Novel Benzotropolone and 2*H*-Furo[3,2-*b*]benzopyran-2-one Pigments from *Tricholoma aurantium* (Agaricales)

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Dedicated to Professor Werner Tochtermann on the occasion of his 65th birthday

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The bright orange-red color of the toadstool *Tricholoma aurantium* is due to the benzotropolone pigment aurantricholone (**1**). The compound is at least partially present as the calcium salt. Minor pigments are the yellow 2*H*-furo[3,2-*b*]benzopyran-2-one derivatives aurantricholides A (**7a**) and B (**7b**), which exhibit strong green fluorescences. Their structures have been established by total syntheses.

Introduction

The fruit bodies of *Tricholoma aurantium* (Schff. ex Fr.) Ricken (German: Orangeroter Ritterling) are found under spruce trees and other conifers in limestone areas of Europe and North America. The mushroom attracts attention by its vivid red-orange to brownish-orange cap and the rusty-orange scales on its belted stalk. In wet weather, the pigments form orange droplets that stain the fingers when picking the mushrooms. It is interesting to note that the orange caps are often splashed with olive-green, which is consistent with the observation that the crude pigments form a dark-green to brownish resin on standing.

Previous investigations of this toadstool led to the isolation of several diterpenoids with the novel 2,3-seconeodolastane skeleton.[1–3] The disagreeable flour-like odor of the fruit bodies is largely due to (*E*)-2-nonenal.[4] We describe herein a chemical investigation of the pigments from *T. aurantium*.

Isolation of the Pigments

It was found that the extraction of the pigments from *T. aurantium* could best be achieved using mushrooms that had been lyophilized immediately after collection. Extraction with acetone followed by methanol yielded a crude pigment that could only be purified by chromatography on Sephadex LH-20 using a 1:1 dichloromethane/methanol mixture as eluent. All attempts to elute the pigments from columns of silica-based materials, polyamide, acetylated polyamide, or Diaion HP 20 led to decomposition. The gel chromatographic separation yielded four main fractions: a brown lipid fraction, a terpenoid fraction, a yellow fraction showing intense green fluorescence, and an orange-red fraction that was found to contain the main pigment aurantricholone (**1**).[1] The fluorescent pigments were named aurantricholide A (**7a**) and aurantricholide B (**7b**). From 27.7 kg of fresh, predominantly young fruit bodies, 87 mg of pure ruby-red aurantricholone (3 mg/kg) was obtained by repeated Sephadex chromatography. The amount of aurantricholides isolated varied considerably between different batches of the collected fungi but never totalled more than a few mg. The two aurantricholides were separated and purified by further Sephadex chromatography. They proved to be rather unstable, being prone to decomposition even on standing in organic solvents.

The aurantricholides A and B were also isolated from *Tricholoma fracticum* (Britz.) Kreis. (= *T. batschii* Gulden), whereas *T. focale* (Fr.) Ricken yielded only small amounts of aurantricholide B.

Aurantricholone (1)

Aurantricholone was obtained as a dark solid with a green metallic lustre. The compound dissolves readily in water, but is only poorly soluble in organic solvents. The ruby-red solutions in methanol or acetone show UV/Vis maxima at 268, 305, and 440 nm. On addition of alkali the color changes irreversibly to green and black, whereas with acids yellow compounds of undetermined structure are formed. Since aurantricholone was not reduced with either zinc/acetic acid or sodium dithionite, a quinone structure could be ruled out.

The molecular mass of aurantricholone was determined from the parent-ion peak at m/z = 576 upon FAB mass spectrometric analysis. Reaction of the pigment with acetic anhydride/pyridine yielded a triacetyl derivative **3**, thereby indicating the presence of at least three hydroxy groups. Methylation of aurantricholone with dimethyl sulfate and potassium carbonate produced a mixture of compounds, from which the pentamethyl ether **4** could be isolated by preparative HPLC. The molecular composition of this ether was determined as $C_{38}H_{30}O_{10}$ (m/z = 646.1859) by high-

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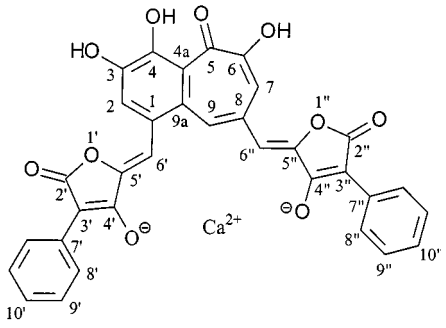
resolution EI-MS. This indicates a molecular formula of $C_{33}H_{20}O_{10}$ for aurantricholone.

The ^{1}H-NMR spectrum of aurantricholone in [D_6]acetone shows signals attributable to 15 aromatic protons. In addition, a signal due to a strongly chelated hydroxy proton is observed at δ = 15.1. The signals are much better resolved in CD_3OD, due to the better solubility of the pigment in this solvent (Table 1). Apart from five singlets due to isolated protons, six multiplets could be discerned, attributable to the protons of two unsubstituted phenyl rings. According to the chemical shifts of the *ortho*-protons at δ = 8.16/8.18, each of the phenyl rings must be attached to an electron-withdrawing substituent.

Table 1. ^{1}H- and ^{13}C-NMR data of **1** (calcium salt) in CD_3OD (600 and 150.9 MHz, respectively)

	δ_C [ppm] (*J* [Hz])		δ_H [ppm] (*J* [Hz])
C-1	126.2 d (6.5)		
C-2	123.2 Dd (161.7, 5.3)	2-H	7.97 s
C-3	144.2 d (3.4)		
C-4	149.2 d (6.9)		
C-4a	118.9 d (8.8)		
C-5	183.0 d (8.4)		
C-6	152.4 d (7.3)		
C-7	116.7 Ddd (156.4, 10.9, 6.9)	7-H	8.28 s
C-8	129.1 s		
C-9	131.7 Ddd (154.5, 7.5, 6.5)	9-H	8.13 s
C-9a	128.4 d (7.2)		
C-2'/C-2''	172.8 s		
	173.2 s		
C-3'/C-3''	90.9 m		
	91.5 m		
C-4'	176.1 d (2.7)		
C-4''	176.6 d (2.3)		
C-5'/C-5''	147.1 m		
	147.5 m		
C-6'	98.5 Dd (163.2, 4.2)	6'-H	6.85 s
C-6''	103.7 Ddd (158.7, 8.0, 3.8)	6''-H	6.42 s
C-7'/C-7''	132.6 t (7.3)		
C-8'/C-8''	124.0 Dt (159.4, 6.5)	8'-H/8''-H	8.16 d (7.6)
	124.1 Dt (160.5, 7.2)		8.18 d (7.6)
C-9'/C-9''	125.9 Dd (156.4, 7.2)	9'-H/9''-H	7.30 dd (7.6, 7.2)
	125.9 Dd (156.4, 7.2)		7.32 dd (7.6, 7.2)
C-10'/C-10''	122.3 Dt (157.6, 7.4)	10'-H/10''-H	7.07 t (7.2)
	122.4 Dt (157.6, 7.4)		7.10 t (7.2)



1 (calcium salt)

The ^{13}C-NMR spectrum (Table 1) features a set of five close pairs of signals that could be ascribed to two almost identical C_5 moieties bearing the phenyl groups. The remaining eleven carbon signals are due to the core unit of the molecule. In addition to five hydroxy groups, aurantricholone contains five further oxygen atoms, which could be ascribed to two lactone groups (δ = 173.2/172.8) and a carbonyl function (δ = 183.0). The chemical shift of the carbonyl group together with the strongly chelated OH group (δ_H = 15.15) and the NMR data of the remaining 10 carbon atoms indicated a 3,4,6-trihydroxybenzocyclohepten-5-one chromophore as the core unit. This was supported by selective ^{1}H-decouplings in the ^{1}H-coupled ^{13}C-NMR spectrum that led to the correlations indicated in Figure 1. The excellent agreement of the NMR data of aurantricholone (**1**) with those of the benzotropolone nucleus of theaflavin (**2**)[5–7] confirmed the presence of a 1,8-disubstituted 2,3-benzotropolone system.

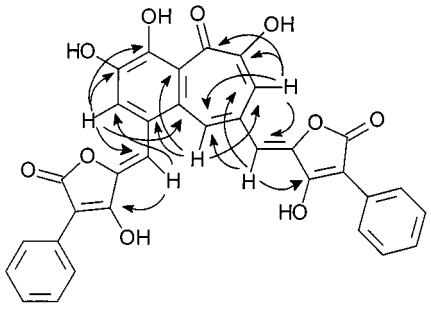
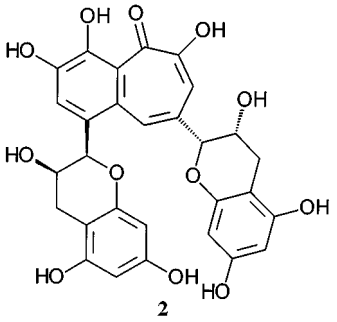


Figure 1. C–H correlations from selective ^{1}H-decouplings in the ^{1}H-coupled ^{13}C-NMR spectrum of aurantricholone (**1**) (coupling constants $J_{C,H}$ are given in Table 1)

Strong cross-peaks between the protons at C-6' and C-6'' of the pulvinone residues and 9-H (δ = 8.13) of the



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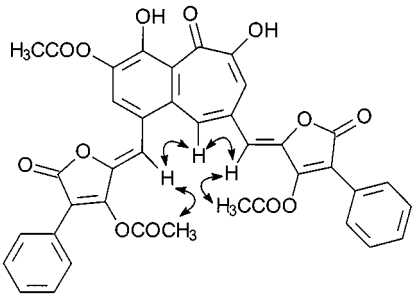

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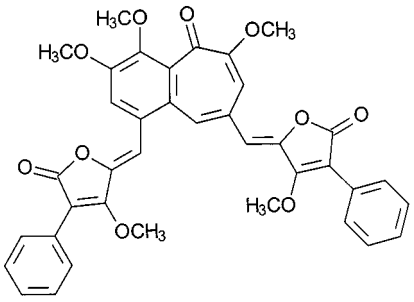

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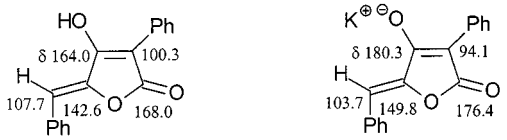
benzotropolone nucleus in the NOESY spectrum of aurantricholone (**1**) provided evidence for the 1,8-attachment of the side chains. The NOESY spectrum of the triacetate showed the same correlations as well as cross-peaks between the acetoxy groups at the butenolide rings and the corresponding protons at C-6' and C-6'', as indicated in formula **3**. This established the (*Z*)-configuration of the exocyclic pulvinone double bonds.

In the ^{13}C-NMR spectrum of **1**, the signals due to C-4' and C-3'' appear at considerably lower field (δ = 176.1/176.6) than in that of the hydroxybutenolide **5** (δ = 164.0) (Figure 2).[8–10] Since a similar downfield shift of the C-3 signal to δ = 180.3 is observed when **5** is converted into its potassium salt **6**, the OH groups at the pulvinone rings of the natural pigment must be ionized.[11]

Neutron activation analysis of aurantricholone isolates with subsequent γ-spectroscopy indicated the presence of substantial amounts of calcium ions. Zinc ions were found to be present at much lower concentration, whereas alkali metal and other metal ions were found only in trace amounts. It could therefore be concluded that in the toadstool aurantricholone is present at least partially in the form



5 **6**

Figure 2. ^{13}C-NMR data of (*Z*)-5-benzylidene-4-hydroxy-3-phenyl-5*H*-furan-2-one (**5**) and its potassium salt (**6**) (in CD_3OD)

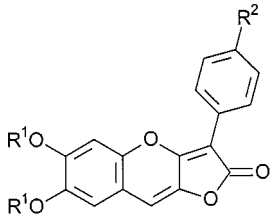
of its calcium salt. This finding is in accord with the water solubility of the orange pigment.

The ^{13}C-NMR data for the pulvinone rings of aurantricholone triacetate **3** and permethyl ether **4** were in good agreement with those of the acetate and methyl ether of the simple hydroxybutenolide **5**.

Aurantricholides A (7a) and B (7b)

The aurantricholides A (**7a**) and B (**7b**) are orange, unstable solids that could be isolated only in very small amounts. A solution of **7a** in methanol exhibits a bright yellow-green fluorescence and shows UV maxima at 204, 269, and 411 nm. In the ^{1}H-NMR spectrum ([D_6]acetone), signals due to a phenyl ring are seen at δ = 7.27 (t, 1 H), 7.44 (dd, 2 H), and 8.21 (d), besides three further aromatic singlets at δ = 7.00, 7.04, and 7.07. The chemical shift of the two *ortho*-protons, δ = 8.21, is indicative of attachment of the phenyl residue to a strongly deshielding moiety. The high-resolution mass spectrum shows a molecular ion at *m*/*z* = 294.0525, in accord with a molecular formula of $C_{17}H_{10}O_5$. Since the ^{1}H-NMR spectrum of **7a** exhibits signals due to eight aromatic protons, the pigment must contain two OH groups, which is consistent with the formation of a bis(trimethylsilyl) derivative on treatment of **7a** with *N,O*-bis(trimethylsilyl)trifluoroacetamide and chlorotrimethylsilane (TMSCl) in pyridine.

A comparison of the UV spectra of aurantricholide A (**7a**) and aurantricholide B (**7b**) indicated a slight bathochromic shift of the long-wavelength maximum to 417 nm for the latter compound. The ^{1}H-NMR spectrum of **7b** in [D_6]acetone features signals attributable to a *p*-hydroxyphenyl residue (δ = 6.93 and 8.07, each d, 4 H) and additional signals due to three isolated protons at δ = 6.96, 6.98, and 7.03.



	7a	**7b**	**8a**	**8b**
R^1	H	H	Ac	Ac
R^2	H	OH	H	OAc

Silylation of aurantricholide B (**7b**) yielded a tris(trimethylsilyl) ether, which indicated the presence of three hydroxy groups. The molecular mass of the silyl derivative was determined from the parent-ion peak at *m*/*z* = 526.1672 ($C_{26}H_{34}Si_3O_6$) in the high-resolution EI-MS. The molecular composition of aurantricholide B could therefore be assigned as $C_{17}H_{10}O_6$. Acetylation of **7b** yielded a triacetyl derivative **8b**, which exhibited three consecutive ketene fragmentations from the molecular ion in its EI-MS.

Considering the high degree of unsaturation in the aurantricholides (15 double bond equivalents), the ^{1}H-NMR data, the UV spectra, and the IR absorptions (KBr) at $\tilde{\nu}$ = 1735, 1719, and 1588 cm^{-1}, the 2*H*-furo[3,2-*b*]benzopyran-2-one structures **7a** and **7b** can be suggested for aurantricholides A and B, respectively.[12]

The proposed structures were confirmed by total syntheses starting from the phosphorus ylides **10a** and **10b** (Scheme 1).[8] Condensation of **10a** or **10b** with 2,4,5-trimethoxybenzaldehyde (**9**) afforded the benzylidene derivatives **11a** and **11b**, respectively, as the more stable (*Z*)-isomers. Irradiation of compounds **11** in chloroform solution by means of a high-pressure mercury lamp yielded complex mixtures of products, from which the furobenzopyranones **12a** and **12b** could be isolated in very low yields (1%) by radial thin-layer chromatography. Up to 70% of the starting materials **11** could be recovered. The structure of **12a** was confirmed by X-ray structure analysis (Figure 3).[13] Cleavage of the *O*-methyl groups with BBr_3 yielded the aurantricholides A (**7a**) and B (**7b**), which were shown to be identical to the natural products by HPLC comparison and the correspondence of their mass and NMR spectra.

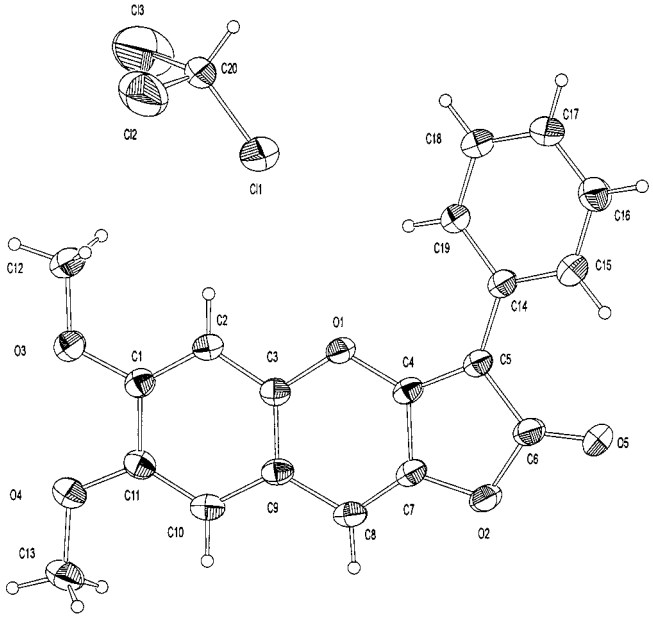
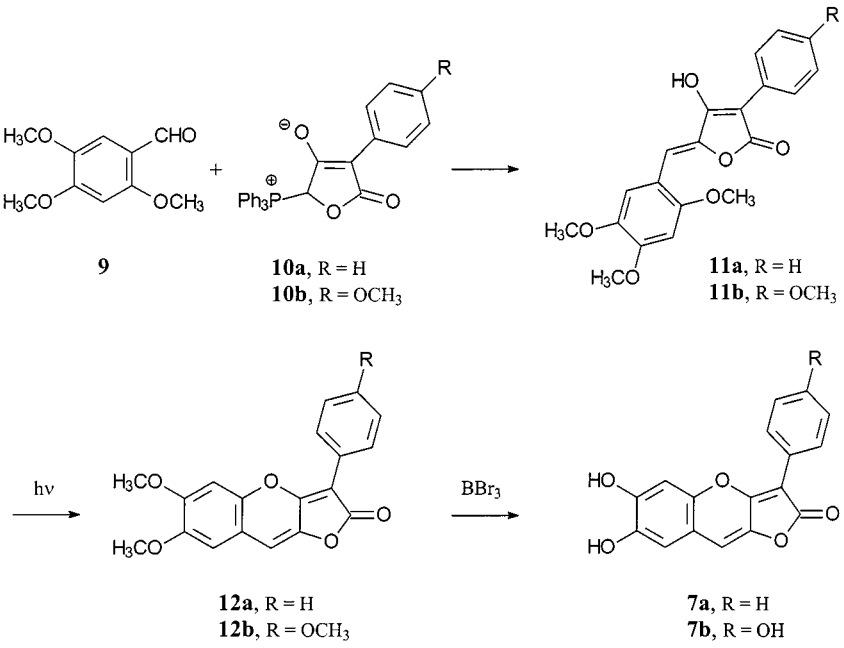


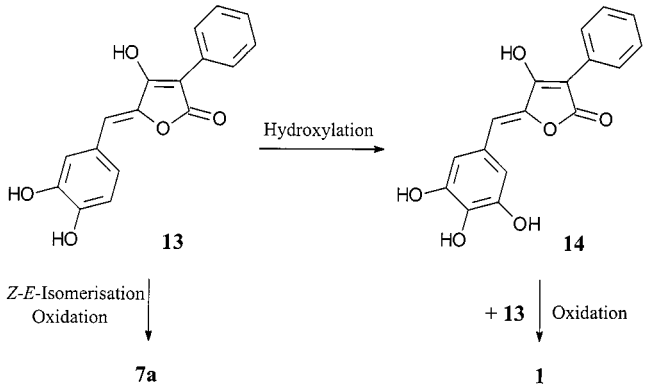
Figure 3. Crystal structure of furobenzopyranone **12a**·$CHCl_3$

Discussion

The pigments of *Tricholoma aurantium* constitute an interesting group of natural products, which may originate from 3,4-dihydroxypulvinone (**13**) as a common precursor (Scheme 2).



Scheme 1. Syntheses of aurantricholides A (**7a**) and B (**7b**)



Scheme 2. Proposed biogenetic relationship of the *Tricholoma* pigments **7a** and **1**[16]

Aurantricholone (**1**), like other 3,4,6-trihydroxybenzocyclohepten-5-ones, could be formed by oxidative coupling of catechol **13** with the corresponding pyrogallol derivative **14**.[14–16] A precedent for this process is the formation of theaflavin (**2**) from (–)-epicatechin and (–)-epigallocatechin.[17] On the other hand, conversion of pulvinone **13** to its (*E*)-isomer followed by oxidative closure of the pyran ring[16] could account for the formation of aurantricholide A (**7a**).

The occurrence of aurantricholides in *Tricholoma aurantium*, *T. fracticum*, and *T. focale* suggests a close chemotaxonomic relationship of these species.[1] A benzotropolone derivative, fomentariol, has previously been isolated from the wood-rotting polypore *Fomes fomentarius*. It is formed by oxidative condensation of 2,3,4-trihydroxycinnamyl alcohol.[18]

Experimental Section

General: Melting points (uncorrected): Reichert hot-stage apparatus. – UV/Vis (MeOH): Perkin–Elmer Lambda 16. – IR: Perkin–Elmer 1420. – NMR: Bruker AMX-600 and ARX-300. – MS and FAB-MS: Finnigan MAT 95Q; EI spectra were obtained at 70 eV. – TLC: Silica gel plates (Merck 60 F_{254}); solvent system I: toluene/ethyl formate/formic acid, 10:5:3 (v/v); solvent system II: CH_2Cl_2/MeOH, 15:1. – Gel chromatography: Sephadex LH-20 (Pharmacia). Radial TLC separations were performed on a Chromatotron 7924T (Harrison Research, Palo Alto). – Analytical HPLC: Waters/Millipore (pump and gradient controller 600E, injector U6 K, photodiode array detector 990+), Knauer Vertex column 4 × 250 mm, Nucleosil C18, 5 μm (Macherey–Nagel); mobile phase: a solvent gradient, starting after 5 min, from 100% 0.05 M HOAc to 100% MeOH in 30 min; flow rate 1 mL/min. – Preparative HPLC: Waters/Millipore (two 590 EF pumps, gradient controller 680, injector U6 K, Knauer variable-wavelength detector with a superpreparative flow cell), Knauer Vertex column 16 × 250 mm, Lichrosorb DIOL, 7 μm (Merck) with guard column 16 × 30 mm; flow rate 6.8 mL/min; detection at 280 nm.

Tricholoma aurantium was collected in the autumn of 1993 and in the following years near Liptingen (Schwäbische Alb), *T. fracticum* near Karlstadt/Main and Niederehe (Eifel), and *T. focale* in the Leutasch (Tyrol).

Isolation of the Pigments: Fresh fruit bodies of *T. aurantium* (1.5 kg) were lyophilized immediately after collection. The freeze-dried material (140 g) was crushed and exhaustively extracted with acetone (3 × 3 L) and thereafter with methanol (2 × 3 L). The combined acetone extracts were filtered and concentrated in vacuo. The orange-brown residue (3.8 g) was then applied to a short Sephadex LH-20 column (20 × 5 cm). Rapid elution (4 mL/min) with CH_2Cl_2/MeOH (1:1, v/v) yielded four colored fractions in the order: brown, yellow-orange (broad), fluorescent green, and orange-red.

To the residue obtained from the combined MeOH extracts, water (75 mL) was added and the mixture was extracted several times with EtOAc (total 1 L). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to yield a dark-brown residue (3.21 g). Separation of the components on Sephadex LH-20 as described for the acetone extract yielded colored fractions in the same order. Evaporation of the solvents from the orange-red fractions of the acetone and MeOH extracts afforded 18 mg and 10 mg of crude pigments, respectively, which were purified by further chromatography on a Sephadex LH-20 column (25 × 2.5 cm) eluting with MeOH (HPLC grade, flow rate 0.3 mL/min) to yield aurantricholone (**1**) in the form of its calcium salt (8 mg).

The fractions exhibiting a green fluorescence were combined and purified by three consecutive Sephadex LH-20 separations eluting with CH_2Cl_2/MeOH (1:1) and MeOH (2×). Aurantricholide A (**7a**) (0.3 mg) was eluted first, followed by aurantricholide B (**7b**) (0.1 mg). In a similar manner, small amounts of **7a/7b** could be obtained from *Tricholoma fracticum*, while *T. focale* afforded only **7b**. In total, ca. 27.7 kg of *T. aurantium* was worked-up in batches of 1–2 kg yielding about 87 mg of aurantricholone (**1**) and 2–3 mg of the aurantricholides A (**7a**) and B (**7b**).

Aurantricholone ("Calcium Salt") (1): Dark amorphous solid with greenish lustre. – M.p. > 250 °C (dec.). – TLC: R_f = 0.46 (system I); R_f = 0 (system II). – UV: λ_{max} (lg ε) = 203 nm (4.612), 268 (4.517), 305 (4.528), 440 (4.293). – IR (KBr): $\tilde{\nu}$ = 3430 cm^{-1}, 2925, 2856, 1712, 1700, 1653, 1636, 1576, 1559, 1505, 1444, 1429, 1332, 1261, 1240, 1153, 1085, 1075, 954, 906, 781, 697. – ^{1}H NMR (600 MHz, [D_6]acetone): δ = 6.21 (s, 1 H, 6''-H), 6.89 (dd, *J* = 7.0, 7.0 Hz, 1 H, 10''-H), 6.93 (dd, *J* = 7.0, 7.0 Hz, 1 H, 10'-H), 6.93 (s, 1 H, 6'-H), 7.14 (dd, *J* = 7.5, 7.0 Hz, 2 H, 9''-H), 7.18 (dd, *J* = 7.5, 7.0 Hz, 2 H, 9'-H), 7.70 (s, 1 H, 2-H), 8.17 (s, 1 H, 9-H), 8.39 (d, *J* = 7.5 Hz, 2 H, 8''-H), 8.42 (d, *J* = 7.5 Hz, 2 H, 8'-H), 8.71 (s, 1 H, 7-H), 15.15 (s, 1 H, 4-OH). – ^{1}H NMR (CD_3OD): See Table 1. – ^{13}C NMR: See Table 1. – (–)-FAB-MS (matrix glycerol); *m/z* (%): 599 (6), 598 (19), 597 (40) [M^+ + Na – H], 577 (18), 576 (44), 575 (100) [M^+ – H], 559 (5), 413 (4), 357 (7), 283 (4), 275 (6), 187 (11), 183 (28), 153 (8), 117 (38), 91 (16).

Aurantricholone Triacetate (3): To a solution of **1** (7.4 mg) in pyridine (2 mL) was added Ac_2O (2 mL). After 3 h at 25 °C, the mixture was concentrated in vacuo and the remaining orange oil was purified by chromatography on Sephadex LH-20 eluting with MeOH. Yield: 3.3 mg of **3** (37%). Orange oil. – R_f = 0.10 (system II). – UV: λ_{max} (lg ε) = 203 nm (4.656), 267 (4.520), 390 (4.444). – IR (KBr): $\tilde{\nu}$ = 3430 cm^{-1}, 2917, 2842, 1740, 1728, 1708, 1635, 1578, 1565, 1508, 1443, 1430, 1368, 1315, 1220, 1205, 1155, 1060, 1015, 950, 907, 898, 780, 702. – ^{1}H NMR (CD_3OD): δ = 2.30 (s, 3 H, $COCH_3$), 2.34 (s, 3 H, $COCH_3$), 2.37 (s, 3 H, $COCH_3$), 6.40 (s, 1 H, 6''-H), 6.82 (s, 1 H, 6'-H), 7.09 (dd, *J* = 7.4, 7.4 Hz, 1 H, 10''-H), 7.12 (dd, *J* = 7.4, 7.4 Hz, 1 H, 10'-H), 7.31 (dd, *J* = 7.5, 7.4 Hz, 2 H, 9''-H), 7.33 (dd, *J* = 7.5, 7.4 Hz, 2 H, 9'-H), 7.83 (s, 1 H, 7-H), 7.92 (s, 1 H, 9-H), 8.13 (d, *J* = 7.5 Hz, 2 H, 8''-H), 8.16 (s, 1 H, 2-H), 8.16 (d, *J* = 7.5 Hz, 2 H, 8'-H). – ^{13}C NMR (CD_3OD): δ = 20.40 ($COCH_3$), 20.70 ($COCH_3$), 20.83 ($COCH_3$), 93.71, 94.14, 98.58 (C-6'), 105.16 (C-6''), 123.68 (C-7), 125.32 (C-10''), 125.43

(C-10'), 126.93 (C-8''), 127.03 (C-8'), 128.89 (C-9''), 128.91 (C-9'), 130.08 (C-2), 132.14 (C-9), 132.53, 133.20, 133.32, 134.73, 135.60, 135.68, 141.23, 144.93, 149.07, 150.98, 152.18, 169.84 ($COCH_3$), 169.94 ($COCH_3$), 170.51 ($COCH_3$), 175.79, 175.99, 179.38, 179.81, 185.97. – (+)-FAB-MS (matrix thioglycerol); *m/z*: 703 [M^+ + H].

Aurantricholone Pentamethyl Ether (4): To **1** (4.5 mg) in dry acetone (4 mL) were added dimethyl sulfate (1.5 mL) and a small amount of anhydrous K_2CO_3. The mixture was refluxed, which resulted in a color change from red to yellow within 15 min. Heating was continued for a further 4 h, then the mixture was filtered and the solvent was removed in vacuo. The remaining oil was diluted with water (5 mL) and extracted with $CHCl_3$ (3×). The combined organic layers were purified by flash chromatography on silica gel eluting with a solvent gradient from hexane to EtOAc. The yellow fractions obtained were then separated by preparative HPLC (eluent A = *n*-hexane; eluent B = *t*BuOMe/propan-2-ol, 5:1; gradient from 20% to 80% B within 40 min). Yield: 0.8 mg (16%) of **4**. Yellow-orange crystals. – M.p. 94–96 °C. – R_f = 0.56 (system I), R_f = 0.96 (system II). – UV: λ_{max} (lg ε) = 203 nm (4.270), 328 (4.057), 399 (3.902). – IR (KBr): $\tilde{\nu}$ = 3460 cm^{-1}, 2955, 2920, 2842, 1765, 1752, 1722, 1712, 1668, 1655, 1632, 1597, 1493, 1460, 1452, 1372, 1315, 1290, 1262, 1210, 1145, 1045. – ^{1}H NMR ([D$_6$]acetone): δ = 3.86 (s, 3 H), 3.90 (s, 3 H), 3.93 (s, 3 H), 3.97 (s, 3 H), 4.03 (s, 3 H), 6.34 (s, 1 H), 6.70 (s, 1 H), 6.90 (s, 1 H), 7.42 (d, *J* = 7.3 Hz, 1 H), 7.43 (d, *J* = 7.3 Hz, 1 H), 7.46 (dd, *J* = 7.3, 7.3 Hz, 2 H), 7.47 (dd, *J* = 7.3, 7.3 Hz, 2 H), 7.57 (d, *J* = 7.3 Hz, 2 H), 7.59 (d, *J* = 7.3 Hz, 2 H), 7.81 (s, 1 H), 7.88 (s, 1 H). – ^{13}C NMR ([D$_6$]acetone): δ = 56.5, 56.7, 61.7, 61.8, 62.6, 104.6 (C-6'), 104.9 (C-6''), 111.2 (C-7), 118.6 (C-2), 127.6, 128.4 (C-9), 125.6, 129.0 (C-9' and C-9''), 129.3 (C-10' and C-10''), 129.8, 131.1, 131.1, 131.2 (C-8''), 131.2 (C-8'), 134.7, 143.6 (C-5' and C-5''), 151.0 (C-3), 153.5 (C-4), 157.7 (C-6), 164.4 (C-4'' and C-4'), 168.4 (C-2'), 168.6 (C-2''), 188.2 (C-5). – EI-MS; *m/z* (%): 676 (13), 646 (100) [M^+], 618 (12) [M^+ – CO], 575 [M^+ – CO – C_2H_3O], 511 (4), 420 (8), 415 (8), 255 (6), 105 (6), 100 (25), 83 (8), 72 (9), 69 (10), 58 (22), 55 (10). – $C_{38}H_{30}O_{10}$: calcd. 646.1839; found 646.1859 (HR-EIMS).

(*Z*)-5-Benzylidene-4-hydroxy-3-phenylfuran-2(5*H*)-one (Potassium Salt) (6): KOH (56 mg, 1 mmol) was added to a solution of (*Z*)-5-benzylidene-4-hydroxy-3-phenylfuran-2(5*H*)-one (**5**)[8] (264 mg, 1 mmol) in MeOH (20 mL) and the mixture was stirred for 16 h at 25 °C. Evaporation of the solvent yielded the yellow salt **6**, which was dried in vacuo. Yield: 287 mg (0.95 mmol, 95%). – M.p. 197 °C. – ^{1}H NMR (300 MHz, CD_3OD): δ = 6.31 (s, 1 H), 7.08 (t, *J* = 7.4 Hz, 1 H), 7.25–7.42 (m, 5 H), 7.82 (d, *J* = 7.5 Hz, 2 H), 8.17 (d, *J* = 7.3 Hz, 2 H). – ^{13}C NMR (75 MHz, CD_3OD): δ = 94.05 (C-3), 103.66 (C-6), 125.29, 126.96 (2 C), 128.66, 128.89 (2 C), 129.75 (2 C), 131.24 (2 C), 135.80, 136.14, 149.85 (C-5), 176.44 (C-2), 180.26 (C-4).

Aurantricholide A (7a): Orange-colored solid. The yellow solution in MeOH exhibits an intense green fluorescence. – M.p. 272 °C (dec.). – R_f = 0.48 (system I). – Analytical HPLC: T_t = 34.94 min. – UV: λ_{max} ($\varepsilon_{rel.}$) = 270 nm (78), 412 (100), 482 (15). – IR (KBr): $\tilde{\nu}$ = 3429 cm^{-1} (br.), 2925 (m), 2854 (w), 1736 (m), 1721 (m), 1612 (s), 1514 (w), 1495 (w), 1459 (w), 1449 (w), 1300 (s), 1192 (w), 776 (w), 659 (w), 659 (w), 643 (w), 568 (m). – ^{1}H NMR ([D$_6$]acetone): δ = 7.00 (s, 1 H), 7.04 (s, 1 H), 7.07 (s, 1 H), 7.27 (dd, *J* = 7.3, 7.3 Hz, 1 H, 4'-H), 7.44 (dd, *J* = 7.7, 7.3 Hz, 2 H, 3'-H), 8.21 (d, *J* = 7.7 Hz, 2 H, 2'-H). – ^{1}H NMR (CD_3OD): δ = 7.02 (s, 1 H), 7.09 (br. s, 2 H), 7.34 (dd, *J* = 7.3, 7.3 Hz, 1 H, 4'-H), 7.49 (dd, *J* = 7.7, 7.3 Hz, 2 H, 3'-H), 8.18 (d, *J* = 7.7 Hz, 2 H, 2'-H). – ^{13}C NMR ([D$_6$]acetone): δ = 94.81, 103.68 (correlated to δ_H = 7.04), 106.03 (correl. to δ_H = 7.07), 112.54 (correl. to δ_H = 7.00), 112.78, 126.96 (C-2'), 128.35 (2 C), 129.79, 139.96, 143.35, 144.92, 147.33, 157.34, 166.24. – $C_{17}H_{10}O_5$: calcd. 294.0528; found 294.0525 (HR-EIMS).

Silylation of 7a: To a solution of **7a** (0.1 mg) in pyridine (0.1 mL) was added a 99:1 mixture of *N,O*-bis(trimethylsilyl)trifluoroacetamide and Me_3SiCl (0.3 mL). The mixture was heated for 30 min at 60 °C and then directly submitted to mass spectroscopic analysis. EI-MS (DI, 160 °C); *m/z* (%): 439 (5), 438 (14) [M^+], 399 (11), 149 (13), 80 (6), 79 (100), 78 (11), 52 (41), 51 (26), 50 (27). – $C_{23}H_{26}Si_2O_5$: calcd. 438.1319; found 438.1297 (HR-EIMS).

Aurantricholide B (7b): Orange-colored solid. The yellow solution in MeOH exhibits an intense green fluorescence. – M.p. > 320 °C (dec.). – R_f = 0.39 (system I). – UV: λ_{max} (lg ε) = 204 nm (4.543), 267 (4.187), 416 (4.323). – ^{1}H NMR ([D$_6$]acetone): δ = 6.94 (d, *J* = 8.8 Hz, 2 H), 6.97 (s, 1 H), 7.09 (s, 1 H), 7.13 (s, 1 H), 8.06 (d, *J* = 8.8 Hz, 2 H), 8.61 (s, 2 H, OH).

Silylation of 7b: Procedure as described for **7a**. EI-MS (DI, 160 °C); *m/z* (%): 527 (1), 526 (2) [M^+], 149 (10), 95 (4), 93 (10), 80 (6), 79 (100), 78 (11), 75 (28), 58 (5), 52 (52), 51 (24), 50 (17). – $C_{26}H_{34}Si_3O_6$: calcd. 526.1663; found 526.1672 (HR-EIMS).

(*Z*)-4-Hydroxy-5-(2,4,5-trimethoxybenzylidene)-3-phenylfuran-2-(5*H*)-one (11a): To a solution of 2,4,5-trimethoxybenzaldehyde (**9**) (2.94 g, 15 mmol) and EtONa (1.02 g, 15 mmol) in EtOH (20 mL) was added 2,5-dihydro-2-oxo-3-phenyl-5-triphenylphosphoniumfuran-4-olate (**10a**)[8] (4.36 g, 10 mmol) and the mixture was stirred for 1–2 h under argon at 25 °C until a clear, dark solution was obtained. This solution was then slowly poured into conc. HCl (30 mL), diluted with water (200 mL), and the mixture was kept at 4 °C overnight. The resulting suspension was filtered and the collected precipitate was recrystallized from MeOH to yield **11a** as yellow needles. Yield: 2.18 g (62%). – M.p. > 125 °C (dec.). – UV: λ_{max} ($\varepsilon_{rel.}$) = 206 nm (100), 230 (53), 264 (48), 302 (47), 336 (85). – IR (KBr): $\tilde{\nu}$ = 3860 cm^{-1} (br.), 3070 (w), 2860 (w), 1708 (vs), 1623 (s), 1280 (s), 1205 (vs), 1030 (s), 998 (m), 815 (w), 773 (w), 685 (m). – ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 3.84 (s, 3 H), 3.90 (s, 3 H), 3.92 (s, 3 H), 6.77 (s, 1 H), 6.99 (s, 1 H), 7.29 (t, *J* = 7.7 Hz, 1 H), 7.42 (dd, *J* = 7.7, 7.2 Hz, 2 H), 7.79 (s, 1 H), 7.98 (d, *J* = 7.2 Hz, 2 H). – ^{13}C NMR (75.5 MHz, [D$_6$]acetone): δ = 56.20, 56.97, 57.00, 98.22, 101.74, 102.31, 114.16, 115.54, 128.02, 128.53 (2 C), 129.00 (2 C), 131.01, 141.08, 144.43, 152.92, 154.58, 163.60, 168.47. – EI-MS; *m/z* (%): 354 (100) [M^+], 208 (18), 193 (13), 181 (29) [$C_{10}H_{13}O_3^+$], 145 (6), 89 (3). – $C_{20}H_{18}O_6$ (354.36): calcd. C 67.79, H 5.12; found C 67.32, H 5.47.

(*Z*)-4-Hydroxy-5-(2,4,5-trimethoxybenzylidene)-3-(4-methoxyphenyl)furan-2(5*H*)-one (11b): Prepared from **9** (2.94 g, 15 mmol) as described above. Recrystallization from MeOH afforded **11b** as orange needles. Yield: 1.12 g (29%). – M.p. 192 °C. – UV: λ_{max} ($\varepsilon_{rel.}$) = 206 nm (100), 254 (61), 294 (44), 304 (47), 384 (90). – IR (KBr): $\tilde{\nu}$ = 3405 cm^{-1} (br.), 2918 (w), 2820 (w), 1700 (m), 1605 (s), 1510 (vs), 1272 (s), 1249 (vs), 1027 (m). – ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 3.81 (s, 3 H), 3.82 (s, 3 H), 3.87 (s, 3 H), 3.89 (s, 3 H), 6.73 (s, 1 H), 6.92 (s, 1 H), 6.97 (d, *J* = 9.1 Hz, 2 H), 7.76 (s, 1 H), 7.93 (d, *J* = 9.1 Hz, 2 H). – ^{13}C NMR (75.5 MHz, [D$_6$]acetone): δ = 55.50, 56.18, 56.94, 56.99, 98.21, 101.68, 101.77, 114.28, 114.47 (2 C), 115.52, 123.31, 129.84 (2 C), 141.22, 144.40, 152.73, 154.43, 159.81, 162.21, 168.71. – EI-MS; *m/z* (%): 384 (100) [M^+], 208 (14), 193 (13), 181 (27) [$C_{10}H_{13}O_3^+$], 147 (7), 119 (5). – $C_{21}H_{20}O_7$ (384.38): calcd. C 65.62, H 5.24; found C 65.88, H 5.31.

6,7-Dimethoxy-3-phenyl-2*H*-furo[3,2-*b*]benzopyran-2-one (12a): A suspension of **11a** (1.0 g, 2.8 mmol) in $CHCl_3$ (400 mL) at 60 °C was irradiated with light from a high-pressure mercury lamp fitted

with a Solidex glass filter ($\lambda > 289$ nm). After 8 h, the solvent was evaporated and the residue was filtered through a short column of silica gel (petroleum ether/EtOAc, 2:1). Purification by radial TLC (petroleum ether/EtOAc, 10:1 1:1) and recrystallization from MeOH afforded **12a** as yellow crystals. Yield: 10 mg (1%). – M.p. 224 °C. – $R_f = 0.16$ (SiO_2, petroleum ether/EtOAc, 3:1). – UV: λ_{max} ($\varepsilon_{rel.}$) = 208 nm (100), 270 (52), 406 (62). – IR (KBr): $\tilde{\nu}$ = 3425 cm^{-1} (br.), 2921 (w), 1730 (s), 1621 (w), 1601 (m), 1519 (m), 1433 (m), 1286 (vs), 1246 (m), 1222 (m), 1008 (w), 731 (w), 691 (w), 649 (w). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.93 (s, 3 H), 3.98 (s, 3 H), 6.79 (s, 1 H), 6.88 (s, 1 H), 7.05 (s, 1 H), 7.31 (t, J = 7.5 Hz, 1 H), 7.46 (dd, J = 7.2, 7.5 Hz, 2 H), 8.17 (d, J = 7.2 Hz, 2 H). – ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 56.41, 56.46, 96.24, 100.61, 105.32, 108.82, 112.98, 126.52 (2 C), 127.44, 128.55 (2 C), 129.28, 140.88, 145.47, 146.98, 150.36, 157.04, 166.78. – EI-MS; *m/z* (%): 322 (100) [M^+], 278 (4), 261 (4), 145 (15), 129 (6). – $C_{19}H_{14}O_5$: calcd. 322.0833; found 322.0842 (HR-EIMS).

6,7-Dimethoxy-3-(4-methoxyphenyl)-2*H*-furo[3,2-*b*]benzopyran-2-one (12b): Prepared as described above from furanone **11b** (1.0 g, 2.6 mmol). Radial thin-layer chromatography and subsequent recrystallization from MeOH afforded **12b** as orange crystals. Yield: 11 mg (1.2%). – M.p. 262 °C. – $R_f = 0.61$ (SiO_2, petroleum ether/EtOAc, 1:1). – UV: λ_{max} (ε_{rel}) = 202 nm (100), 263 (26), 412 (2). – IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1}, 2963, 1734, 1601, 1515, 1288, 1262, 1221, 1087, 1024, 801, 568. – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.87 (s, 3 H), 3.95 (s, 3 H), 4.00 (s, 3 H), 6.75 (s, 1 H), 6.88 (s, 1 H), 7.02 (d, J = 8.9 Hz, 2 H), 7.05 (s, 1 H), 8.14 (d, J = 8.9 Hz, 2 H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.38, 56.51, 56.54, 96.32, 100.71, 104.68, 108.99, 113.14, 114.13 (2 C), 121.96, 128.03 (2 C), 141.15, 145.53, 146.93, 150.29, 155.93, 158.97, 167.09. – EI-MS; *m/z* (%): 352 (100) [M^+], 337 (20), 308 (5), 176 (5), 147 (5). – $C_{20}H_{16}O_6$: calcd. 352.0947; found 352.0941 (HR-EIMS).

Synthesis of 6,7-Dihydroxy-3-phenyl-2*H*-furo[3,2-*b*]benzopyran-2-one (Aurantricholide A) (7a): A solution of **12a** (10 mg, 0.03 mmol) in CH_2Cl_2 (3 mL) was cooled to –78 °C under argon atmosphere, whereupon a 1 M solution of BBr_3 in CH_2Cl_2 (0.3 mL, 0.3 mmol) was added by means of a syringe. The resulting suspension was allowed to warm to room temperature overnight and then diluted with EtOAc. The organic phase was washed sequentially with aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$, dilute HCl, and water. It was then dried (Na_2SO_4) and the solvent was evaporated in vacuo. The crude product was purified by column chromatography on Sephadex LH-20 eluting with methanol. Yield: 7 mg (80%) of **7a** as orange crystals. The UV, ^{1}H-NMR, and MS data of **7a** were in full agreement with those of natural aurantricholide A. A direct HPLC comparison of the two products confirmed that they were identical.

Synthesis of 6,7-Dihydroxy-3-(4-hydroxyphenyl)-2*H*-furo[3,2-*b*]benzopyran-2-one (Aurantricholide B) (7b): Prepared from **12b** (10 mg, 0.028 mmol) as described above. Yield: 5 mg (56%). – IR (KBr): $\tilde{\nu}$ = 3425 cm^{-1} (br.), 1744 (m), 1710 (m), 1662 (s), 1606 (s), 1587 (s), 1514 (m), 1458 (m), 1368 (w), 1299 (s), 1238 (m), 1178 (m), 1112 (w), 933 (w), 836 (w), 568 (m). – The UV, ^{1}H-NMR, and MS data of **7b** were in full agreement with those of natural aurantricholide B. A direct HPLC comparison of the two products confirmed that they were identical.

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