Calostomal, a Polyene Pigment from the Gasteromycete *Calostoma cinnabarinum* (Boletales)

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Z. Naturforsch. **2007**, *62b*, 129 – 131; received August 16, 2006

The North American stalked puffball *Calostoma cinnabarinum* (Boletales) owes its red-orange colour to the heptaene pigment calostomal. Its structure has been determined by ^{1}H and ^{13}C NMR spectroscopy of the corresponding methyl ester as all-*trans*-16-oxohexadeca-2,4,6,8,10,12,14-heptaenoic acid (**1**). Neither pulvinic acids nor other typical Boletales pigments could be detected in this fungus. The structural relationship of **1** to other polyene pigments from fungi is discussed.

Key words: Calostoma, Boletales, Polyenes, Mushroom Pigments, Chemotaxonomy

Introduction

Calostoma cinnabarinum Corda (Stalked Puffball-in-aspic) is a striking red-orange puffball widespread in the eastern USA and Asia. It is easily recognized by its gelatinous transparent exoperidium positioned on a stalk, which is soon disrupted by the developing spore sac, exposing the red inner parts. The phylogenetic position of *Calostoma* has recently been clarified by comparison of nuclear and mitochondrial ribosomal DNA sequences [1, 2]. The results of these investigations indicate a close relationship of *Calostoma* with certain genera of the order Boletales, especially *Gyroporus, Scleroderma* and *Pisolithus* (*Sclerodermatineae* Binder & Bresinsky). Since fungi of this order are chemotaxonomically well defined by the occurrence of hydroxylated pulvinic acids and biosynthetically related shikimate-derived pigments [3 – 5], we became interested in an investigation of the *Calostoma* pigments.

Results and Discussion

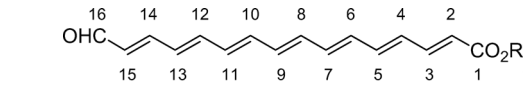
For our studies, air-dried fruit bodies of *C. cinnabarinum* were repeatedly extracted with ethyl acetate. Evaporation of the bright yellow extracts yielded the crude pigment as an orange solid, sparingly soluble in organic solvents. A second fraction of this compound could be obtained by methanol extraction of the residue remaining from the treatment with ethyl acetate. HPLC analyses revealed that both extracts contain one major pigment, named calostomal (**1**), which exhibited UV/vis maxima at λ_{max} = 444 and 422 nm. The IR spectrum of the pigment showed strong absorptions in the C=O/C=C region at ν = 1694, 1665, 1639, 1620 and 1588 cm^{-1}. The EI MS of calostomal exhibited a molecular ion at *m/z* = 256 corresponding to the molecular formula $C_{16}H_{16}O_3$. A fragment ion peak at *m/z* = 227 [$M^+ - 29$] indicated the presence of an aldehyde group.

Table 1. NMR data of compound **2** in $CDCl_3$ at 290 K.

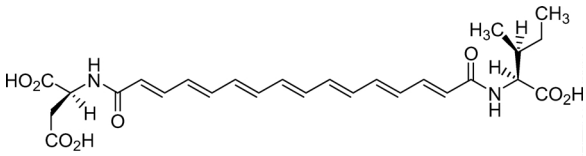
No	δ_H (multiplicity, *J* in Hz)	δ_C
1	–	167.4
2	5.84 (d, 15.3)	120.9
3	7.26 (dd, 15.3, 11.3)	144.3
4	6.31 (dd, 14.6, 11.3)	133.0
5	6.55 (dd, 14.6, 11.3)	140.4
		133.9*
6,7,8,9	6.35 – 6.40 (m)	134.7*
		136.7*
		138.3*
10	6.47 (dd, 15.0, 11.3)	130.7
11	6.34 (m)	135.8
12	6.65 (dd, 14.6, 11.3)	142.3
13	6.42 (dd, 15.0, 11.3)	131.1
14	7.07 (dd, 15.0, 11.3)	151.4
15	6.10 (dd, 15.0, 8.0)	131.3
16	9.51 (d, 8.0)	193.3
CH_3	3.68 (s)	51.6

* Assignments interchangeable.

Because of its low solubility, calostomal (**1**) was converted into the lemon yellow methyl ester by treatment with diazomethane. The ^{1}H NMR spec-



1, R = H
2, R = CH_3



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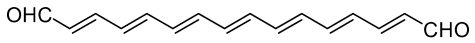

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trum of calostomal methyl ester (Table 1) exhibited a methyl singlet at δ_H = 3.68, a doublet for an aldehyde proton at δ_H = 9.51 (J = 8.0 Hz) and ten well separated multiplets for olefinic methine protons with coupling constants of J = 14.6–15.3 Hz, indicating an all-*trans*-relationship. Four additional methine groups appeared as an overlapping multiplet. From these data calostomal methyl ester could be defined as methyl (all-*trans*)-16-oxohexadeca-2,4,6,8,10,12,14-heptaenoate (**2**). The structure is supported by the ^{13}C NMR spectrum (Table 1), which showed signals for the ester methyl group at δ_C = 51.6, fourteen olefinic methine carbons between δ_C = 120.9 and 151.4 and the ester and aldehyde carbonyls at δ_C = 167.4 and 193.3, respectively. Calostomal (**1**) is a new compound, whereas the methyl ester **2** has been reported before as an intermediate in the total synthesis of a *Xanthomonas* pigment [6].

In conclusion, none of the typical Boletales pigments like pulvinic acids, pulvinic acid dimers, cyclopentenones or grevillins [3] could be detected in *C. cinnabarinum*. Calostomal (**1**) is structurally closely related to the polyene part of the boletocrocins (*e. g.* **3**), a unique group of amino acid conjugates responsible for the bright colour of *Boletus laetissimus* and *B. rufo-aureus* [7]. In addition, the 2*H*-azepine alkaloids of the bolete *Chalciporus piperatus* are formed from an aminoketone precursor derived from a polyunsaturated heptaketide chain [8]. The chemotaxonomic relevance of these findings for the inclusion of *Calostoma* into the order Boletales awaits further investigations. Verpacrocin (**4**), the dialdehyde corresponding to calostomal, has been isolated from cultures of the Ascomycete *Verpa digitaliformis* [9].

Experimental Section

General

TLC: System A: Silica gel 60 F_{254} aluminum foils (Merck); toluene/HCO_2Et/HCO_2H, 10 : 5 : 3 (v/v/v); system B: RP-18 plates (Merck), MeCN/H_2O/HCO_2H, 70 : 100 : 0.1 (v/v/v). HPLC: Waters 600 E Pump and System Controller with Photodiode Array Detector 990+. Macherey & Nagel Vertex columns 4 × 250 mm, packing material Nucleosil 100 C18, 5 μm. Eluent A: H_2O/MeCN 9 : 1 + 0.5% TFA; eluent B: MeCN. Linear gradient: 0 min: A 100%, 50 min: B 100%, 60 min: B 100%; flow rate 1 mL/min, detection range 200 – 600 nm. UV: Hewlett-Packard 8452 A-Adiode spectrophotometer. IR: Bruker FT IR spectrophotometer IFS 45. NMR: Bruker AMX 600 spectrometer (^1H at 600.13, ^{13}C at 150.93 MHz), chemical shifts in δ rel. to $CDCl_3$ (δ_H = 7.26, δ_C = 77.7) as internal standard. HR EIMS: Finnigan MAT 95Q instrument using EI at 70 eV.

Fungal material

Calostoma cinnabarinum was collected in August 1996 near Coweeta Hydrologic Station, Otto, North Carolina, USA (leg. et det. N. Arnold). The fresh fungi were gently dried in a stream of air.

Extraction and isolation

The air-dried fruit bodies were separated from the stalks. The resulting material (7.5 g) was then ground and extracted with 800 mL portions of EtOAc until the extracts remained colourless. All procedures were carried out under argon in the dark. The combined solutions were concentrated under reduced pressure at 20 °C to a volume of 600 mL and then repeatedly washed with H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was triturated several times with hexane and hexane/EtOAc (1 : 1, v/v) to yield an orange-yellow solid (8.6 mg), sparingly soluble in common solvents. After the EtOAc extraction, the remaining fungal material was still red. It was mixed with sea sand, ground with a pistil and extracted exhaustively with 400 mL portions of MeOH. The solvent was removed under reduced pressure at 20 °C and the residue dissolved in EtOAc. Treating this solution as described above yielded an additional sample (5.8 mg) of calostomal (**1**) as an orange-yellow solid. Combined yield 14.4 mg (0.19% of dry weight); TLC (system A): R_f = 0.37, blue-green colour with conc. H_2SO_4; HPLC (RP-18): R_t = 14.6 min. – UV/vis (MeOH): λ_{max} (lg ε) = 248 (5.77, sh), 290 (5.70), 304 (5.70), 416 (6.26), 437 (6.25) nm. – IR (KBr): ν = 3430 (s, br), 2923 (m), 2853 (m),

1800 (w), 1694 (w), 1665 (m), 1639 (m), 1620 (m), 1588 (m), 1540 (w), 1386 (w), 1146 (ss), 1112 (m), 1013 (m) cm^{-1}. – EIMS, *m/z* (%) = 256 (100) $[M]^+$, 227 (10) $[M-CHO]^+$, 213 (48), 185 (22), 171 (18), 149 (25), 129 (35), 115 (21), 97 (19), 85 (24), 73 (87), 71 (27), 69 (25), 61 (18), 60 (56), 57 (57), 55 (33).

*Methylation of calostomal (**1**)*

1 (5 mg) was suspended in MeOH (2 mL) under argon and stirred in an ice bath. Then a solution of diazomethane in Et_2O was added dropwise until the gas production ceased (*ca*. 6 mL). The stirring was continued at 0 °C for additional 30 min, resulting in a clear solution. After addition of a few drops of AcOH, the solvent was carefully removed under reduced pressure. The remaining solid was suspended in H_2O and extracted with $CHCl_3$. The yellow organic phase was dried (Na_2SO_4) and concentrated to yield calostomal methyl ester (**2**) (4.1 mg, 78 %). TLC (system B): R_f = 0.80, blue-green colour with conc. H_2SO_4. – See Table 1 for NMR data. – EIMS: *m/z* (%) = 270 (100) $[M]^+$, 211 (5) $[M-CO_2CH_3]^+$, 209 (5) $[M-CHO-CH_3OH]^+$, 181 (10), 169 (5), 167 (10), 166 (7), 165 (11), 153 (8), 141 (13), 129 (17), 128 (14), 117 (14), 116 (8), 115 (20), 105 (9), 91 (25). – HR EIMS: *m/z* (%) = 270.1257 (calcd. 270.1256 for $C_{17}H_{18}O_3$.

Acknowledgements

We thank Dr. Norbert Arnold, Halle, for mycological assistance and Dr. Peter Spiteller for NMR measurements. The work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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