Structure and Absolute Configuration of the Fungal Ansabenzoquinone Rhizopogone

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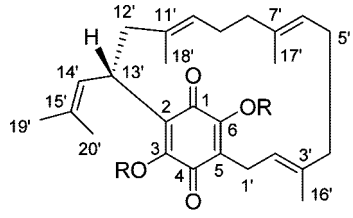
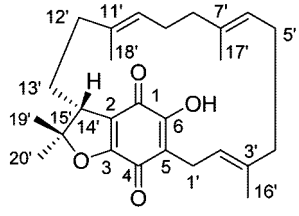
The main pigment of the basidiomycete *Rhizopogon pumilionus* is the [13]paracyclophane derivative rhizopogone (**1**). The structures of **1** and the accompanying 2-acetoxyrhizopogone (**5**) were determined by spectroscopic studies, including 1D and 2D NMR measurements. The absolute configuration of **1** was assigned by comparison of its CD spectrum with that of secotridentoquinone (**4**). The structures of **1** and tridentoquinone (**3**) suggest a common biosynthesis originating from 2-geranylgeranyl-6-hydroxy-1,4-benzoquinone (**9**). This supports the close taxonomic relationship of the genera *Rhizopogon* and *Suillus* (Boletales).

The fungal genus *Rhizopogon* Fries (Basidiomycota) comprises more than 100 hypogeous species.[1] One of the rarest in Europe is *R. pumilionus* (Ade) Bataille (German: Latschen-Wurzeltrüffel), a truffle-like fungus, first described in 1909 by Ade[2] and then not recorded or its existence even doubted for several decades. Serendipitously, we recovered this species from the Leutasch region of the Tyrolean Alps,[3] where it is associated with *Pinus mugo*. The orange-ochre sporocarps of *R. pumilionus* are 1 to 2.5 cm in diameter and mature at least partly underground. In this paper we give a detailed account of the isolation, structure elucidation, and determination of the absolute configuration of the main pigment, named rhizopogone (**1**), an unusual meroterpenoid, structurally related to the ansa compound tridentoquinone (**3**)[4] from the bolete *Suillus tridentinus*.



R = H: Rhizopogone (**1**)
R = Ac: Rhizopogone Diacetate (**2**)



Tridentoquinone (**3**)

(biogenetic numbering)

Results and Discussion

Extraction of fresh fruit bodies of *R. pumilionus* with methanol or acetone gave an intense orange solution. In addition to polar substances, TLC showed a nonpolar orange spot that turned violet upon exposure to ammonia vapor. Purification of the crude extract by gel permeation chromatography on Sephadex LH-20 and subsequent removal of polar impurities yielded pure rhizopogone (**1**). Seven fruit bodies yielded 25 mg of **1**, corresponding to 1% of dry weight.

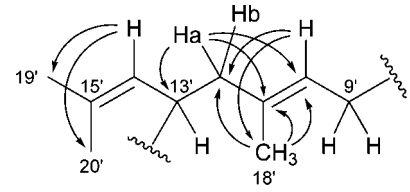


Figure 1. Partial structure A with selected long-range CH-correlations.

Rhizopogone showed UV/vis maxima at 301 and 440 nm (sh), which, in combination with the violet color reaction with ammonia, pointed to a 2,5-dihydroxybenzoquinone chromophore.[5] The HR-EIMS exhibited a molecular ion at *m/z* 410.2445, corresponding to the molecular formula $C_{26}H_{34}O_4$. Rhizopogone is thus isomeric with tridentoquinone (**3**), with which it shares the strong molecular ion and negligible isoprene unit fragmentations, a behavior characteristic of meroterpenoid ansabenzoquinones.[6] The ^{13}C NMR spectrum of rhizopogone (see Experimental Section) exhibited signals for five methyls, six methylenes, one aliphatic methine, and four −CH=C< double bonds. This indicated a regular geranylgeranyl chain connected at C-1′ and one of the methylene positions with the 2,5-dihydroxybenzoquinone ring to form an ansa structure. The terminal $(H_3C)_2C{=}CH-$ unit could be easily discerned and extended to partial structure A by COSY experiments and the COLOC[7] and HMBC relationships given in Figure 1. The methine proton H-13′ appeared as ddd at δ_H 4.01 (J = 12.3, 4.2, and 9.0 Hz) due to vicinal couplings with the nonequivalent neighboring methylene protons and the vinyl proton H-14′ (δ 5.52). From these results and the evidence given below, structure **1** could be proposed for rhizopogone.

In accord with this structure were the NMR signals for the diastereotopic protons at C-1′, which appeared as two doublet of doublets at δ 3.03 and 3.16 (J = 13.2/7.0 and 13.2/9.0 Hz, respectively), in excellent agreement with the corresponding values for **3** [δ 3.02 and 3.18 (J = 13.8/8.1 and 13.8/7.4 Hz)].[4a] Cross-peaks in the COLOC and HMBC spectra between CH_2-12′ and C-2 as well as between the 1′-protons and C-5 confirmed the attachment of the geranylgeranyl moiety to the corresponding positions of the dihydroxybenzoquinone ring.

All quaternary signals in the ^{13}C NMR spectrum of rhizopogone (**1**) were present except those of C-1, C-3, C-4, and C-6, a common phenomenon for 2,5-dihydroxybenzoquinones, caused by rapid intramolecular proton transfer.[8] These signals could, however, be detected after conversion of **1** into its diacetate **2**. This resulted in

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[#] Crystal structure determination.

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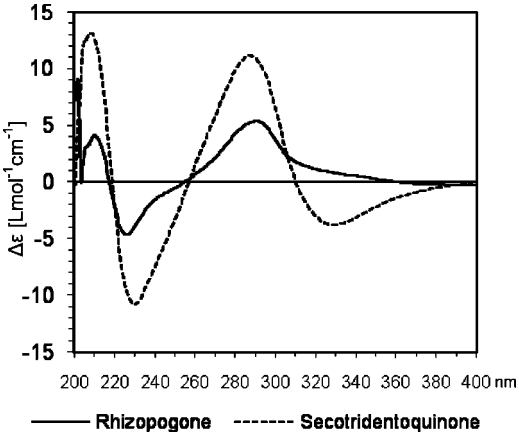
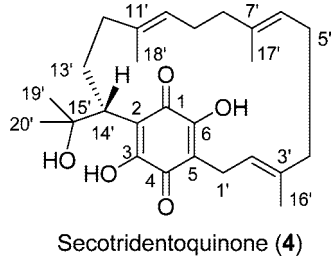


Figure 2. CD spectra of rhizopogone (**1**) and secotridentoquinone (**4**).

a downfield shift of approximately 19 ppm for the signals of C-2 and C-5.[9] Interestingly, the NMR spectra of the diacetate exhibited a 2-fold set of signals. This may be explained by atropdiastereomer formation, due to the stereogenic center at C-13′ and the rotationally restricted 2,5-diacetoxybenzoquinone ring. In **1**, rapid intramolecular proton exchange in the 2,5-dihydroxybenzoquinone moiety[8] renders this unit "achiral", thus explaining the singular set of signals observed in the NMR spectra.

In order to determine the absolute configuration of rhizopogone (**1**), we correlated this [13]paracyclophane derivative with tridentoquinone (**3**), whose absolute configuration has recently been established as 14′*R* by X-ray structure analysis of the corresponding (−)-camphanoate.[4a] Treatment of **3** with aqueous alkali afforded secotridentoquinone (**4**) without affecting the stereogenic center.[4b] Since ansaquinone **4** and rhizopogone (**1**) possess the same arrangement of the stereogenic center relative to the dihydroxybenzoquinone chromophore and differ only in the size of their ansa rings, the experimental agreement of the CD curves depicted in Figure 2 indicates the same absolute configuration for both compounds. The 13′*S* configuration can thus be assigned to rhizopogone (**1**).



Secotridentoquinone (**4**)

Crystallization of secotridentoquinone (**4**) from ethanol yielded crystals suitable for single-crystal X-ray structure analysis, in which both individual atropdiastereomers were visible (Figure 3).

Rhizopogone (**1**) was accompanied by small amounts of a second pigment, **5**, to which the molecular composition $C_{28}H_{36}O_6$ could be assigned by HREIMS (*m*/*z* 468.2537). This suggested a structure in which one of the protons of **1** is replaced by an acetoxy group. Accordingly, the EIMS of **5** showed a fragment ion at *m*/*z* 408, formed by elimination of acetic acid from the molecular ion, and in the ^{1}H NMR spectrum of pigment **5** the acetoxy group was recognized by a methyl singlet at δ 2.09. In addition, the typical signals of the ansa ring and partial structure A (Figure 1) were present, excluding an acetoxy substituent in the aliphatic region of the molecule. Comparison of the ^{1}H NMR spectrum of **5** with that of the parent pigment **1** revealed a close similarity of the CH_2-1′ (δ 3.01, 3.22 vs 3.03, 3.16) and CH-2′ (δ 5.09 vs 5.14) signals, whereas the signals corresponding to partial structure A were shifted

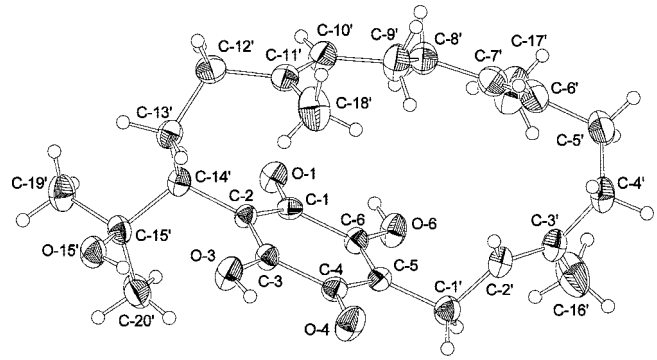
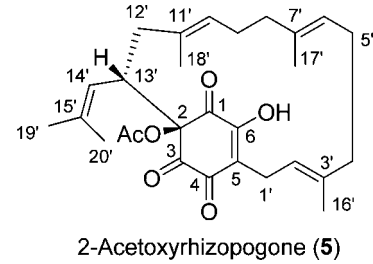


Figure 3. ORTEP plot derived from a single-crystal X-ray analysis of secotridentoquinone (**4**) (only one of the two atropdiastereomers is shown).

toward higher field. Thus, the signal of the central 13′-proton appeared as a pseudoquartet at δ 3.75 (**1**: 4.01), and the 9 Hz doublet of the olefinic 14′-proton experienced a strong high-field shift toward δ 4.84 (**1**: 5.52). To account for these changes, the acetoxy substituent had to be at C-2 of the quinone ring, leading to structure **5** for the minor pigment. The change in hybridization at C-2 results in a different conformation of the ansa cycle, which explains the differences in the H,H-coupling constants in the ^{1}H NMR spectrum compared to those of rhizopogone (**1**). The relative configuration given in formula **5** was proposed by assuming an acetoxylation of **1** from the less hindered side.



2-Acetoxyrhizopogone (**5**)

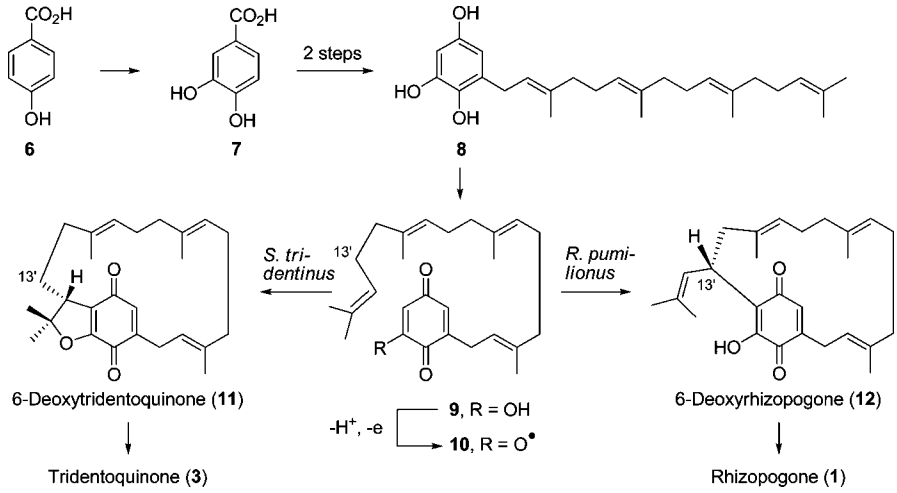
The specific incorporation of ^{13}C-labeled hydroxybenzoic acids **6** and **7** into tridentoquinone (**3**) suggested 6-geranylgeranyl-2-hydroxy-1,4-benzoquinone (**9**) as the key intermediate in the biosynthesis of this *Suillus* pigment (Scheme 1).[4a] Oxidative cycloaddition of the corresponding hydroxyquinone radical **10** to the terminal double bond could furnish 6-deoxytridentoquinone (**11**), the immediate precursor of **3**.[10] The formation of rhizopogone (**1**) may be explained by radical **10** abstracting a hydrogen atom from the 13′-methylene group, followed by addition of the resulting allyl radical[11] to the hydroxybenzoquinone ring.[12] The 6-deoxyrhizopogone (**12**) formed could then be hydroxylated[4a] to give rhizopogone (**1**).

Isolation of the closely related ansaquinones **1** and **3** from *Rhizopogon pumilionus* and *Suillus tridentinus* underlines the close taxonomic relationship of these genera. This had already been deduced from the common occurrence of hydroxypulvinic acids and related Boletales pigments[13] in *Rhizopogon* and *Suillus* and has been verified by molecular genetic investigations.[14] Interestingly, these unique ansaquinones have so far been detected only in one species of each genus, despite the fact that each genus contains more than 100 species.[15] The presence of the unique ansabenzoquinone **1** in *R. pumilionus* removes any doubt[1a] about this fungus being a valid species. Since **1** can be easily detected by TLC on silica gel (yellow-orange spot, violet color with ammonia vapor), this simple test allows for the reliable identification of *R. pumilionus*.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on Bruker AMX 600, Bruker ARX 300, or Varian VXR 400S spectrometers. All spectra were measured at room temperature in

Scheme 1. Proposed Biosynthesis of the Fungal Ansaquinones **1** and **3**



$CDCl_3$, using the residual solvent signal as an internal standard (δ_H = 7.26, δ_C = 77.1). Chemical shifts are given in ppm (δ) values. The melting point was determined with a melting point microscope (Reichert Thermovar) and was not corrected. IR spectra were recorded on a Perkin-Elmer 1420 FTIR spectrometer. UV/vis spectra were recorded on a Perkin-Elmer spectrophotometer Lambda 25. CD spectra were measured on a Jasco J-715 spectropolarimeter. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. EIMS and HREIMS were taken on Finnigan MAT 90 and 95Q spectrometers (70 eV). Gel permeation column chromatography was performed on Sephadex LH-20 (Pharmacia) without applying pressure. High-speed countercurrent chromatography (HSCCC) was performed with an apparatus from P.C. Inc., Potomac, MD, consisting of a multilayer coil, a counterweight/triple coil, and a Rainin Dynamax SD-200 pump. Preparative HPLC was performed with a Merck Hitachi L-6200 intelligent pump, a 655A variable wavelength UV monitor, and a D-2000 integrator. Column: Knauer Nucleosil C_6H_5, flow 3 mL/min. Gradient A: 0.2% aqueous NH_4OAc−MeCN, 9:1, to MeCN within 60 min (linear). Analytical TLC was performed with Kieselgel 60 F_{254} (Merck); solvent system A: toluene−HCO_2Et−HCO_2H, 10:5:3. Preparative TLC was performed on Kieselgel 60 F_{254} glass plates (Merck), 20 × 20 cm, thickness 0.5 mm.

Fungal Material. *R. pumilionus* (Ade) Bataille was collected on July 28, 1997, from the Puitalm (1550−1600 m altitude) in the Leutasch region, Tyrol, Austria. Leg. W. S., det. A. Bresinsky.

Extraction and Isolation. Seven freshly collected, unground fruit bodies of *R. pumilionus* (wet weight 12.5 g) were shaken with acetone (40 mL) and 2 drops of 2 N HCl for 1 h at room temperature, which resulted in an intense orange solution. After filtration through a pad of glass wool, the solvent was removed under reduced pressure (30 °C) and the residue (132 mg) was subjected to gel permeation chromatography (Sephadex LH-20, column 20 × 1.5 cm, eluent MeOH). The fractions containing rhizopogone (**1**) were concentrated under reduced pressure. The dry residue was dissolved in a small volume of toluene and filtered. To the filtrate were added EtOAc (30 mL) and a few drops of AcOH, and the resulting solution was washed with water (2 × 20 mL), concentrated, and dried under reduced pressure to yield 25.3 mg of **1** (1% of dry weight). The remaining fractions were subjected to HSCCC (mobile phase: *n*-hexane, 4 volume parts; stationary phase: AcOH/MeOH, 1:1 volume parts; 80 mL column; forward rotation mode 825 rpm; flow 1.5 mL/min). 2-Acetoxyrhizopogone (**5**) (1 mg, 0.04% of dry weight) accumulated in the stationary phase and was purified by preparative HPLC (gradient A).

Rhizopogone (1): red solid; R_f 0.78 (solvent system A), + NH_3 violet; mp 110 °C; $[\alpha]_D^{20}$ +28 (*c* 0.155, $CHCl_3$); UV/vis (MeOH + 1% AcOH) λ_{max} (log ε) 301 (3.67), 440 (sh) (2.38) nm; (MeOH + 1% NEt_3) λ_{max} (log ϵ) 313 (3.85), 531 (2.98) nm; CD (*c* 0.042 mM, MeOH) λ ($\Delta\varepsilon$) 209 (+4.2), 225 (−4.6), 289 (+5.4) nm; IR (KBr) ν_{max} 3320, 2940, 2860, 1650, 1640, 1445 cm^{-1}; ^1H NMR (600 MHz) δ 7.67 (6-OH), 7.58 (3-OH), 5.52 (1H, br d, J = 9.0 Hz, H-14′; at 300 MHz: dsept, J = 9.0, 1.5 Hz), 5.14 (1H, br dd, $J \approx$ 9, 7 Hz, H-2′), 4.92 (1H, br t, $J \approx$ 6 Hz, H-10′), 4.83 (1H, br t, $J \approx$ 6 Hz, H-6′), 4.01 (1H, ddd, J = 12.3, 9.0, 4.2 Hz, H-13′), 3.16 (1H, dd, J = 13.2, 9.0 Hz, H-1′a), 3.03 (1H, dd, J = 13.2, 7.0 Hz, H-1′b), 2.47 (1H, dd, J = 12.3, 12.3 Hz, H-12′a), 2.12−1.83 (4H, m, H-4′, H-5′, H-8′, H-9′), 2.09 (1H, dd, J = 12.3, 4.2 Hz, H-12′b; partially obscured by overlapping methylene signals), 1.70 (3H, s, H-16′), 1.69 (3H, s, H-19′), 1.66 (3H, s, H-20′), 1.57 (3H, s, H-18′), 1.46 (3H, s, H-17′); ^{13}C NMR (151 MHz) δ 136.0 (qC, C-3′), 135.2 (qC, C-7′), 133.0 (qC, C-11′), 132.7 (qC, C-15′), 127.9 (CH, C-10′), 125.3 (CH, C-14′), 123.3 (CH, C-6′), 121.1 (CH, C-2′), 117.6 (qC, C-2), 115.3 (qC, C-5), 43.6 (CH_2, C-12′), 40.2 (CH_2, C-8′), 39.0 (CH_2, C-4′), 33.1 (br, CH, C-13′), 26.2 (CH_2, C-9′), 25.7 (CH_3, C-19′), 24.6 (CH_2, C-5′), 21.5 (CH_2, C-1′), 18.0 (CH_3, C-20′), 16.05 (CH_3, C-17′), 15.95 (CH_3, C-18′), 15.87 (CH_3, C-16′), assigned by long-range CH-correlations (COLOC,[7] optimized for J = 8.3 Hz [↔] and HMBC, optimized for J = 7.3 Hz [⇔]) showing the ^2J correlations *H*-1′a ↔ *C*-5, *H*-1′a ⇔ *C*H-2′, *H*-1′b ↔ *C*-5, *H*-1′b ⇔ *C*H-2′, *C*H-2′ ↔ *C*H$_2$-1′, *H*-12′a ⇔ *C*-11′ and *C*H-13′, *C*H$_3$-16′ ↔ C-3′, *C*H$_3$-17′ ↔ *C*-7′, *C*H$_3$-18′ ↔ *C*-11′, *C*H$_3$-19′ ↔ *C*-15′, *C*H$_3$-20′ ↔ *C*-15′ in addition to the ^3J correlations *H*-1′a ⇔ *C*-3′, *H*-1′b ⇔ *C*-3′, *C*H-2′ ↔ *C*H$_3$-16′, *C*H-6′ ↔ *C*H$_2$-8′ and *C*H$_3$-17′, *C*H-10′ ↔ *C*H$_2$-12′ and *C*H$_3$-18′, *H*-12′a ⇔ *C*-2 and *C*H-10′ and *C*H-14′, *H*-12′b ↔ *C*-2, *C*H-14′ ↔ *C*H$_3$-19′ and *C*H$_3$-20′, *C*H$_3$-16′ ↔ *C*H-2′ and *C*H$_2$-4′, *C*H$_3$-17′ ↔ *C*H-6′ and *C*H$_2$-8′, *C*H$_3$-18′ ↔ *C*H-10′ and *C*H$_2$-12′, *C*H$_3$-19′ ↔ *C*H-14′ and *C*H$_3$-20′, *C*H$_3$-20′ ↔ *C*H$_3$-19′, 6-O*H* ↔ *C*-5; signals for C-1, C-3, C-4, C-6 were not visible due to line broadening;[8] EIMS *m*/*z* 410 [M^+] (44), 395 [$M^+ - CH_3$] (16), 367 (2), 327 (9), 299 (11), 259 [$C_{15}H_{15}O_4^+$] (42), 220 [$C_{12}H_{12}O_4^+$] (100), 189 [$C_{14}H_{21}^+$] (23), 147 (6), 135 (8), 119 (8), 105 (7), 91 (5), 79 (5), 67 (4); HREIMS *m*/*z* 410.2445 (calcd for $C_{26}H_{34}O_4$, 410.2457).

Rhizopogone Diacetate (2). To a solution of rhizopogone (**1**, 6.1 mg, 14.8 μmol) in Ac_2O (0.2 mL) was added pyridine (6 μL, 74 μmol). After stirring for 1 h at 40 °C, the volatiles were removed and the residue was dried under reduced pressure. Purification by preparative TLC (eluent: PE−EtOAc, 7:1) yielded **2** as a yellow oil (3.8 mg, 7.7 μmol, 52%): ^1H NMR (600 MHz, $CDCl_3$) δ 5.50 (0.5H, br d, J = 9.0 Hz, H-14′†), 5.15 (0.5H, br m, H-14′‡), 5.04−4.93 (2H, m, H-2′†,‡, H-10′†,‡), 4.89 (0.5H, m, H-6′‡), 4.86 (0.5H, m, H-6′†), 4.15 (0.5H, ddd, J = 11.7, 7.5, 4.0 Hz, H-13′‡), 3.86 (0.5H, ddd, J = 11.7, 9.0, 4.0 Hz, H-13′†), 3.29 (0.5H, dd, J = 12, 9 Hz, H-1′a‡), 3.15 (0.5H, dd, J = 12.9, 6.8 Hz, H-1′a†), 3.05 (0.5H, dd, J = 12.9, 9.0 Hz, H-1′b†), 2.95 (0.5H, dd, J = 12, 7 Hz, H-1′b‡), 2.54 (0.5H, ψ-t, $J \approx$ 12.5 Hz, H-12′a†), 2.36, 2.35, 2.33, 2.31 (each 1.5H, 2 × $COCH_3^\dagger$ and 2 × $COCH_3^\ddagger$), 2.20−1.80 (9.5H, m, 4 × CH_2, H-12′a‡, H-12′b†,‡), 1.69 (s, 1.5H, CH_3-16′‡), 1.67 (s, 1.5H, CH_3-19′†), 1.66 (s, 1.5H, CH_3-16′†), 1.65 (s, 1.5H, CH_3-19′‡), 1.58 (s, 1.5H, CH_3-20′†), 1.56 (s, 1.5H, CH_3-18′†), 1.55 (s, 1.5H, CH_3-18′‡), 1.53 (s, 1.5H, CH_3-20′‡), 1.52 (s, 1.5H, CH_3-17′‡), 1.50 (s, 1.5H, CH_3-17′†); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.04, 180.02 br, 179.6, 179.4 (all qC, C-1†,‡ and C-4†,‡), 167.63 br, 167.62, 167.43, 167.38 br (all qC, 2 × *C*O$CH_3^\dagger$ and 2 × *C*O$CH_3^\ddagger$), 148.8, 148.1 (both qC, C-3†,‡ and C-6†,‡, 2 signals obscured), 138 br (qC, C-3′‡), 137.3 (qC, C-3′†), 136.3 (qC, C-2†,‡, 1 signal obscured), 135.8 (qC, C-7′†; signal for C-7′‡ obscured), 135 br (qC, C15′‡), 134.1 (qC, C-5‡), 133.9 (qC, C-5†), 133.6 (qC, C-15′†), 131.7 br (2 carbons, qC, C-11′†,‡), 129.2 (CH, C-10′†), 129.0 br (CH, C-10′‡), 124.7 (CH,

C-14′†), 124.0 (CH, C-14′‡), 122.8 (CH, C-6′†), 122.5 br (CH, C-6′‡), 119.5 (CH, C-2′†), 118.9 br (CH, C-2′‡), 45.3 (CH_2, C-12′‡), 44.3 (CH_2, C-12′†), 39.2 (CH_2, C-8′†), 39.0 (CH_2, C-4′†), 38.9 (CH_2, C-4′‡), 38.7 (CH_2, C-8′‡), 35.6 (CH, C-13′†), 32.5 (CH, C-13′‡), 26.6 br (CH_2, C-5′‡ or C-9′‡), 25.9 (CH_3, C-19′†), 25.6 (CH_2, C-5′† or C-9′†), 25.5 (CH_3, C-19′‡), 24.7 br (CH_2, C-9′‡ or C-5′‡), 24.2 (CH_2, C-9′† or C-5′†), 22.90 (CH_2, C-1′†), 22.86 br (CH_2, C-1′‡), 20.59, 20.48, 20.45, 20.36 (all CH_3, 2 × $COCH_3$† and 2 × $COCH_3$‡), 18.4 br (CH_3, C-20′‡), 17.9 (CH_3, C-20′†), 17.0 (CH_3, C-17′‡), 16.3 (CH_3, C-16′‡), 16.12 (CH_3, C-17′†), 16.07 (CH_3, C-18′†), 15.91 (CH_3, C-18′‡), 15.85 (CH_3, C-16′†), †,‡: atropdiastereomers; EIMS *m/z* 494 [M^+] (32), 479 (33), 452 [M^+ − ketene] (29), 437 (36), 410 [**1**$^+$] (45), 395 [**1**$^+$ − CH_3] (25), 259 [$C_{15}H_{15}O_4^+$] (58), 220 [$C_{12}H_{12}O_4^+$] (100), 189 [$C_{14}H_{21}^+$] (52), 81 (23), 69 [$C_5H_9^+$] (17), 43 (76); HREIMS *m/z* 494.2662 (calcd for $C_{30}H_{38}O_6$, 494.2668).

Secotridentoquinone (4). Preparation was from tridentoquinone (**3**) as described in ref 4b. Final purification by gel permeation chromatography on Sephadex LH-20 (eluent MeOH): orange solid; for mp, $[\alpha]_D$, UV, IR, MS data, see ref 4b; CD (*c* 0.060 mM, MeOH) λ ($\Delta\varepsilon$) = 208 (+13.1), 229 (−10.8), 287 nm (+11.1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47, 7.70 (each 1H, s, br, 2 × OH), 5.16 (1H, br dd, J = 9, 6 Hz, H-2′), 4.89 (1H, br t, J = 6 Hz), 4.79 (1H, br t, J = 5.5 Hz, H-6′ and H-10′), 3.20 (1H, dd, J = 13.8, 6.0 Hz, H-1′a), 3.05 (1H, dd, J = 13.8, 9.3 Hz, H-1′b), 2.81 (1H, dd, J = 12.2, 3.0 Hz, H-14′), 2.20−1.70 (12H, m, 6 × CH_2), 1.66 (s, 3H, H-16′), 1.52, 1.45 (each s, 3H, H-17′ and H-18′), 1.30 (s, 3H, H-19′), 1.15 (s, 3H, H-20′); ^{13}C NMR (151 MHz, $CDCl_3$) δ 136.2 (qC, C-3′), 134.6, 134.2 (both qC, C-7′ and C-11′), 125.7, 123.6 (both CH, C-6′ and C-10′), 120.8 (CH, C-2′), 116.3 (qC, C-2), 116.0 (qC, C-5), 74.0 (qC, C-15′), 45.4 (CH, C-14′), 39.1 (CH_2, C-4′), 38.8, 38.6 (each CH_2, C-8′ and C-12′), 29.5 (CH_3, C-20′), 27.9 (CH_3, C-19′), 26.5, 24.1, 22.4 (each CH_2, C-5′, C-9′, C-13′), 21.4 (CH_2, C-1′), 16.3 (CH_3), 15.3 (CH_3, C-16′), 14.9 (CH_3), signals for C-1, C-3, C-4, and C-6 were not visible due to broadening.[8]

X-ray Crystal Structure Analysis of Secotridentoquinone (4).[16] The data were collected on an Enraf-Nonius CAD4 diffractometer at 293(2) K using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods using SHELXS-97[17a,c] and refined by full-matrix least-squares on F^2 using SHELXL-97.[17b,c] The structure was displayed using ZORTEP.[18] Red stick (0.27 × 0.33 × 0.53 mm), $C_{26}H_{36}O_5$, M_r = 428.57, orthorhombic system, space group $P2_12_12_1$, Z = 8, a = 8.7058(10) Å, b = 11.5062(15) Å, c = 49.029(17) Å, V = 4911.3(19) Å^3, D_{calcd} = 1.159 g/cm^3, $F(000)$ = 1856, μ = 0.079 mm^{-1}, 6697 collected reflections (2.16° ≤ θ ≤ 21.67°, 0 ≤ h ≤ 9, −11 ≤ k ≤ 0, −50 ≤ l ≤ 50), 5725 independent reflections (R_{int} = 0.0175), goodness-of-fit on F^2 S = 1.019, R_1 = 0.0632 and wR_2 = 0.1077 for all reflections, R_1 = 0.0371 and wR_2 = 0.0893 for 4416 observed reflections [$I > 2\sigma(I)$], refining 575 parameters and no restraints, semiempirical absorption correction from ψ-scans (T_{min} = 0.9724, T_{max} = 1.0000), final electron density between −0.141 and 0.118 e Å^{-3}.

2-Acetoxyrhizopogone (5): UV (MeOH) λ_{max} 296 nm; CD (*c* ≈ 0.2 mM, MeOH) λ ($\Delta\varepsilon$) 244 (+5.8), 290 (+1.7, shoulder), 307 (+0.1), 334 (+1.5) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.13 (1H, dd, J = 10, 1.5 Hz, H-10′#), 5.09 (1H, dd, J = 10, 6 Hz, H-2′), 4.84 (1H, d, J = 9 Hz, H-14′), 4.76 (1H, t, J = 6 Hz, H-6′#), 3.75 (1H, ψ-q, J = 9 Hz, H-13′), 3.22 (1H, dd, J = 13.0, 10.0 Hz, H-1′a), 3.01 (1H, dd, J = 13.0, 6.0 Hz, H-1′b), 2.19−1.92 (≈ 8H, m, H-12′a, and 3.5 × CH_2), 2.09 (3H, s, $COCH_3$), 1.82 (1H, m, 0.5 × CH_2), 1.75, 1.72 (each 3H, s, CH_3-19′ and CH_3-20′), 1.67 (3H, s, CH_3-16′), 1.6 (1H, m, H-12′b, obscured by H_2O signal), 1.52, 1.40 (each 3H, s, CH_3-17′ and CH_3-18′), chemical shifts and integrals of nonassigned CH_2 protons are tentative, # assignments interchangeable; EIMS *m/z* 468 [M^+] (100), 408 [M^+ − AcOH] (10), 393 [M^+ − AcOH − CH_3] (19), 327 (12), 313 (12), 299 [$C_{18}H_{19}O_4^+$] (30), 259 [$C_{15}H_{15}O_4^+$] (74), 220 [$C_{12}H_{12}O_4^+$] (42), 187 (21), 149 (19), 135 (19), 119 (31), 111 (19), 91 (35), 81 (39), 69 (42), 55 (40); HREIMS *m/z* 468.2537 (calcd for $C_{28}H_{36}O_6$, 468.2511).

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Supporting Information Available: Photographs of *R. pumilionus*, UV/vis spectra of **1**; 1D and 2D NMR spectra and EIMS spectra of compounds **1**, **2**, and **5**; 1D NMR spectra of **4**; bond lengths and bond angles for both atropdiastereomers of **4** present in the crystal (X-ray crystal structure analysis). This information is available free of charge via the Internet at http://pubs.acs.org.

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(16) Crystallographic data for the structure **4** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC-266984). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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