DOI: 10.1002/ejoc.200900718

Cristatomentin, a Green Pigment of Mixed Biogenetic Origin from *Albatrellus cristatus* (Basidiomycetes)

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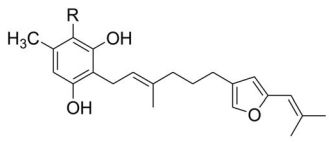
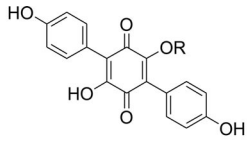
Keywords: Natural products / Meroterpenoids / Quinones / Furans / Fungi

The structure of cristatomentin (**5**), the green pigment of the toadstool *Albatrellus cristatus*, has been elucidated by MS studies and a comparison of the NMR spectrum of **5** with the data for cristatic acid (**1**) and albatrellin (**6**). The furylbenzoquinone chromophore of **5** may be derived from **1** and 2-*O*-acetylatromentin (**4**), which co-occur with cristatomentin in the fungus.

Nitrogen-free, green pigments are rare in nature.[1] In the course of our investigations of mushroom pigments, we became interested in the green tint of fruit bodies from *Albatrellus cristatus* (Schaeff.) Kotl. & Pouzar (German: Grüner Kammporling), a terrestrial polypore easily recognized by its yellowish green pileus. In a previous investigation, we isolated cristatic acid (**1**) as a major colourless constituent from this toadstool.[2] The compound exhibited remarkable cytotoxic activities. With the exception of the reddish brown atromentin (**3**),[2,3] the pigments of *A. cristatus* remained unknown. We now report the identification of 2-*O*-acetylatromentin (**4**)[4] as a yellow pigment of *A. cristatus* and the structural elucidation of cristatomentin (**5**), the compound responsible for the green colouration of fresh fruit bodies.



cristatic acid (**1**): R = CO_2H
cristatin (**2**): R = H



atromentin (**3**): R = H
2-*O*-acetylatromentin (**4**): R = Ac

For the isolation of the pigments, the powdered, freeze-dried toadstools were first extracted with hexane to remove cristatic acid (**1**). Extraction of the residue with acetone and gel chromatography of the pigments on Sephadex LH-20 followed by chromatography on acetylated polyamide-6 afforded 2-*O*-acetylatromentin (**4**), identified by comparison with an authentic sample.[4b] The more polar cristatomentin (**5**) and atromentin (**3**) were extracted with methanol and separated by repeated chromatography on Sephadex LH-20. Cristatomentin was finally obtained in pure form by reversed-phase MPLC. The pigment gave a lime-green solution in chloroform,[5] which exhibited UV/Vis absorptions at λ_{max} = 413 and 615 nm. In methanol the maxima were shifted to 256, 400 and 577 nm.

The ^{1}H NMR spectrum of cristatomentin contains the typical signals of cristatic acid (**1**) (Table 1) except that of the α-proton at the furan ring, which allowed the assignment of partial structure **A** (Figure 1). As indicated in Figure 1, several of the signals in the meroterpenoid part of **5** experience distinct upfield shifts, pointing to shielding of the corresponding protons by the aromatic portion of the molecule. Partial structure **A** is supported by the ^{13}C NMR spectrum, which exhibits all 23 carbon signals and matches the corresponding signals for the terpenoid part of albatrellin (**6**), a blue pigment from *Albatrellus flettii*[6] (Table 1). The NMR spectroscopic data for the orsellinic acid terminus are in excellent agreement with the corresponding data for cristatic acid (**1**).[2c]

In addition to the signals for partial structure **A**, the ^{1}H NMR spectrum of cristatomentin indicates the presence of two AA′BB′ systems at δ_H = 6.73/7.02 and 6.87/7.37 ppm (J = 8.8 Hz), which can be assigned to two 4-hydroxyphenyl residues. This suggests a structural relationship with atromentin (**3**), supported by signals for two quinone carbonyl signals at δ_C = 184.5 and 186.1 ppm in the ^{13}C NMR spectrum and the base peak at m/z = 310 [$C_{18}H_{14}O_5$] in the high-resolution EI-MS, which corresponds to the leuco form of 2-deoxyatromentin (**10**)[7] (see below). Since the NMR spectroscopic data exclude a connection of partial structure **A** with the aromatic part of the molecule through one of the 4-hydroxyphenyl rings, the second half of crista-

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Table 1. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data for the meroterpenoid part of cristatomentin (**5**), cristatic acid (**1**),[2c] and albatrellin (**6**).[6a]

Position	**5** (^{1}H NMR)[a]	**1** (^{1}H NMR)[b]	**6** (^{1}H NMR)[c]	**5** (^{13}C NMR)[a]	**1** (^{13}C NMR)[b]	**6** (^{13}C NMR)[c]
1′	–	–	–	164.4	164.6	154.7
2′	–	–	–	113.2	113.9	110.3
3′	–	–	–	159.8	160.9	154.7
4′	–	–	6.18 s	106.1 br.	105.7	108.9
5′	–	–	–	142.4	141.9	137.4
6′	6.34 s	6.19 s	6.18 s	110.9	111.3	108.9
7′	2.45 s	2.47 s	2.18 s	24.1	24.3	21.1
8′	3.29 d	3.25 d	3.32 d	22.6	22.7	22.1
9′	5.24 t	5.22 t	5.17 tm	124.2	124.8	122.1
10′	–	–	–	134.3	134.7	137.9
11′	1.85 t	1.98 t	obscured	40.2	40.1	39.1
12′	1.38 m	1.63 tt	1.64 m	28.5	29.2	27.8
13′	1.93 t	2.30 t	2.21 t	25.7	24.9	25.4
14′	–	–	–	130.2	127.8	131.3
15′	6.11 s	6.02 s	6.17 s	111.7	110.0	110.9
16′	–	–	–	157.9	154.9	154.9
17′	5.88 m	5.96 m	6.00 m	115.0	115.8	114.1
18′	–	–	–	136.5	135.0	137.3
19′	1.80 s	1.86 s	1.89 s	27.1	27.0	27.2
20′	1.74 s	1.94 s	1.95 s	20.2	20.1	20.3
21′	–	7.06 s	–	141.3	138.1	140.3
22′	1.69 s	1.75 s	1.75 s	16.1	16.0	16.2
23′	–	–	–	175.4 br.	175.9	–

[a] Recorded at 400 and 100 MHz, respectively, in [D$_6$]acetone. [b] Recorded at 600 and 150.9 MHz, respectively, in CD_3OD. [c] Recorded at 600 and 150.9 MHz, respectively, in $CDCl_3$.

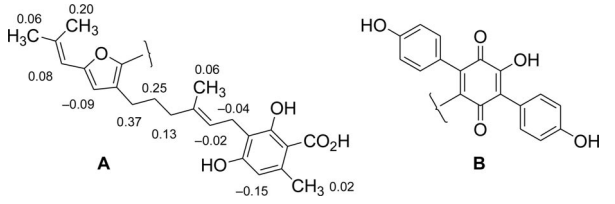
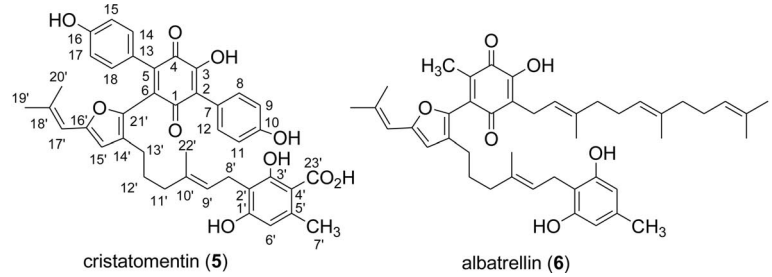


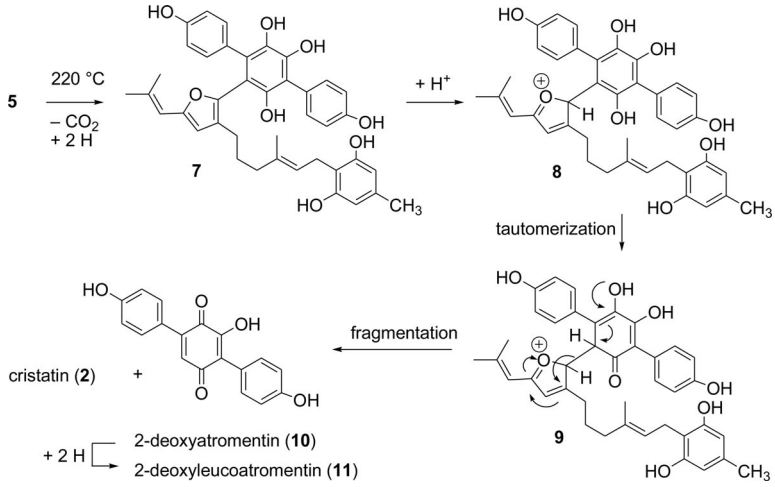
Figure 1. Partial structures **A** and **B** [the numbers given on **A** indicate the change in chemical shift ($\Delta\delta_H$) for the ^{1}H NMR signals of cristatomentin (**5**) relative to the corresponding signals for cristatic acid (**1**)].

tomentin is represented by structure **B** (Figure 1). A combination of both partial structures yields constitution **5** for cristatomentin, which is supported by the agreement of the chemical shift values for the carbon atoms connecting both moieties in cristatomentin (**5**) (C-6: δ_C = 134.3 ppm; C-21′: δ_C = 141.3 ppm) and albatrellin (**6**) (C-6: δ_C = 135.5 ppm; C-21′: δ_C = 140.3 ppm).[6a]

The EI-MS of cristatomentin (**5**) shows that the compound suffers thermal decarboxylation[2a,6a] and yields a pseudo molecular ion peak at *m*/*z* (%) = 646 (4) in addition to a more intense peak at *m*/*z* (%) = 648 (16); [M + 2] ions are characteristic for benzoquinones and arise by reduction of the quinone in the inlet system.[8] Two fragment ions at *m*/*z* (%) = 340 (10) [$C_{22}H_{28}O_3$] and 310 (100) [$C_{18}H_{14}O_5$] correspond to the molecular compositions of cristatin (**2**) and 2-deoxyleucoatromentin (**10**), respectively, and indicate thermal cleavage of decarboxyleucocristatomentin (**7**; *m*/*z* = 648) in the inlet system (Scheme 1). Protonation of **7** could yield the highly stabilized cation **8**, which, after tautomerization to cation **9**, fragments into cristatin (**2**) and 2-deoxyatromentin (**10**). The latter is then reduced to leuco compound **11**, responsible for the base peak at *m*/*z* = 310.

Additional fragment ions at *m*/*z* (%) = 136 (41) [$C_9H_{12}O$], 137 (70) [$C_8H_9O_2$], and 149 (67) [$C_{10}H_{13}O$] are formed from the molecular ion of cristatin (**2**) by cleavage of the side chain by McLafferty rearrangement, benzylic cleavage at the aromatic terminus, and cleavage between C-11′ and C-12′, respectively.[2a] The (–)-FAB MS of **5**[9] exhibits ion peaks at *m*/*z* = 691 [M$^-$ + H] and 647 [M$^-$ + H – CO_2], which confirm the presence of the carboxy group.

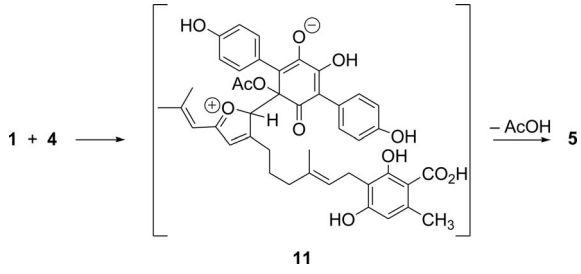




Scheme 1. Proposal for the thermal fragmentation of decarboxyleucocristatomentin (**7**) in the inlet system of the mass spectrometer.

The proposed structure of cristatomentin (**5**) explains the shielding of the protons in the cristatic acid chain by the terphenylquinone moiety. Molecular models indicate that the steric interaction between the furan ring and the neighbouring hydroxyphenyl residue forces the latter to adopt an out-of-plane conformation, thereby exposing the nearby side-chain protons and the protons of the 20′-methyl group to the shielding effect of the aromatic system (Figure 1).

We have recently shown that albatrellin (**6**) is formed by addition of cristatin (**2**) to 2-farnesyl-3-hydroxy-5-methyl-1,4-benzoquinone.[6a] An analogous reaction between cristatic acid (**1**) and 2-*O*-acetylatromentin (**4**) would afford adduct **11**, which could subsequently lose acetic acid to form the furylbenzoquinone **5** (Scheme 2).[10] This sequence can be considered as a reversal of the fragmentation depicted in Scheme 1. It should be noted that the atromentin half of cristatomentin is biosynthesized via 4-hydroxyphenyl pyruvate on the shikimate pathway,[11] whereas the cristatic acid half originates from the tetraketide orsellinic acid and the sesquiterpene farnesol. *Albatrellus cristatus* thus uses three different metabolic pathways for the synthesis of its green pigment.



Scheme 2. Proposal for the formation of cristatomentin (**5**).

Experimental Section

General: Evaporation of the solvents was performed under reduced pressure by using a rotary evaporator. Column chromatography: Sephadex LH-20 (Pharmacia), acetylated polyamide-6 (Polyamide SC-6AC, 50–160 μm, Macherey–Nagel). Analytical TLC: Silica gel 60 F_{254} aluminum foils (Merck); solvent system A (v/v): toluene/HCO_2Et/HCO_2H (10:5:3); B: $CHCl_3$/MeOH (5:1). MPLC: Duramat CFG pump, Lobar prepacked column, packing material LiChropreb RP-8. UV/Vis: Varian Cary 17 spectrophotometer. IR: Perkin–Elmer 1420. NMR: Bruker WM 400, in [D_6]acetone with the solvent peak as internal standard. EI-MS: A.E.I. MS 50 with data system DS 50, direct inlet (DI) at 70 eV and 180 °C. MS (FAB): VG analytical ZAB 1S′′ mass spectrometer with reversed geometry, thioglycerol matrix, xenon, 7 keV.

Mushrooms: *A. cristatus* was collected in September 1981, 1983, and 1988 in Germany near Hilberath (Eifel) and Kaltenbrunn (Bavaria).

Isolation Procedure: Freshly collected, lyophilized fruit bodies of *A. cristatus* (114 g) were pulverized and exhaustively extracted with petroleum ether (60–80 °C) until no more cristatic acid (**1**) could be detected in the extracts (pink colour with HCl vapours on a silica gel TLC plate). The combined extracts were concentrated under reduced pressure to furnish **1** as fine needles, which could be recrystallized from hexane. The total yield of **1**[2a] was 3.42 g (3%), however, much lower yields (ca. 0.5%) were obtained with aged mushrooms. The fungal material from the petroleum ether extraction was kept under reduced pressure to remove the residual solvent. Then the powder was exhaustively extracted with MeOH until the extracts remained colourless. The combined MeOH extracts were concentrated and, after addition of some water for removal of the mannitol, extracted with EtOAc (4 × 150 mL). The combined organic phases were dried (Na_2SO_4), and the resulting residue was prepurified by gel chromatography on Sephadex LH-20 with MeOH. The green pigment **5** was eluted in the final fractions in front of atromentin (**3**). Further enrichment of **5** was achieved by repeated chromatography on Sephadex LH-20 with MeOH and MeOH/$CHCl_3$ (1:1). Traces of **3** and other pigments were removed by MPLC on RP-8 phase with MeOH/water (5:1). Chromatography of the residue on Sephadex LH-20 with MeOH/$CHCl_3$ (1:1) yielded **5** (4.3 mg, 0.004%) as a lime green, solidifying oil. Pigment **5** dissolves in acetone or $CHCl_3$ (+ some MeOH) with a green and in pure MeOH with a greenish brown colour. Brownish colours were also observed during chromatography of **5** on Sephadex LH-20 or RP-8 with MeOH or MeOH/H_2O. For the isolation of 2-*O*-acetylatromentin (**4**), the lyophilized, powdered fruit bodies of *A. cristatus* (20 g) were washed with petroleum ether as described above. The resulting residue was extracted with acetone (200 mL)

at room temperature overnight, and the solid was separated by centrifugation. After repeating this procedure two times, the combined extracts were concentrated and chromatographed on Sephadex LH-20 [eluent: acetone/MeOH (3:2)] to yield the crude orange-yellow pigment. Chromatography on acetylated polyamide-6 (eluent: acetone) furnished **4** (ca. 20 mg, 0.1%) as an orange-red solid [R_f (TLC) = 0.39 (system A)], identical with an authentic sample.[4b]

Cristatomentin (5): R_f (TLC) = 0.46 (system A), 0.39 (system B), green spot. UV/Vis (MeOH): λ_{max} (logε) = 256 (4.31), 268 (sh, 4.23), 400 (3.39), 577 (2.77) nm. UV/Vis ($CHCl_3$):[5] λ_{max} (ε_{rel}) = 413 (1.0), 615 (0.48) nm. IR (KBr): $\tilde{\nu}$ = 3360 (s, br.), 2960 (sh), 2930 (s), 2850 (sh), 1700 (m), 1645 (s), 1605 (s), 1585 (sh), 1510 (s), 1450 (m), 1370 (m), 1320 (s), 1270 (s), 1170 (s), 1125 (m), 1050 (w), 835 (w) cm^{-1}. ^1H NMR (400 MHz, [D_6]acetone): δ = 1.38 (m, $J \approx$ 7 Hz, 2 H, 12′-H), 1.69 (s, 3 H, 22′-H), 1.74 (s, 3 H, 20′-H), 1.80 (s, 3 H, 19′-H), 1.85 (t, $J \approx$ 7 Hz, 2 H, 11′-H), 1.93 (t, $J \approx$ 7 Hz, 2 H, 13′-H), 2.45 (s, 3 H, 7′-H), 3.29 (d, J = 7 Hz, 2 H, 8′-H), 5.24 (t, J = 7 Hz, 1 H, 9′-H), 5.88 (m, 1 H, 17′-H), 6.11 (s, 1 H, 15′-H), 6.34 (s, 1 H, 6′-H), 6.73, 7.02 (AA′BB′ system, J = 8.8 Hz, 4 H, 15/17-H and 14/18-H), 6.87, 7.37 (AA′BB′ system, J = 8.8 Hz, 4 H, 9/11-H and 8/12-H) ppm; at T = 220 K the signals of the 5 hydroxy groups became visible: δ = 8.80, 9.45, 9.90, 10.05, and 13.5 (2-OH) ppm. ^{13}C NMR (100 MHz, [D_6]acetone): see Table 1 plus additional signals at δ = 115.2 (C-15/17), 115.4 (C-9/11), 120.0 (C-13), 125.0 (C-7), 132.4 (C-14/18), 133.2 (C-8/12), 134.3 (C-6), 155.0 (C-16), 156.3 (br., C-3), 158.6 (C-10), 184.5 (br., C-4), 186.1 (C-1) ppm. The signals of C-2 and C-5 were obscured. The assignments of the methyl, methylene and methine signals were confirmed by an HMQC experiment. EI-MS: m/z (%) = 648 (16) [M + 2 H – CO_2]$^+$, 646 (4) [M – CO_2]$^+$, 340 (10) [$C_{22}H_{28}O_3$], 310 (100) [$C_{18}H_{14}O_5$], 308 (2), 282 (1) [$C_{17}H_{14}O_4$], 280 (2), 203 (30) [$C_{14}H_{19}O$], 177 (15) [$C_{11}H_{13}O_2$], 175 (47) [$C_{11}H_{11}O_2$], 150 (10) [$C_{10}H_{14}O$], 149 (67) [$C_{10}H_{13}O$], 148 (20) [$C_{10}H_{12}O$], 137 (70) [$C_8H_9O_2$], 136 (41) [$C_9H_{12}O$], 107 (13) [C_7H_7O], 83 (21) [C_5H_7O]. $C_{40}H_{38}O_8$ [M – CO_2]$^+$: calcd. 646.2567; found 646.2545. MS [(–)-FAB, 1,4-dithioerythrose + NaCl]:[9] m/z (%) = 713 (5.1) [M + Na]$^-$, 691 (3.7) [M + H]$^-$, 647 (2.3) [M + H – CO_2]$^-$.

Acknowledgments

This work was financially supported by the Bundesministerium für Bildung und Forschung and the Fonds der Chemischen Industrie. We thank Dr. Bert Steffan and Dr. Ingrid Josten for recording the NMR spectra, and Dr. Kay Greenfield, Brisbane, Australia, for improving the manuscript.

[1] R. L. Edwards, V. Fawcett, D. J. Maitland, R. Nettleton, L. Shields, A. J. S. Whalley, *J. Chem. Soc., Chem. Commun.* **1991**, 1009–1010, and references therein.
[2] a) L. Zechlin, M. Wolf, W. Steglich, T. Anke, *Liebigs Ann. Chem.* **1981**, 2099–2105; b) synthesis: A. Fürstner, T. Gastner, *Org. Lett.* **2000**, *2*, 2467–2470; c) the high-field ^1H and ^{13}C NMR spectroscopic data of cristatic acid (**1**) were kindly provided by Dr. Peter Spiteller and Mr. Robert Jäger, TU München.
[3] a) A. Bresinsky, A. Rennschmid, *Ber. Dtsch. Bot. Ges.* **1971**, *84*, 313–329; b) H. Besl, A. Bresinsky, *Z. Pilzkd.* **1977**, *43*, 311–322.
[4] a) A. Kämmerer, H. Besl, A. Bresinsky, *Plant Systemat. Evol.* **1985**, *150*, 101–117; b) H. Besl, A. Bresinsky, G. Geigenmüller, R. Herrmann, C. Kilpert, W. Steglich, *Liebigs Ann. Chem.* **1989**, 803–810; c) M. Gill, W. Steglich, *Prog. Chem. Org. Nat. Prod.* **1987**, *51*, 25.
[5] The sample was dissolved in a small amount of methanol and then diluted with chloroform.
[6] a) B. Koch, W. Steglich, *Eur. J. Org. Chem.* **2007**, 1631–1635; b) D. N. Quang, T. Hashimoto, Y. Arakawa, C. Kohchi, T. Nishizawa, G.-I. Soma, Y. Asakawa, *Bioorg. Med. Chem.* **2006**, *14*, 164–168; c) X.-L. Yang, C. Qin, F. Wang, Z.-J. Dong, J.-K. Liu, *Chem. Biodivers.* **2008**, *5*, 484–489.
[7] C. Takahashi, K. Yoshihira, S. Natori, M. Umeda, *Chem. Pharm. Bull.* **1976**, *24*, 613–620.
[8] See, for example: a) R. T. Aplin, W. T. Pike, *Chem. Ind. (London)* **1966**, 2009; b) S. Ukai, K. Hirose, A. Tatematsu, T. Goto, *Tetrahedron Lett.* **1967**, *8*, 4999–5002; c) J. Heiss, K.-P. Zeller, A. Rieker, *Org. Mass Spectrom.* **1969**, *2*, 1325–1334; d) P. C. Beaumont, R. L. Edwards, *J. Chem. Soc. C* **1969**, 2398–2403; e) H. Budzikiewicz, C. Djerassi, D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, **1967**, p. 118. Strong [M + 2] ions were also observed in the case of albatrellin (**6**) and related benzoquinones, see ref.[6a]
[9] Under (–)-FAB conditions **5** is reduced to the leuco compound (m/z = 692 [M + 2 H]), from which the negative ions are derived by loss of H^+, optionally with exchange of H against Na, or fragmentations.
[10] The possibility that cristatomentin (**5**) is formed in the toadstool from **1** and **4** without the help of enzymes cannot be ruled out and needs further investigations.
[11] M. Gill, W. Steglich, *Prog. Chem. Org. Nat. Prod.* **1987**, *51*, 17–18.

Received: June 28, 2009
Published Online: November 30, 2009

