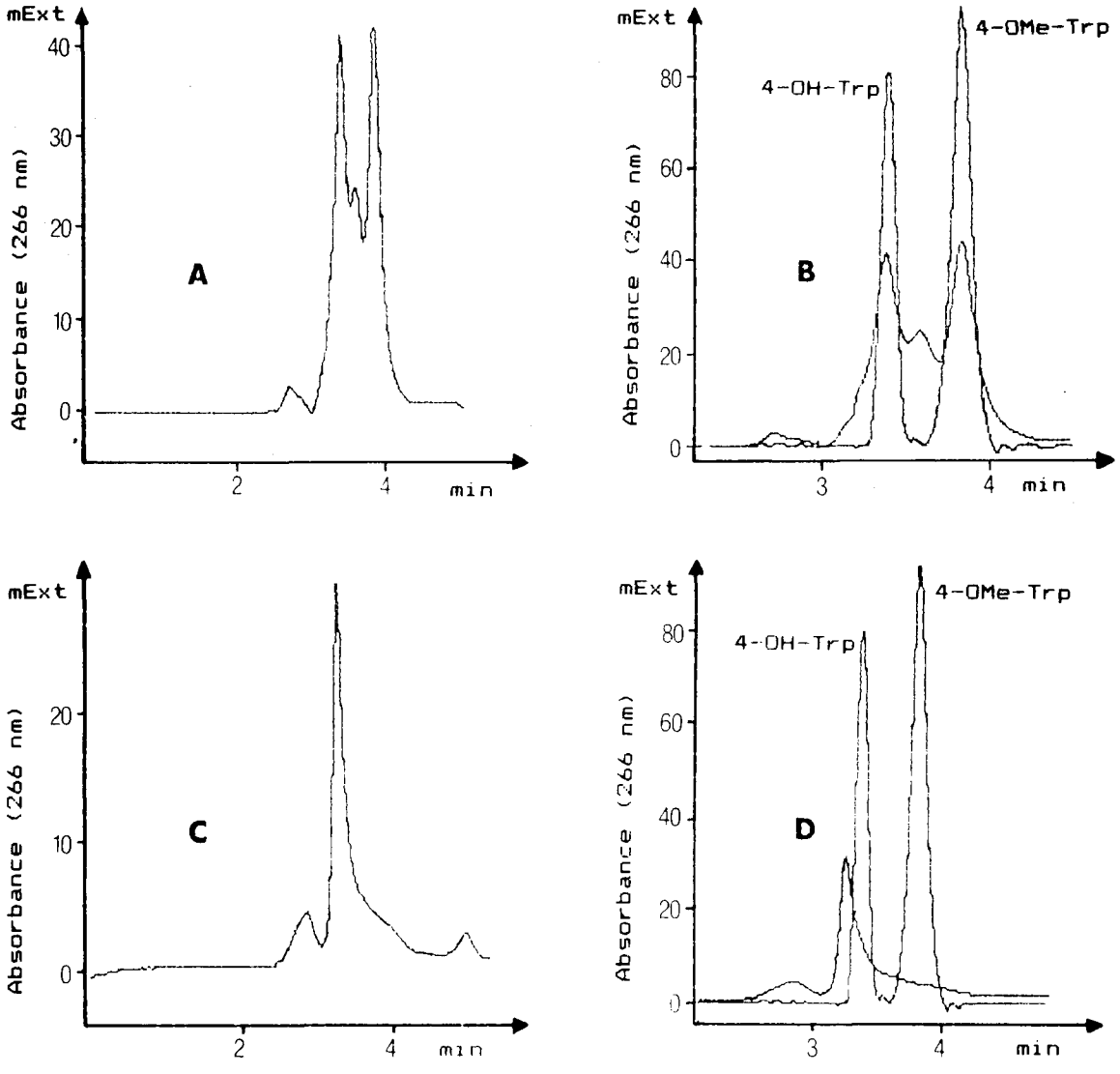


Figure 3. HPLC analysis of purified hydrolysates. Methanolic *Cortinarius speciosissimus* extracts were processed in order to liberate 4-OH-Trp (**6a**) and 4-OMe-Trp (**6b**) from cortinarins. One hydrolysis experiment was performed after addition of synthetic **6a** and **6b** (fig. 3 A, recovery 45% and 14% resp.). The identity of the tryptophanes **6a**/**6b** is shown by the superimposition of *A* with a chromatogram of the pure amino acids (*B*). In authentic hydrolysates no **6a**/**6b** could be detected (*C*, *D*).

were chosen in such a way that also amatoxins from *Amanita phalloides* were absorbed, and these were used for comparison in preliminary experiments. Under these conditions adsorption of cortinarins should also occur, since they are far more basic than the amatoxins owing to their content of lysine and ornithine.

By this ion-exchange chromatography, accompanying sugars were removed. This is important, because during acidic hydrolysis of proteins glucose decreases the rate of recovery of tryptophane even under reducing conditions[27].

Peptide-containing extracts were enriched by cation exchange chromatography, desulphurized with Raney nickel and hydrolyzed. Hydrolysates were purified on cellulose and analyzed with HPLC.

According to reported values for cortinarins[15] (A: 0.47%; B: 0.60%; C: 0.20% of *C. sp.* dry weight), 0.11% 4-OH-Trp and 0.13% 4-OMe-Trp were expected after reductive work-up and hydrolysis as described above. In sample A_1, both amino acids were absent (expected amounts were 7.40 mg **6a**, 8.70 mg **6b**). Only in fraction A_2, where **6a** and **6b** were added as references, were they detectable (found 0.38 mg **6a**, recovery 45%; 0.15 mg **6b**, recovery 14%).

Hence, we did not succeed in detecting cortinarins. If cortinarins had been present, the original concentrations of cortinarins A–C in *C. speciosissimus* cannot have exceeded a maximal concentration of 0.02–0.04% in the dry mass, if we consider potential losses during the work-up and the detection limit for **6a**/**6b**. This statement con-

tradicts data given by Tebbett and Caddy who found amounts more than 20-fold higher.

Conclusion

Our analysis of the fluorescent zones with the chromatographic behavior of the so-called cortinarins revealed only steroids. We were unable to find any substance related to cortinarins in any extract of mushrooms from various locations collected over a period of nine years. As, in addition, the structure elucidation [15] of **4b**/**5b** is doubtful in decisive parts, the structure assignment of Tebbett and Caddy must be incorrect.

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Announcements

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First Announcement and Call for Papers

Plant-animal interactions. Besides contributions to the main topic of the congress, which will be published, other papers on all aspects of tropical ecology are welcome.

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