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Studies on the Structure and Biosynthesis of Tridentoquinone and Related Meroterpenoids from the Mushroom *Suillus tridentinus* (Boletales)

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Dedicated to Professor Herbert Mayr on the occasion of his 60th birthday

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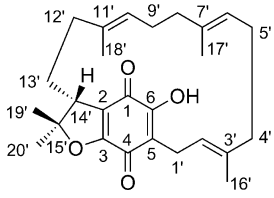
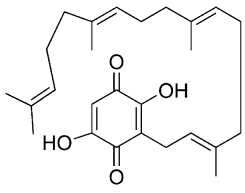
Tridentoquinone (**1**), the main pigment of *Suillus tridentinus*, is accompanied by the known meroterpenoid bolegrevilol (**3**) and a dimer, tridentorubin (**5**). The absolute configuration of **1** was unambiguously established by a single-crystal X-ray analysis of the corresponding (–)-camphanoate. The structure of **5** was elucidated by 2D NMR techniques including a 2D INADEQUATE experiment. Feeding experiments with [1-^{13}C]-labeled 4-hydroxybenzoic acid (**6***) and 3,4-dihydroxybenzoic acid (**7***) proved the incorporation of these precursors into all three metabolites. Tridentoquinone (**1**) was monolabeled at C-1 suggesting the formation of the ansa ring by oxidative cyclisation of 2-geranylgeranyl-6-hydroxybenzoquinone (**10**). This was supported by the isolation of the expected intermediate, deoxytridentoquinone (**11**), from the mushroom extract. Tridentorubin (**5**) may be formed by addition of precursor **10** to tridentoquinone (**1**). This hypothesis is backed up by the in vitro formation of an analogous product **13** from **1** and 1,4-benzoquinone.

Introduction

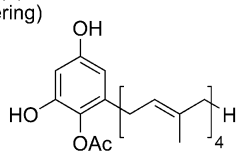
Suillus tridentinus (Bres.) Singer (German: Rostroter Lärchen-Röhrling) is an edible mushroom easily recognized by its beautifully orange pores and cinnamon brown cap. It is found from July to October on limestone in Alpine larch forests. The fruit bodies contain as main pigment tridentoquinone (**1**),[1] a unique red ansaquinone, which is apparently related to the linear boviquinone-4 (**2**) from *S. bovinus*.[2] Up to 35 mg of quinone **1** can be isolated from a single fruit body of *S. tridentinus*. In the present publication we describe a reinvestigation of the constituents of this fungus and studies on their biosynthesis.



tridentoquinone (**1**)
(biogenetic numbering)



boviquinone-4 (**2**)



bolegrevilol (**3**)

Results and Discussion

Isolation of Tridentorubin and Bolegrevilol

During our studies on the biosynthesis of tridentoquinone (**1**), we isolated two additional meroterpenoids. Chromatography of the crude EtOAc extract on acetylated polyamide-6 with hexanes yielded a mixture of tridentoquinone (**1**) and a new compound, named tridentorubin (**5**), which could be efficiently separated by high speed counter current chromatography (HSCCC). Consecutive elution of the polyamide column with EtOAc yielded bolegrevilol (**3**), already known from other *Suillus* and *Gomphidius* species.[3]

Absolute Configuration of Tridentoquinone

Originally, the (14′*S*) configuration was deduced for tridentoquinone (**1**) based on the weak anomalous dispersion

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of oxygen in X-ray diffraction studies.[1] In order to confirm this assignment, we synthesized several derivatives of **1** containing either a heavy atom or a chiral residue of known absolute configuration attached to the enolic hydroxy group. Finally, crystallisation of the (–)-camphanoate **4** from ethanol at –20 °C yielded suitable crystals for a single-crystal X-ray structure analysis. The molecular structure shown in Figure 1 established the absolute configuration as (14′*R*). Thus, the original (14′*S*) assignment is wrong and has to be corrected. The ^{13}C NMR signals of tridentoquinone were unambiguously attributed by 2D NMR experiments, including HMBC, HMQC, and INADEQUATE techniques.

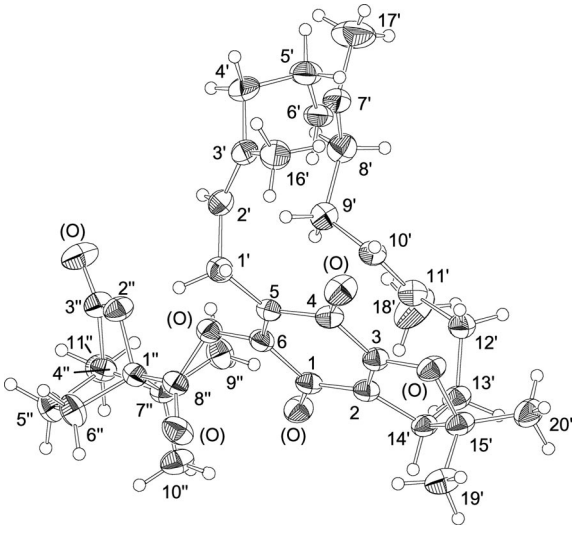
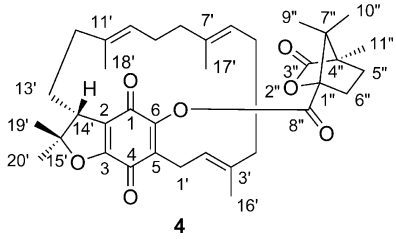


Figure 1. ORTEP plot derived from a single-crystal X-ray analysis of camphanoate **4**.



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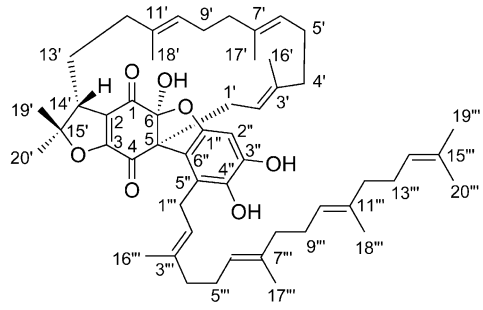
Tridentorubin

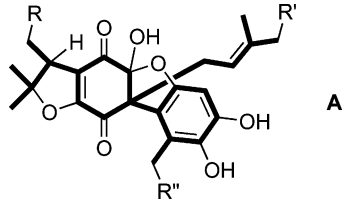
Tridentorubin (**5**) was isolated as a red oil showing UV/Vis maxima at 300 and 420 nm. The compound caused an orange spot on TLC that exhibited no colour change upon exposure to NH_3, excluding the presence of a hydroxyquinone moiety. In the HR-EIMS a molecular ion at *m*/*z* = 806.5126 corresponds to the molecular formula $C_{52}H_{70}O_7$, indicating a dimeric structure. This is supported by the mass spectrum, in which two prominent fragment ions at *m*/*z* = 410 ($C_{26}H_{34}O_4$) and 398 ($C_{26}H_{38}O_3$) can be recognized that match the molecular ions of tridentoquinone and (geranylgeranyl)benzenetriol, respectively. The tridentoquinone ion gives rise to characteristic fragments at *m*/*z* = 395, 329, 261, 259, 220, 209, 208, 207, 147, 121 and a prominent fragment ion at *m*/*z* = 81.[4] In contrast, the (geranylgeranyl)benzenetriol ion is responsible for intense fragment ions at *m*/*z* = 329, 261, 193, 139, 137 and the base ion at *m*/*z* = 69 (100%), resulting from sequential loss of isoprene units from the parent ion *m*/*z* = 398.[4]



tridentorubin (**5**)
(biogenetic numbering)

The ^{13}C NMR spectrum of tridentorubin (**5**) exhibits signals for 10 methyl, 14 methylene, 9 methine, and 19 quaternary carbon atoms. The methine carbon atoms could be further divided into 7 olefinic CH, one aromatic CH at δ_C = 97.1, and a characteristic CH signal at δ_C = 51.0 ppm (δ_H = 2.98, dd, *J* = 10.2, 2.6 Hz). The latter indicates that **5** still contains the dihydrofuran ring of tridentoquinone (**1**), in which the corresponding methine signal occurs at δ_C = 48.1 ppm (δ_H = 3.06, t, *J* = 4.9 Hz). In comparison to the two quinone carbonyl signals of **1** at δ = 179.6 and 180.4 ppm, the C=O signals of **5** are shifted towards lower field (δ = 188.2, 191.6 ppm), indicating a fundamental change in the benzoquinone part of the molecule.

In order to gain more structural information, a 2D INADEQUATE experiment[5] was carried out with 280 mg of tridentorubin (**5**). From the observed C–C connectivities, a functionalized benzofuro[5,6-*b*]benzofuran system **A** could be firmly identified as central core of the molecule (Figure 2). Considering the unaccounted atoms and the breakdown of tridentorubin into tridentoquinone and (geranylgeranyl)benzenetriol in the mass spectrum, partial structure **A** can be extended to formula **5**, which is in accordance with the ^1H and ^{13}C NMR spectroscopic data and the COSY and COLOC spectra. The assumption that tridentorubin (**5**) has the same absolute configuration as the main pigment tridentoquinone (**1**) leads to the (*R*) configuration for C-14′.



A

Figure 2. Central core **A** of tridentorubin (**5**) as determined by a 2D INEDAQUATE experiment.

Previous Investigations on the Biosynthesis of *Suillus* Meroterpenoids

Previous studies on the biosynthesis of boviquinone-4 (**2**) in *S. bovinus* revealed that the quinone ring is formed from 4-hydroxybenzoic acid (**7**) via 3,4-dihydroxybenzoic acid (**8**).[6,7] Rapid tautomerisation of the 2,5-dihydroxybenzoquinone **2** obtained after feeding the [1-^{13}C]-labeled precursors leads to an equal distribution of the label between positions 2 and 4. Guided by the structural similarity of tridentoquinone to boviquinone-4, expressed in formulas **1** and **2**, we originally assumed that ansaquinone **1** is formed from dihydroxyquinone **2** by oxidative cycloaddition of the terminal polyprenyl double bond to the hydroxyquinone unit. A boviquinone-4 epoxide was considered as plausible intermediate.[7,8] In order to obtain experimental proof for this suggestion, feeding experiments with ^{13}C-labeled precursors were carried out.

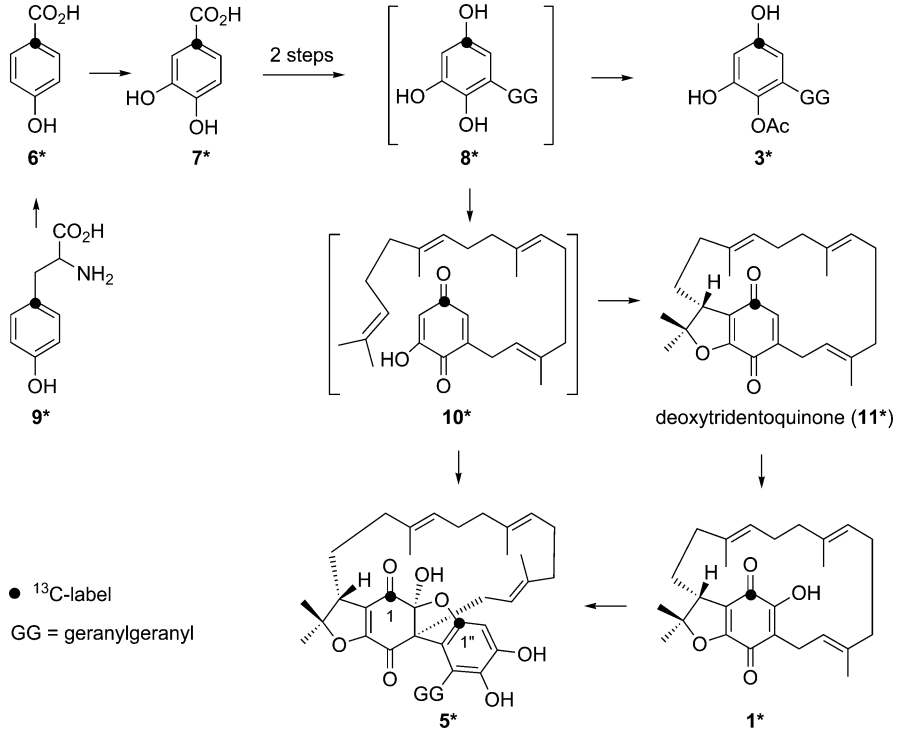
Feeding of [^{13}C]-Labeled Precursors to Fruit Bodies of *S. tridentinus*

Administration of 4-hydroxy-[1-^{13}C]benzoic acid[9] (**6***) or 3,4-dihydroxy-[1-^{13}C]benzoic acid[10] (**7***) to young fruit bodies of *S. tridentinus* in their natural environment led to 4–5% incorporation of these precursors into tridentoquinone (**1***), bolegrevilol (**3***), and tridentorubin (**5***) (for details see Table 1). Importantly, only the carbonyl group at C-1 in tridentoquinone (**1***) was labeled with ^{13}C (Scheme 1). This excludes boviquinone-4 (**2**) as "symmetrical" precursor of **1** and renders our original hypothesis as invalid, which would have resulted in equal labeling of positions C-1 and C-3. The labeled carbonyl group in tridentoquinone corresponds to C-1 of 3,4-dihydroxybenzoic acid, suggesting 6-(geranylgeranyl)benzene-1,2,4-triol (**8**) as possible intermediate. This triol could then either be acetylated to yield bolegrevilol (**3**) or oxidized to hydroxybenzoqui-

Table 1. Feeding experiments with fruit bodies of *S. tridentinus*.

Precursor	Amount per fruit body	Number of fruit bodies	Solvent or solubilizer	^{13}C-Enrichment of **1**
6*[a]	40 mg	5	water	3.9% (C-1)
6*	25 mg	8	acetone	4.0% (C-1)[b]
6*	10 mg	1	cyclodextrin[c]	4.3% (C-1)
7*	33 mg	3	DMSO	5.4% (C-1)
15a*/15b*	40 mg	1	water	0.96% (C-1), 0.79% (C-4)
8*	20 mg	2	Et_2O	0%
8*	10 mg	2	liposomes	0%
14*	20–40 mg	9	Et_2O	0%
14*	10 mg	3	liposomes	0%
16*	10 mg	3	liposomes	0%
17	10 mg	5	cyclodextrin[c]	**20** (0.5 mg)
18	10 mg	4	cyclodextrin[c]	**20** (2.0 mg)

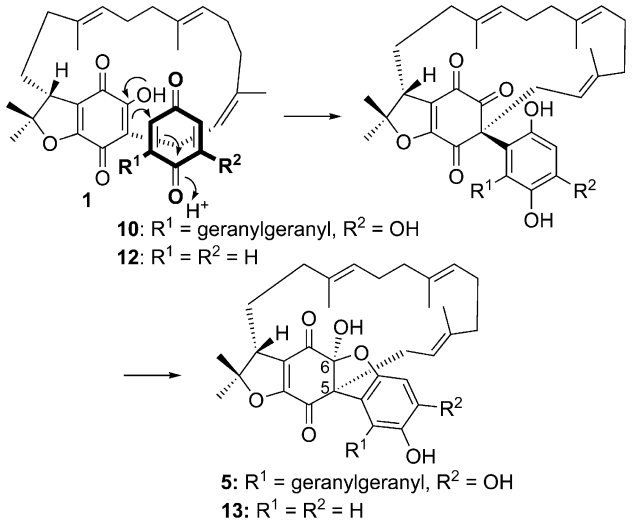
[a] Ammonium salt. [b] Bolegrevilol (**3***) and tridentorubin (**5***) showed ^{13}C-enrichments of 5.4% (C-4), resp. 3.3% (C-1) and 5.9% (C-1′′). [c] 2-Hydroxypropyl-β-cyclodextrin (Aldrich), average degree of substitution: 7, in water.



Scheme 1. Labeling pattern of the meroterpenoids **1***, **3***, and **5*** after feeding of 4-hydroxy-[1-^{13}C]benzoic acid (**6***) or 3,4-dihydroxy-[1-^{13}C]benzoic acid (**7***) to fruit bodies of *S. tridentinus*.

none **10**, the immediate precursor of deoxytridentoquinone (**11**). The latter could be formed by oxidative cycloaddition of the hydroxyquinone moiety of **10** to the terminal double bond of the side chain, probably by a radical mechanism. Experimental evidence for this type of reaction is known from the literature.[11] Hydroxylation of ansaquinone **11** would then yield tridentoquinone (**1**), in accordance with the labeling results. This suggestion is supported by the isolation of the presumed intermediate **11** from specimens of *S. tridentinus*, which had been applied for feeding experiments.[12] Deoxytridentoquinone (**11**) exhibits a molecular ion at *m*/*z* = 394 and is visible as yellow spot on TLC showing no colour change upon exposure to ammonia vapour. The NMR spectra of **11** strongly resemble those of tridentoquinone (**1**) with the exception of the additional methine signal at δ_H = 6.34 ppm (pseudo-q, $^4J_{H,H} \approx 1.1$ Hz, δ_C = 134.0 ppm) and the absence of the corresponding enol carbon signal.[13] The lack of the 6-hydroxy group causes a downfield shift of the C-5 signal from δ_C = 116.4 (**1**) to 145.4 ppm (**11**).

The labeling pattern of tridentorubin (**5***) can be explained by addition of hydroxyquinone **10*** to tridentoquinone (**1***) as depicted in Scheme 1 and Scheme 2.[14] Interestingly, the incorporation rates for C-1 (3.3%) and C-1" (5.9%) differ, suggesting that labeled **1*** is diluted by the tridentoquinone pool already present in young mushrooms.



Scheme 2. Reaction of tridentoquinone (**1**) with 1-geranylgeranyl-3-hydroxy-1,4-benzoquinone (**10**) and 1,4-benzoquinone (**12**), respectively.

Since attack of the electrophilic quinone **10** on the enol group of tridentoquinone (**1**) can only occur from the plane opposite to the sterically demanding ansa ring, the (5*S*)/(6*S*)-stereochemistry given in formula **5** can be proposed for tridentorubin (Scheme 2). This is supported by a model reaction. Stirring of tridentoquinone (**1**) with 1,4-benzoquinone (**12**) in THF at room temperature led to the formation of a single product **13** in 10–16% yield, visible as an orange spot on the TLC. Refluxing the mixture in THF did not increase the yield, and addition of bases (DBU) or acids (TFA) led only to decomposition. The corresponding NMR signals of compound **13** agreed with those of tridentorubin (**5**), and the analogue exhibited a nearly identical CD spectrum showing that both compounds possess the same absolute and relative configuration.[15]

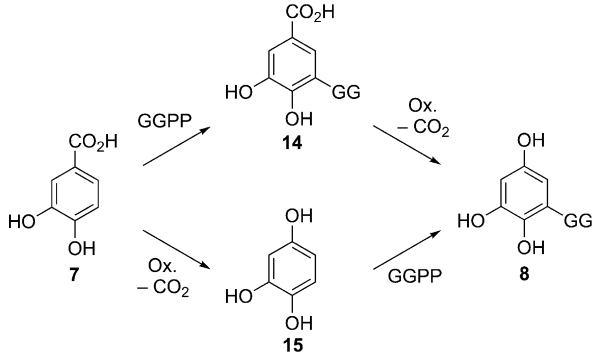
The role of 4-hydroxybenzoic acid (**6**) and 3,4-dihydroxybenzoic acid (**7**) as biosynthetic precursors of the *Suillus* meroterpenoids is highlighted by the detection of their methyl esters in the GC/MS, after treatment of the crude mushroom extract with diazomethane.

That *S. tridentinus* is able to transform tyrosine into 4-hydroxybenzoic acid was demonstrated in a long-term incubation of the submerse culture with DL-[1′-^{13}C]tyrosine (**9***),[10] resulting in 12% enhancement of the C-1 signal of tridentoquinone (**1**) (Table 2). In contrast, feeding of labeled tyrosine to the fruit bodies gave no detectable incorporation, probably due to the low solubility of this amino acid in aqueous media.

Table 2. Feeding experiments with submerse cultures of *S. tridentinus*.

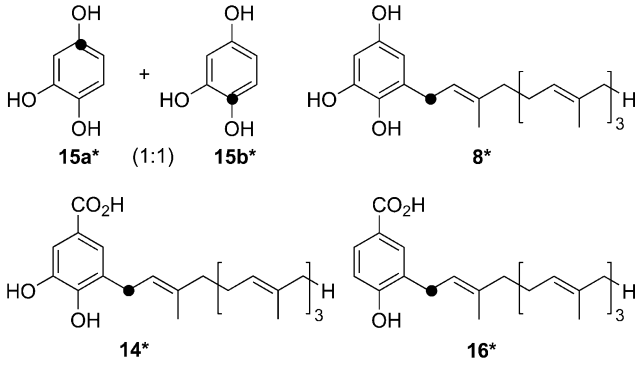
Precursor	Amount of precursor	Solvent	Incubation period	^{13}C Enrichment of **1***
6*	50 mg	acetone	10 weeks	2.8% (C-1)
9*	80 mg	–	5 months	12% (C-1)

For the conversion of 3,4-dihydroxybenzoic acid (**7**) into 6-(geranylgeranyl)benzene-1,2,4-triol (**8**) two alternatives can be considered, differing in the sequence of the polyprenylation and oxidative decarboxylation steps (Scheme 3). To distinguish between these possibilities, the ^{13}C-labeled precursors shown in Scheme 4 were synthesized and administered to young fruit bodies of *S. tridentinus*.



Scheme 3. Two possibilities for the conversion of 3,4-dihydroxybenzoic acid (**7**) into 6-(geranylgeranyl)benzene-1,2,4-triol (**8**).

Feeding a 1:1 mixture of 1- and 4-^{13}C-labeled benzene-1,2,4-triol (**15a*/15b***) gave tridentoquinone (**1***) with equal signal enhancements of C-1 and C-4, corresponding to 1.8% incorporation (Table 1). This finding implies that *S. tridentinus* is able to geranylgeranylate triol **15** with formation of meroterpenoid **8**, the putative precursor of bolegrevilol (**3**) and tridentoquinone (**1**). The low incorporation of **15*** as compared to that of **6*** or **7*** is probably due to losses by enzymatic oxidative polymerization, visible as black spots on the injection sites. Benzene-1,2,4-triol (**15**) is a known metabolite of several *Gomphidius* species,[16] in

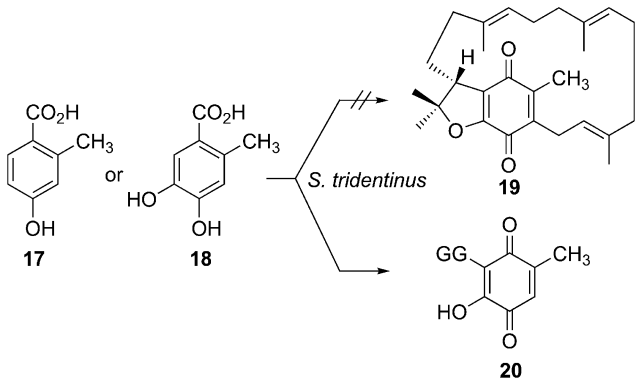


Scheme 4. Additional ^{13}C-labeled precursors used for feeding experiments.

which it occurs together with bolegrevilol (**3**).[3c] Feeding experiments with *G. glutinosus* proved the biosynthetic origin of triol **15** and bolegrevilol (**3**) from 4-hydroxybenzoic acid.[17]

In contrast, administration of 3-[1′-^{13}C]geranylgeranyl-4,5-dihydroxybenzoic acid[18] (**14***) or the potential precursors **8*** and **16***[18] to *S. tridentinus* under varying conditions (solution in ether, solubilization in water by addition of cyclodextrin or package in liposomes) gave no detectable incorporation (Table 1). These negative results can be explained with difficulties of the lipophilic precursors to reach the organelles in which the biosynthesis takes place. This indicates the limits of feeding experiments with isotope labeled precursors and awaits studies on the enzymatic level to reach an unambiguous decision between these two possibilities.

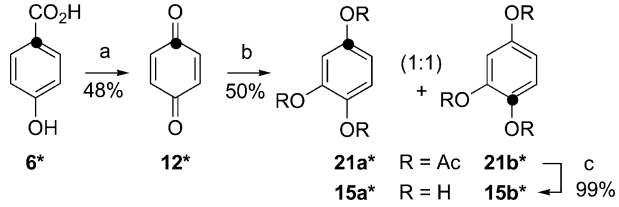
In an additional experiment, 4-hydroxy-2-methylbenzoic acid (**17**) and 4,5-dihydroxy-2-methylbenzoic acid (**18**), respectively, were fed to *S. tridentinus*. We expected that the methyl group would block the final hydroxylation and therefore lead to 6-deoxy-6-methyltridentoquinone (**19**). To our surprise, however, in both experiments small amounts of 2-geranylgeranyl-3-hydroxy-6-methyl-1,4-benzoquinone (**20**) were isolated instead (Scheme 5). Obviously, the "normal" prenylation at C-3 is sterically hindered and the mushroom switches to C-6, a position typical for the polyprenylation in *S. bovinus* and other *Suillus* species.[6]



Scheme 5. Formation of hydroxybenzoquinone **20** after feeding the 2-methylated hydroxybenzoic acids **17** and **18**.

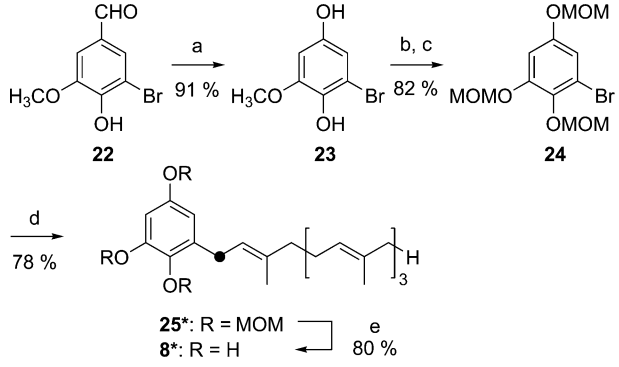
Syntheses of Precursors Used for the Feeding Experiments

The synthesis of a 1:1 mixture of [4-^{13}C]- and [1-^{13}C]benzene-1,2,4-triol (**15a*/15b***) commenced from ring-labeled 4-hydroxybenzoic acid **6***,[9] which on acidic decarboxylation[19] followed by oxidation with Fremy's salt furnished [1-^{13}C]-1,4-benzoquinone (**12***) (Scheme 6). Subsequent Thiele–Winter reaction[20] gave two differently labeled triacetates **21a*/21b***, which on acid-catalyzed methanolysis yielded a 1:1 mixture of [4-^{13}C]- and [1-^{13}C]benzene-1,2,4-triol (**15a*/15b***). The transformation was accomplished in 4 steps with 24% overall yield. In comparison, the preparation of uniformly labeled [4-^{13}C]benzene-1,2,4-triol would have required 7 reaction steps.[10]



Scheme 6. Synthesis of a 1:1 mixture of [4-^{13}C]- and [1-^{13}C]benzene-1,2,4-triol (**15a*/15b***); reagents and conditions: a) 10 N HCl, 150–160 °C, 24 h; then KH_2PO_4 buffer, $ON(SO_3K)_2$, room temp., 2 h; b) Ac_2O, H_2SO_4 c) HCl, MeOH, reflux.

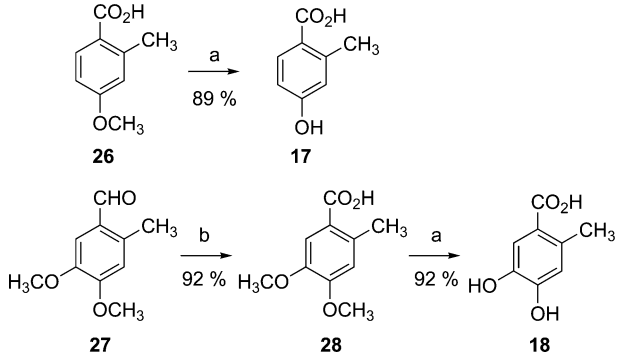
For the preparation of side-chain labeled deacetylbolegrevilol (**8***), 5-bromovanillin[21] (**22**) was subjected to Kabalka's variant of the Dakin reaction[22] (Scheme 7). The resulting bromo-methoxyhydroquinone **23** was deprotected[23] and subsequently treated with chloromethoxymethane to yield the tris-MOM ether **24**.[24] Alkylation of lithiated **24** with [1-^{13}C]geranylgeranyl bromide[18] in the presence of a catalytic amount of Li_2CuCl_4[25] afforded the polyprenyl derivative **25*** in 74–83% yield, which on deprotection under mildly acidic conditions gave 6-[1′-^{13}C](geranylgeranyl)benzene-1,2,4-triol (**8***).



Scheme 7. Synthesis of the side-chain labeled precursor **8***; reagents and conditions: a) $Na_2CO_3 \times 1.5\ H_2O_2$, THF, H_2O; b) BBr_3, CH_2Cl_2; c) MOMCl, *i*PrNEt$_2$, CH_2Cl_2; d) *n*BuLi, THF, –78 °C, 0.25 h; then Li_2CuCl_4 (0.04 equiv.), (*E*,*E*,*E*)-[1-^{13}C]geranylgeranyl bromide,[18] –78 °C → room temp.; e) AcCl, MeOH.

4-Hydroxy-2-methylbenzoic acid (**17**) was obtained by demethylation[23] of 4-methoxy-2-methylbenzoic acid (**26**).[26] 4,5-Dihydroxy-2-methylbenzoic acid (**18**) was pre-

pared by sodium chlorite oxidation[26] of 4,5-dimethoxy-2-methylbenzaldehyde[27] (**27**) and subsequent demethylation (Scheme 8).



Scheme 8. Synthesis of 2-methyl-hydroxybenzoic acids **17** and **18**; reagents and conditions: a) BBr_3, CH_2Cl_2; b) $NaClO_2$, H_2NSO_3H, THF, H_2O.

Conclusions

In conclusion, the specific incorporation of [1-^{13}C]-labeled hydroxybenzoic acids **7*** and **8*** into tridentoquinone (**1**) and tridentorubin (**5**) by fruit bodies of *S. tridentinus* has been demonstrated. The labeling at C-1 and C-1/C-1′′, respectively, is consistent with 2-geranylgeranyl-6-hydroxy-1,4-benzoquinone (**10**) playing a dual role as precursor of 2-deoxytridentoquinone (**11**) as well as of tridentorubin (**5**). In the latter case an addition of **10** to tridentoquinone (**1**) takes place, a reaction,[15] which could be mimicked by a model experiment.

Experimental Section

General: Melting points (uncorrected): Reichert Thermovar hot-stage. Optical rotations: Perkin–Elmer 241. Elemental analyses: Microanalytical Laboratory, Ludwig-Maximilians-Universität München and Universität Bonn, respectively. IR: Perkin–Elmer FT-IR 1000. Intensity of the bands: ss (very strong), s (strong), m (medium), and w (weak). UV/Vis spectra: Perkin–Elmer Lambda 16. CD spectra: Jobin Yvon Instruments S.A. CD-6-Dichrograph. NMR: Bruker WH 90, WM 200, WM 400, ARX 300, and AMX 600, with TMS (indicated) or the solvent peak as an internal standard ($CDCl_3$: δ_H = 7.26, δ_C = 77.1; [D$_6$]DMSO: δ_H = 2.49, δ_C = 39.7. CD_3OD: δ_H = 3.31, δ_C = 49.0; [D$_6$]acetone: δ_H = 2.04, δ_C = 29.8). ^{1}H-coupled ^{13}C NMR: multiplets due to ^1J(C,H) couplings are indicated by capital letters, ^3J and other couplings in small letters. MS: Finnigan MAT 90 and MAT 95Q, A.E.I. MS 30 and MS 50 (direct inlet, 70 eV). GC/EI-MS: Varian 3400, 25 m BP5 column (SGE), with Finnigan MAT 95Q. X-ray diffraction: Enraf–Nonius CAD4 diffractometer at 293(2) K using graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å). Analytical HPLC: Waters M 510 Pump (flow rate: 1 mL/min), System Controller M721 and M730 with Photodiode Array Detector 990+, column: Bischoff nucleosil C_6H_5 (7 μm), 4 x 250 mm; solvent A: 0.2% ammonium acetate buffer (pH 5.7)/MeCN, 9:1; solvent B: MeCN; HPLC system 1: 100% A to 100% B within 60 min. Analytical TLC: Silica gel 60 F_{254} aluminium foils (Merck); solvent system A: toluene/HCO_2H/HCO_2Et, 10:5:3. Flash chromatography: silica gel 60, 40–63 μm (Merck). Column chromatography: acetylated polyamide-6 (Polyamide SC-6AC, 50–160 μm, Macherey–Nagel). Gel permeation chromatography: Sephadex LH-20 (Pharmacia). High-speed countercurrent chromatography (HSCCC) was performed with an apparatus from P.C. Inc., Potomac, MD, USA, consisting of a multi-layer coil, a counter-weight/triple coil and a Rainin's Dynamax® SD-200 pump. The numbering of the carbon skeleton follows biogenetic considerations and is indicated in formulas **1** and **5**. All solvents were distilled prior to use. Air and moisture sensitive compounds were handled under argon using standard Schlenk techniques. THF was distilled under Ar from Na/benzophenone. CH_2Cl_2 was distilled under Ar from Sicapent (Merck).

Mushrooms: The collecting of *S. tridentinus* and the feeding experiments were carried out in September/October 1985–2001 in larch forests near Ehrwald and Nassereith, Tyrol, Austria. *S. tridentinus* was cultured in modified Moser's medium B: aneurin: 50 g, biotin: 1 g, inositol: 50 mg, KH_2PO_4: 0.5 g, $MgSO_4$: 0.5 g, 0.002% (w/v) aq. $ZnSO_4$: 0.5 mL, 1% (w/v) aq. $FeCl_3$: 1 mL, 0.1 M aq. $CaCl_2$: 5 mL, 1% (w/v) aq. $MnSO_4$: 0.5 mL, yeast extract: 0.2 g, maltose: 20 g, glucose: 10 g, peptone: 2 g, demineralised water: ad 1 L.

Isolation and Structural Elucidation

Isolation Procedure: Lyophilized fruit bodies of *S. tridentinus* (33 g) were pulverized and extracted exhaustively with EtOAc. The combined extracts were concentrated and the brown residue taken up in a small amount of EtOAc. Chromatography on acetylated polyamide-6 with *n*-hexane yielded **1** together with **5**. Subsequent elution with EtOAc afforded crude **3**. The *n*-hexane fraction was concentrated and the resulting residue dissolved in *n*-hexane (5 mL). On separation by HSCCC [mobile phase: *n*-hexane, 4 volume parts, stationary phase: AcOH/MeOH, 1:1 volume parts; 230 mL column, forward rotation mode, 900 rpm, flow: 1.5 mL/min] pure **1** (69 mg, yield 0.2% of dry-weight) eluted with the mobile phase first, followed by pure **5** (21 mg, yield 0.06% of dry-weight). The yields of **5** varied considerably, depending on the status of the fungi and the isolation procedure.[15]

Tridentoquinone (1): Yields variable, up to 1.3% of dry-weight. Dark red oil, crystals from hexanes. R_f (TLC) = 0.76 (solvent system A), red spot, + NH_3 blue. R_t (HPLC) = 35.8 min (system 1). CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 289 (0.4), 337 (–0.8) nm. For m.p., $[\alpha]_D$, UV and IR data, see ref.[1] for MS data ref.[4] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.38 (s, 3 H, 19′-H), 1.48 (s, 3 H, 18′-H), 1.52 (s, 3 H, 17′-H), 1.56 (s, 3 H, 20′-H), 1.70 (s, 3 H, 16′-H), 1.60–2.20 (m, 12 H, 4′-H, 5′-H, 8′-H, 9′-H, 12′-H, 13′-H), 3.02 (dd, J = 13.8, 8.1 Hz, 1 H, 1′-H$_A$), 3.06 (t, J = 4.9 Hz, 1 H, 14′-H), 3.18 (dd, J = 13.8, 7.4 Hz, 1 H, 1′-H$_B$), 4.89 (dd, J = 8.1, 4.8 Hz, 1 H, 10′-H), 4.95 ("t", J = 6 Hz, 1 H, 6′-H), 5.16 (t, J = 7.4 Hz, 1 H, 2′-H), 7.24 (s, 1 H, OH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.7 (C-16′), 15.9 (C-18′), 16.7 (C-17′), 21.4 (C-1′), 22.2 (C-20′), 24.2 (C-5′), 24.5 (C-13′), 27.2 (C-9′), 29.5 (C-19′), 35.3 (C-12′), 38.9 (C-4′), 39.0 (C-8′), 48.1 (C-14′), 95.3 (C-15′), 116.4 (C-5), 118.4 (C-2), 120.7 (C-2′), 123.8 (C-6′), 125.9 (C-10′), 134.2 (C-11′), 135.1 (C-7′), 135.5 (C-3′), 152.7 (C-6), 159.7 (C-3), 179.6 (C-1), 180.4 (C-4) ppm. The assignments were confirmed by a 2D INADEQUATE experiment.

Bolegrevilol (3): Crude **3** was purified by chromatography on Sephadex LH-20 (acetone/MeOH, 4:1; subsequently MeOH) to furnish the pure compound as a colourless oil (16 mg, yield 0.05% of dry-weight). R_f (TLC) = 0.49 (solvent system A), colourless spot. R_t (HPLC) = 43.20 min (system 1). For spectroscopic data, see ref.[3a,3b]

Tridentorubin (5): Red oil. R_f (TLC) = 0.67 (solvent system A), orange spot, + NH_3 no colour change. R_t (HPLC) = 45.73 min (system 1). $[\alpha]_D^{20}$ = +1054 (c = 0.5, MeOH). UV/Vis (qualitative, MeOH): 300, 320 (sh), 425 nm. CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 221 (3.9), 288 (–0.4), 310 (1.1) nm. IR (KBr): $\tilde{\nu}$ = 3558 (m), 3389 (s, br), 2965 (s), 2917 (ss), 2853 (s), 1694 (m), 1660 (ss), 1609 (s), 1471 (m), 1453 (s), 1355 (m), 1325 (m), 1304 (m), 1255 (m), 1217 (m), 1159 (m), 1108 (m), 1075 (m), 1036 (m), 986 (w), 962 (w), 910 (w), 849 (m), 734 (w), 704 (w), 573 (w) cm^{-1}. ^1H NMR (600 MHz, [D$_6$]acetone): δ = 1.33 (s, 3 H, 19′-H), 1.48 (s, 3 H), 1.49 (s, 3 H, 20′-H), 1.55 (s, 6 H), 1.56 (s, 6 H), 1.60 (s, 3 H), 1.62 (s, 3 H), 1.80 (s, 3 H), 1.84–2.22 (m, 24 H), 2.86 (dd, J = 13.3, 7.3 Hz, 1 H, 1′-H_A), 2.98 (dd, J = 10.2, 2.6 Hz, 1 H, 14′-H), 3.30 (dd, J = 13.3, 7.3 Hz, 1 H, 1′-H_B), 3.69 (dd, J = 15.5, 6.3 Hz, 1 H, 1‴-H_A), 4.02 (dd, J = 15.5, 6.3 Hz, 1 H, 1‴-H_B), 4.90–4.97 (m, 3 H), 5.07 (m, 2 H), 5.12 (t, J = 6.4 Hz, 1 H), 5.22 (t, J = 5.3 Hz, 1 H, 2‴-H), 6.09 (s, 1 H, OH), 6.23 (s, 1 H, 2″-H), 6.47 (br. s, 1 H, OH) ppm. ^1H NMR (600 MHz, $CDCl_3$): δ = 1.42, 1.52, 1.57 (each s, 3 H), 1.58 (s, 6 H), 1.60 (s, 3 H), 1.61 (s, 3 H), 1.63 (s, 3 H), 1.68 (s, 3 H), 1.81–1.85 (m, 2 H), 1.87 (3 H), 1.90–2.17 (m, 22 H), 2.95 (dd, J = 13.1, 8.0 Hz, 1 H), 2.98 (dd, J = 10.0, 2.5 Hz, 1 H), 3.30 (dd, J = 13.1, 8.0 Hz, 1 H), 3.76 (dd, J = 16.2, 6.2 Hz, 1 H), 3.86 (dd, J = 16.2, 6.2 Hz, 1 H), 4.85–4.91 (m, 2 H), 4.95 (m, 1 H), 5.05–5.10 (m, 3 H), 5.35 (br. t, J = 6.2 Hz, 1 H), 6.35 (s, 1 H), 6.65 (br., 1 OH), 8.52 (br., 1 OH) ppm. ^1H-coupled ^{13}C NMR (151 MHz, [D$_6$]acetone): δ = 15.5 (CH_3), 16.1 (CH_3), 16.3 (CH_3), 16.7 (CH_3), 16.8 (CH_3), 17.7 (CH_3), 22.6 (Q, J = 126 Hz, CH_3-20′), 25.3 (CH_2), 25.8 (CH_3-19‴), 26.5 (CH_2), 26.8 (CH_2), 27.3 (CH_2), 27.4 (CH_2), 27.5 (Tm, J = 120 Hz, CH_2-1‴), 29.0 (Q, J = 117 Hz, CH_3-19′), 30.8 (T, J = 128 Hz, CH_2-13′), 33.3 (T, J = 130 Hz, CH_2-1′), 39.1 (CH_2), 39.4 (CH_2), 40.37 (2 × CH_2), 40.44 (CH_2), 42.2 (T, J = 125.5 Hz, CH_2-4′), 51.0 (D, J = 135.5 Hz, C-14′), 68.2 (d, J = 6.1 Hz, C-5), 94.7 (br. m, C-15′), 97.1 (D, J = 161 Hz, C-2″), 105.2 (d, J = 7.1 Hz, C-6), 116.9 (d, J = 5.1 Hz, C-6″), 119.9 (Dm, J = 149 Hz, CH_{vin}-2′), 124.8 (CH_{vin}), 125.07 (CH_{vin}), 125.10 (CH_{vin}), 125.2 (CH_{vin}), 125.5 (Dq, J = 148, 6 Hz, CH_{vin}-2‴), 127.8 (CH_{vin}), 128.71 (m, C-2), 128.74 (m, C-5″), 131.6 ("septet", J = 6 Hz, C_{vin}-15‴), 134.0 (C_{vin}), 134.7 (m, C_{vin}-3‴), 135.4 (C_{vin}), 135.5 (C_{vin}), 136.2 (C_{vin}), 139.9 (m, C-4″), 140.2 (m, C_{vin}-3′), 146.7 (br. s, C-3″), 151.1 (d, J = 4.0 Hz, C-1″), 163.1 (d, J = 4 Hz, C-3), 188.2 (s, C-1), 191.6 (d, J = 8.1 Hz, C-4) ppm. 1 CH_3 signal obscured (in $CDCl_3$ 7 CH_3 signals visible between δ = 15 and 18.5 ppm). C-C connectivities observed in the 2D INADEQUATE experiment: C-1 ↔ C-2 ↔ C-3 ↔ C-4 ↔ C-5 ↔ C-6 ↔ C-1; C-2 ↔ C-14′ ↔ C-15′ ↔ C-19′, C-20′; C-13′ ↔ C-14′; C-5 ↔ C-1′ ↔ C-2′ ↔ C-3′ ↔ C-4′, C-16′; C-5 ↔ C-6″ ↔ C-1″ ↔ C-2″ ↔ C-3″ ↔ C-4″ ↔ C-5″ ↔ C-6″. EI-MS: m/z (%) = 807 (9) [M + 1]$^+$, 806 (18) [M$^+$], 805 (9), 804 (16),412 (21), 411 (28), 410 (93) $[C_{26}H_{34}O_4]^+$, 398 (26) $[C_{26}H_{38}O_3]^+$, 396 (10), 395 (10), 329 (11), 261 (23), 259 (19), 247 (13), 245 (12), 243 (10), 220 (10), 209 (12), 208 (20), 207 (17), 206 (12), 194 (14), 193 (10), 179 (17), 177 (42), 147 (11), 140 (11), 139 (28), 138 (17), 137 (8), 135 (16), 123 (14), 121 (24), 109 (20), 107 (16), 95 (20), 93 (17), 81 (56), 79 (13), 69 (100). HR EI-MS: m/z = 806.5126 [M$^+$] (calcd. for $C_{52}H_{70}O_7$: 806.5122).

Deoxytridentoquinone (11): 5 lyophilized fruit bodies[12] were worked up as described in the general procedure. The pale yellow fractions from the HSCCC separation were treated with Et_2O and washed several times with water. Concentration of the dried ($MgSO_4$) organic phase yielded **11** (1.5 mg, 0.01% of dry-weight) as a yellow oil. R_f (TLC) = 0.81 (solvent system A), yellow spot, + NH_3: no colour change. ^1H NMR (600 MHz, $CDCl_3$): δ = 1.35, 1.46 (each s, 3 H), 1.55 (s, 6 H), 1.64 (s, 3 H), 1.76–1.89 (m, 4 H), 1.94–2.18 (m, 8 H), 2.81 (dd, J = 15, 7.5 Hz, 1 H, 1′-H_A), 3.02 (t, J = 5.5 Hz, 1 H, 14′-H), 3.37 (dd, J = 15, 7.5 Hz, 1 H, 1′-H_B), 4.82, 4.96 (each t, br., J ≈ 6.3 Hz, 1 H, 6′-H, 10′-H), 5.13 (t, br., J ≈ 7.7 Hz, 1 H, 2′-H), 6.34 (pseudo-q, J ≈ 1.1 Hz, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 15.7, 16.2, 16.3 (each CH_3), 22.1 (CH_3-20′), 24.3, 25.4, 27.4, 27.5 (each CH_2), 29.3 (CH_3-19′), 35.2 (CH_2-12′), 38.9, 39.1 (C-4′ and C-8′), 48.3 (CH-14′), 94.1 (C-15′), 120.5, 123.8 (each CH_{vin}), 124.7 (C-2), 126.3 (CH_{vin}), 133.99 (CH-6), 134.02, 135.7, 137.4 (each C_{vin}), 145.4 (C-5), 156.5 (C-3), 180.6 (C-4), 185.6 (C-1) ppm. EI-MS: m/z (%) = 397 (21), 396 (83), 395 (31), 394 (100) [M$^+$], 379 (30), 366 (8), 351 (8), 313 (30), 271 (17), 259 (15), 257 (14), 245 (80), 243 (38), 231 (40), 229 (28), 217 (22), 215 (25), 205 (20), 203 (20), 201 (18), 192 (54), 191 (62), 177 (45), 147 (14), 121 (19), 119 (15), 109 (13), 107 (16), 105 (17), 95 (15), 93 (21), 91 (27), 81 (36), 79 (24), 77 (19), 69 (21), 67 (28), 57 (20), 55 (35), 53 (18), 41 (47).

(+)-(1″*S*)-*O*-Camphanoyl-(14′*R*)-tridentoquinone (4): To a solution of **1** (0.30 g, 0.73 mmol) and triethylamine (0.11 mL, 0.79 mmol) in dry THF (15 mL) were added at 0 °C (1*S*)-(–)-camphanoyl chloride (0.132 g, 0.61 mmol) and a few crystals of 4-(dimethylamino)pyridine (DMAP). After stirring the mixture at room temp. overnight, water (50 mL) was added and the product extracted with Et_2O (50 mL). The organic phase was washed with water and brine, dried ($MgSO_4$), and flash chromatographed on silica gel (hexanes/EtOAc, 9:1) to yield ester **4** (196 mg, 54%) as an orange oil, which crystallized from EtOH, m.p. 123–125 °C. R_f (TLC) = 0.22 (hexanes/EtOAc, 9:1). $[\alpha]_D^{20}$ = +178.5 (c = 0.07, MeCN). IR (KBr): $\tilde{\nu}$ = 2972 (m), 2932 (m), 1799 (ss), 1678 (s), 1658 (s), 1609 (s), 1256 (m), 1167 (m), 1095 (m), 1042 (s) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.16 (s, 6 H), 1.19 (s, 3 H), 1.37 (s, 3 H, 19′-H), 1.46 (s, 3 H, 18′-H), 1.53 (s, 3 H, 17′-H), 1.58 (s, 3 H, 20′-H), 1.71 (s, 3 H, 16′-H), 1.70–2.25 (m, 15 H, 6 × CH_2, 5″-H_2, 6″-H_{endo}), 2.57 (ddd, J = 13.7, 10.8, 4.3 Hz, 1 H, 6″-H_{exo}), 2.92 (dd, J = 13.7, 7.3 Hz, 1 H, 1′-H_A), 3.06 (t, J = 4.8 Hz, 1 H, 14′-H), 3.27 (dd, J = 13.7, 7.3 Hz, 1 H, 1′-H_B), 4.86–4.96 (m, 2 H, 6′-H, 10′-H), 5.00 (br. t, J = 7 Hz, 1 H, 2′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 9.9 (C-11″), 16.0, 16.1, 16.5, 16.6, 16.7 (each CH_3), 22.2, 22.9 (C-1′ and C-20′), 24.2, 24.8, 27.1, 28.9, 31.2 (C-5′, C-9′, C-13′, C-5″, C-6″), 29.6 (C-19′), 35.5 (C-12′), 38.96, 39.00 (C-4′ and C-8′), 48.5 (CH-14′), 54.9, 55.2 (C-4″ and C-7″), 90.9 (C-1″), 94.9 (C-15′), 118.6 (2×, C-2, C-5), 122.4, 123.6, 126.2 (each CH_{vin}), 133.2, 135.6, 137.9 (each C_{vin}), 149.1 (C-6), 157.1 (C-3), 164.8, 176.5 (C-3″ and C-8″), 177.9, 179.4 (C-1 and C-4) ppm. EI-MS (DI, 245 °C): m/z (%) = 593 (100) [M$^+$ + 2H], 591 (27) [M$^+$], 590 (64), 439 (11), 396 (14), 394 (20), 388 (16). 311 (12), 245 (16), 243 (16), 207 (15), 191 (16), 137 (11), 125 (16), 121 (17), 109 (28), 97 (17), 83 (72).

Tridentorubin Analogue 13: A solution of **1** (0.17 g, 0.41 mmol) and **12** in THF (2 mL) was stirred at room temp. for 12 h. After concentration under reduced pressure, the product was purified by chromatography on Sephadex LH-20 (acetone) to yield **13** (35 mg, 16%) as an orange oil. R_f (TLC) = 0.65 (solvent system A), orange-brown spot. CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 215 (3.4), 287 (–0.6), 311 (1.0) nm. IR (KBr): $\tilde{\nu}$ = 2924 (s), 1698 (m), 1669 (ss), 1614 (s), 1446 (s), 1372 (m), 1255 (w), 1152 (w), 1076 (w), 980 (w), 888 (w), 826 (w) cm^{-1}. ^1H NMR (300 MHz, [D$_6$]acetone): δ = 1.40, 1.52, 1.56, 1.59, 1.62 (each s, 3 H), 1.90–2.15 (m, 12 H), 2.84 (dd, J = 13.3, 7.3 Hz, 1 H, 1′-H_A), 3.00–3.15 (m, 2 H, 1′-H_B, 14′-H), 4.90–5.05 (m, 3 H, 2′-H, 6′-H, 10′-H), 6.75 ("s", 1 H, 5″-H), 6.98 ("s", 2 H, 2″-H, 3″-H) ppm. ^{13}C NMR (75 MHz, [D$_6$]acetone): δ = 15.4, 16.1, 16.7 (each CH_3), 22.6 (CH_3-20′), 25.4 (CH_2), 27.1 (CH_2), 29.0 (CH_3-19′), 31.0 (CH_2-13′), 32.7 (CH_2-1′), 39.1 (CH_2), 39.4 (CH_2), 42.0 (CH_2-4′), 50.8 (CH-14′), 66.5 (C-5), 94.7 (C-15′), 106.3 (C-6), 108.4 (3×, C-3″, C-5″, C-6″),* 118.7 (CH_{vin}-2′), 125.0 (CH_{vin}),

127.7 (CH_{vin}), 129.4 (C-2), 129.9 (C-2′′), 134.0 (C_{vin}), 136.2 (C_{vin}), 140.9 (C_{vin}-3′), 152.8 (2×, C-1′′, C-4′′),[#] 162.5 (C-3), 187.6 (C-1), 190.0 (C-4) ppm. *In $CDCl_3$ 3 separate signals at δ = 111.0, 112.4, and 116.2 ppm. [#]In $CDCl_3$ 2 separate signals at δ = 150.3 and 151.5 ppm. EI-MS: *m*/*z* (%) = 520 (8), 519 (36) [M + 1]$^+$, 518 (100) [M$^+$], 500 (5), 412 (6), 411 (11), 410 (36) [**1**$^+$], 329 (12), 304 (15), 303 (46), 302 (17), 301 (10), 286 (10), 285 (36), 215 (10), 177 (9), 121 (7), 81 (14). HRMS (EI): *m*/*z* = 518.26.2 [M$^+$] (calcd. for $C_{32}H_{38}O_6$: 518.2669).

Synthesis of Precursors

[1-^{13}C]-1,4-Benzoquinone (12*): A mixture of **6***[9] (0.59 g, 4.2 mmol) and 10 N HCl (4 mL) was heated at 150–160 °C for 24 h in a steel vessel. After cooling to room temp. and opening the lid, the stirring was continued for 10 min. The mixture was neutralized with 5 N KOH, followed by addition of KH_2PO_4 (8 g). Then, a solution of potassium nitrosodisulfonate (6.8 g, 25 mmol) in water (200 mL) was added dropwise over 1 h, and the stirring was continued for 2 h. After addition of solid NaCl, the crude product was extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to afford **12*** (0.22 g, 48%) as tawny crystals. R_f (TLC) = 0.89 (hexanes/$CHCl_3$/MeOH, 5:4:1; staining with anisaldehyde). HR EI-MS: *m*/*z* = 109.0241 [M$^+$] (calcd. for $C_5{}^{13}CH_4O_2$: 109.0245).

[1-^{13}C]- and [4-^{13}C]-1,2,4-Triacetoxybenzene (21a*/21b*), 1:1 Mixture: To a stirred mixture of Ac_2O (0.57 mL, 6.1 mmol) and H_2SO_4 (43 mg, 0.35 mmol) was added in portions **12*** (0.19 g, 1.75 mmol), whereby the temperature was kept below 45 °C by ice cooling. After stirring at room temp. for 2 h, water (5 mL) was added, upon which the product crystallized. The crystals were separated, washed with ice-water and dried to yield a 1:1 mixture of **21a*/21b*** (0.23 g, 50%). M.p. 92 °C, R_f (TLC) = 0.73 (hexanes/EtOAc, 1:1). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ = 2.29 (s, 9 H), 6.84–7.33 (m, 3 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ = 20.6 (2 × CH_3), 21.0 (CH_3), 117.1 (d, $^1J_{C,C}$ = 71 Hz), 119.4 (d, $^1J_{C,C}$ = 70 Hz), 123.5 (d, $^1J_{C,C}$ = 71 Hz), 148.09 (d, $^1J_{C,C}$ = 70 Hz), 148.16, 167.8, 168.1, 168.8 ppm. HR EI-MS: *m*/*z* = 253.0663 [M$^+$] (calcd. for $C_{11}{}^{13}CH_{12}O_6$: 253.0667).

[1-^{13}C]- and [4-^{13}C]Benzene-1,2,4-triol (15*a/15*b), 1:1 Mixture: To a solution of **21a*/21b*** (1:1 mixture, 0.23 g, 0.89 mmol) in MeOH (10 mL) were added a few drops of 6 N HCl. The mixture was refluxed for 2 h and then concentrated under reduced pressure to yield a 1:1 mixture of **15a*/15b*** (0.11 g, 99%) as a grey solid. M.p. 133 °C (sublim.), R_f (TLC) = 0.38 (hexanes/EtOAc = 1:1). ^{1}H NMR (400 MHz, D_2O, TMS): δ = 6.05 (m, 1 H), 6.18 (m, 1 H), 6.49 (m, 1 H) ppm.

2-Bromo-6-methoxybenzene-1,4-diol (23): To a mixture of THF (33 mL) and H_2O (13 mL) were added with stirring 5-bromovanillin[21] (**22**, 2.31 g, 10 mmol) and sodium percarbonate ($Na_2CO_3 \times 1.5H_2O_2$, 1.65 g, 10.5 mmol). The stirring was continued until the TLC indicated completion of the reaction (2.5 h). After removal of the volatiles and drying (Na_2SO_4), the residue was flash chromatographed (hexanes/EtOAc, 2:1) to yield **23** as a grey solid (2.00 g, 91%). R_f (TLC) = 0.25 (hexanes/EtOAc, 2:1). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 3.80 (s, 3 H, OCH_3), 6.48 and 6.57 (each d, *J* = 2.6 Hz, 1 H, 3-H and 5-H), 7.45 and 8.04 (each s, br., 1 H, OH) ppm. ^{13}C NMR (75 MHz, [D$_6$]acetone): δ = 56.5 (OCH_3), 100.5 (C-5), 109.0 (C-2), 110.9 (C-3), 138.2 (C-1), 149.5, 151.5 (C-4 and C-6) ppm. EI-MS: *m*/*z* (%) = 220 (8) [M$^+$ (^{81}Br)], 219 (94), 218 (9) [M$^+$ (^{79}Br)], 217 (100), 205 (56), 203 (54), 177 (37), 175 (37), 95 (7), 69 (13), 53 (16), 43 (4).

1-Bromo-2,3,5-tris(methoxymethoxy)benzene (24): To a suspension of **23** (1.03 g, 4.68 mmol) in dry CH_2Cl_2 (10 mL), maintained at 0 °C in a Schlenk flask, BBr_3 (1.8 mL, 18.7 mmol) was added. The mixture was warmed to room temp. and then put into an ultrasonic bath until a clear solution resulted (10 min). After stirring for 4 h, the solution was cooled to 0 °C and MeOH (10 mL, purged with Ar) was added. The mixture was refluxed for 0.5 h and then concentrated under reduced pressure. The resulting residue was suspended in CH_2Cl_2 (10 mL) under an argon atmosphere and treated with chloromethoxymethane (6 M in EtOAc,[24] 4.7 mL, 28 mmol) and *N*,*N*-ethyldiisopropylamine (6.4 mL, 37 mmol). After stirring the mixture overnight, ammonia (2 N, 50 mL) was added and the crude product extracted with Et_2O (3 × 50 mL). The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (hexanes/EtOAc, 5:1) afforded **24** (1.29 g, 82%) as a colourless oil. R_f (TLC) = 0.30 (hexanes/EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.46, 3.48, 3.65 (each s, 3 H, OCH_2OCH_3), 5.09, 5.10, 5.16 (each s, 2 H, OCH_2OCH_3), 6.81, 6.93 (each d, *J* = 2.8 Hz, 1 H, 4-H and 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 56.2, 56.4, 58.0 (each OCH_2OCH_3), 94.9, 95.4, 99.0 (each OCH_2OCH_3), 105.4 (C-4), 113.5 (C-6), 117.8 (C-1), 139.2 (C-2), 151.4, 154.2 (C-3 and C-5) ppm. EI-MS: *m*/*z* (%) = 338 (5) [M$^+$ (^{81}Br)], 336 (5) [M$^+$ (^{79}Br)], 262 (12), 260 (12), 257 (2) [M – Br]$^+$, 232 (10), 230 (10), 181 (2), 45 (100) [C_2H_5O]$^+$. $C_{12}H_{17}BrO_6$ (337.17): calcd. C 42.75, H 5.08, Br 23.70; found C 42.61, H 5.05, Br 23.88.

1-(*E*,*E*,*E*)-[1′-^{13}C]Geranylgeranyl-2,3,5-tris(methoxymethoxy)-benzene (25*): In a Schlenk flask, which had been flushed with Ar and charged with a solution of **24** (0.202 g, 0.60 mmol) in dry THF (3 mL), *n*BuLi (0.38 mL, 0.65 mmol, 1.7 M in hexanes) was added dropwise at –78 °C. The mixture was stirred for 15 min at the same temperature prior to the addition of Li_2CuCl_4[25a] (0.10 mL, 0.02 mmol, 0.2 M in THF [Aldrich]). After stirring for additional 10 min, a solution of (*E*,*E*,*E*)-[1-^{13}C]geranylgeranyl bromide[18] (0.177 g, 0.5 mmol) in THF (1.7 mL) was added over 0.5 h. The stirring was continued for 2 h at –78 °C, and then the mixture was warmed to room temp. Saturated aqueous NH_4Cl (10 mL) was added followed by 2 N ammonia (20 mL), and the crude product was extracted with Et_2O (3 × 25 mL). The combined organic phases were washed with water and brine and dried (Na_2SO_4). Flash chromatography (hexanes/EtOAc, 6:1) afforded **25*** (0.207 g, 78%) as a colourless oil. R_f (TLC) = 0.34 (hexanes/EtOAc, 6:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.60 (s, 9 H, 3 × CH_3), 1.68 (s, 3 H, 16′-H), 1.71 (s, 3 H, 17′-H), 1.92–2.17 (m, 12 H, 6 × CH_2), 3.39 (dd, $^1J_{C,H}$ = 128, $^3J_{H,H}$ = 7.1 Hz, 2 H, 1′-H), 3.47, 3.50, 3.60 (each s, 3 H, OCH_2OCH_3), 5.04, 5.09, 5.17 (each s, 2 H, OCH_2OCH_3), 5.04–5.17 (m, 3 H, 3 × CH_{vin}), 5.25–5.36 (m, 1 H, 2′-H), 6.53 (dd, $^3J_{C,H}$ = 4.4, $^4J_{H,H}$ = 2.9 Hz, 1 H, 6-H), 6.72 (d, *J* = 2.9 Hz, 1 H, 4-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.1 (2×, C-18′, C-19′), 16.3 (d, $^3J_{C,C}$ = 3.8 Hz, C-17′), 17.7 (C-20′), 25.8 (C-16′), 26.74, 26.78, 26.84 (each CH_2, C-5′, C-9′, C-13′), 28.6 (^{13}C-1′), 39.7–39.9 (3 × CH_2, C-4′, C-8′, C-12′), 56.0, 56.3, 57.5 (each OCH_2OCH_3), 95.0, 95.3, 99.3 (each OCH_2OCH_3), 103.4 (C-4), 110.3 (C-6), 122.4 (d, $^1J_{C,C}$ = 43.7 Hz, C-2′), 124.2, 124.4, 124.5 (3 × CH_{vin}), 131.3 (C-15′), 134.9, 135.2 (2 × C_{vin}), 136.67 (d, $^1J_{C,C}$ = 43.5 Hz, C-1), 136.71 (C_{vin}), 139.7 (C-2), 150.3, 153.8 (d, $J_{C,C}$ = 4.7 Hz, C-3, C-5) ppm. Numbering of the geranylgeranyl chain carbon atoms as in ref.[18] EI-MS: *m*/*z* (%) = 532 (4), 531 (12) [M$^+$], 499 (1), 454 (1), 266 (4), 222 (6), 196 (8), 184 (7), 147 (9), 135 (9), 121 (9), 109 (8), 107 (8), 95 (8), 93 (8), 81 (18) [C_6H_9]$^+$, 69 (53) [C_5H_9]$^+$, 45 (100) [C_2H_5O]$^+$, 41 (10) [C_3H_5]$^+$. HRMS (EI): *m*/*z* = 531.3626 [M$^+$] (calcd. for $C_{31}{}^{13}CH_{50}O_6$: 531.3641). $C_{32}H_{50}O_6$ (530.75):[28] calcd. C 72.42, H 9.50; found C 72.12, H 9.50.

6-(*E*,*E*,*E*)-[1′-^{13}C](Geranylgeranyl)benzene-1,2,4-triol (8*): In a Schlenk flask, flushed with argon and charged with a solution of **25*** (106 mg, 0.20 mmol) in MeOH (2 mL), acetyl chloride (29 μL, 0.40 mmol) was added. After completion of the reaction (3 h, TLC monitoring), H_2O (25 mL) was added and the crude product extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (2 × 15 mL) and concentrated under reduced pressure. Flash chromatography (hexanes/acetone, 2:1) afforded **8*** (64 mg, 80%) as a brownish, waxy solid, R_f (TLC) = 0.34 (hexanes/acetone, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.61 (s, 9 H, 3 × CH_3), 1.69 (s, 3 H, 16′-H), 1.75 (s, 3 H, 17′-H), 1.93–2.20 (m, 12 H, 6 × CH_2), 3.28 (dd, $^1J_{C,H}$ = 127, $^3J_{H,H}$ = 7.2 Hz, 2 H, 1′-H), 4.90–5.17 (m, 4 H, 3 × CH_{vin}, 1 × OH), 5.24–5.34 (m, 1 H, 2′-H), 5.76 (br. s, 1 H, OH), 6.17 (dd, $^3J_{C,H}$ = 4.4, $^4J_{H,H}$ = 2.8 Hz, 1 H, 5-H), 6.32 (d, J = 2.8 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.07, 16.14 (C-18′, C-19′), 16.2 (d, $^3J_{C,C}$ = 3.8 Hz, C-17′), 17.7 (C-20′), 25.8 (C-16′), 26.4, 26.7, 26.8 (each CH_2, C-5′, C-9′, C-13′), 29.9 (^{13}C-1′), 39.6–39.9 (3 × CH_2, C-4′, C-8′, C-12′), 101.2 (C-3), 107.8 (C-5), 121.4 (d, $^1J_{C,C}$ = 42.9 Hz, C-2′), 123.7, 124.2, 124.5 (each CH_{vin}), 128.8 (d, $^1J_{C,C}$ = 43.5 Hz, C-6), 131.4 (C-15′), 135.2, 135.8 (2×, each C_{vin}), 138.9 (C-1), 145.2, 149.4 (d, $J_{C,C}$ = 4.7 Hz, C-2, C-4) ppm. Numbering of the geranylgeranyl chain carbon atoms as in ref.[18] EI-MS: *m*/*z* (%) = 400 (12), 399 (42) [M$^+$], 331 (2), 330 (2), 263 (5), 259 (8), 248 (7), 207 (9), 195 (25), 180 (26), 178 (27), 149 (10), 148 (10), 141 (23), 140 (58), 139 (46), 135 (17), 123 (21), 121 (18), 109 (25), 107 (15), 95 (23), 93 (17), 81 (52) [C_6H_9]$^+$, 69 (100) [C_5H_9]$^+$, 55 (16), 41 (32) [C_3H_5]$^+$.

4-Hydroxy-2-methylbenzoic Acid (17): To a suspension of **26**[26] (1.66 g, 10 mmol) in dry CH_2Cl_2 (20 mL) was added BBr_3 (2.1 mL, 22 mmol) at 0 °C. After stirring for 5 h at room temp., the mixture was cooled to 0 °C and quenched by dropwise addition of water. Then, water (75 mL) and EtOAc (100 mL) were added, and under vigorous stirring the mixture was treated with 2 N NaOH until the aqueous phase remained alkaline. After acidification with 2 N HCl, the organic phase was separated and the aqueous phase extracted with EtOAc (2 × 75 mL). The combined organic phases were washed with water and brine, dried (Na_2SO_4) and concentrated under reduced pressure to yield **17** (1.36 g, 89%) as a grey solid. R_f (TLC) = 0.62 (hexanes/EtOAc, 1:1). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 2.53 (s, 3 H, CH_3), 6.73 (dd, $J \approx 9$, 2.5 Hz, 1 H, 5-H), 6.75–6.76 (m, 1 H, 3-H), 7.91 (d, $J \approx 9$ Hz, 1 H, 6-H), 8.92 (br. s, 1 H, OH) ppm. ^{13}C NMR (75 MHz, [D$_6$]acetone): δ = 22.3 (CH_3), 113.4 (C-5), 119.1 (C-3), 121.3 (C-1), 134.4 (C-6), 144.2 (C-2), 161.6 (C-4), 168.9 (CO_2H) ppm. EI-MS: *m*/*z* (%) = 153 (7), 152 (100) [M$^+$], 135 (76), 134 (54), 107 (27), 106 (15), 77 (16), 51 (5).

4,5-Dimethoxy-2-methylbenzoic Acid (28): Aldehyde (**27**)[27] (3.00 g, 16.7 mmol) was oxidized following the procedure described in ref.[26] Yield 3.03 g (92.5%). M.p. 143–144 °C. R_f (TLC) = 0.16 (hexanes/EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.62 (s, 3 H, CH_3), 3.90, 3.92 (each s, 3 H, OCH_3), 6.70, 7.60 (each s, 1 H, 3-H and 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.1 (CH_3), 55.96, 56.03 (each OCH_3), 114.1, 114.3 (each CH, C-3, C6), 119.8 (C-1), 136.5 (C-2), 146.6 (C-5), 152.7 (C-4), 173.0 (CO_2H) ppm. EI-MS: *m*/*z* (%) = 197 (11), 196 (100) [M$^+$], 181 (19), 179 (5), 151 (7), 163 (3), 150 (7), 135 (16), 107 (5), 77 (4), 65 (4), 51 (2), 39 (4). $C_{10}H_{12}O_4$ (196.20): calcd. C 61.22, H 6.16; found C 60.82, H 6.16.

4,5-Dihydroxy-2-methylbenzoic Acid (18): 28 (1.57 g, 8.00 mmol) was demethylated with BBr_3 (2.5 mL, 25.6 mmol) following the procedure described for **17**. Yield 1.24 g (92%). R_f (TLC) = 0.33 (hexanes/EtOAc, 1:1). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 2.45 (s, 3 H, CH_3), 6.72, 7.53 (each s, 1 H, 3-H and 6-H), 7.99, 8.37 (each s, br., 1 H, OH), 10.50 (br. s, 1 H, CO_2H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 21.3 (CH_3), 118.4, 118.8 (each CH, C-3, C-6), 120.2 (C-1), 132.4 (C-2), 142.8 (C-5), 149.2 (C-4), 168.4 (CO_2H) ppm. EI-MS: *m*/*z* (%) = 168 (100) [M$^+$], 151 (30), 150 (17), 123 (48), 121 (50), 77 (10), 51 (8), 43 (5).

Feeding Experiments

Feeding Experiments with Fruit Bodies: A solution (0.05–0.3 mL) of the respective precursor was injected via syringe into the stalk of young specimens of *S. tridentinus*. The mushrooms were harvested after 3–9 d, frozen in liquid N_2, and stored at –20 °C until work-up. The experimental details and results of the feeding experiments are given in Table 1. In the case of cyclodextrin preparations, the precursors were mixed with 2-hydroxypropyl-β-cyclodextrin,[29] average degree of substitution was 7 (1 equiv., $M_{average}$ = 1541, Aldrich) and an amount of water equal to the amount of the cyclodextrin (w/w). The mixture was stirred until a clear viscous liquid was obtained, which was administered to the mushrooms. Liposomes were prepared by the film method.[30]

Feeding Experiments with Submerse Cultures: Mycelium cultures[31] of *S. tridentinus* were grown on cotton pads in modified Moser's medium B (250 mL, heat sterilisation). After 7–14 d, a solution of **6*** in acetone (0.5 mL) was added via sterile filtration (cellulose acetate, 0.22 μm). **9*** was added as a solid before heat sterilisation. For the isolation of **1***, the culture broth was removed by filtration and discarded. The residual mycelium was minced and exhaustively extracted with EtOAc. The combined organic phases were dried (Na_2SO_4) and concentrated. Further purification of **1*** followed the isolation procedure from mushrooms. Due to the short HSCCC column used (80 mL), **1*** (2 mg) eluted with the mobile phase.

Isolation of 2-Geranylgeranyl-3-hydroxy-6-methyl-1,4-benzoquinone-4 (20) after Feeding of 17 and 18: The acids **17** and **18** were administered to young fruit bodies as detailed in Table 1, and the resulting benzoquinone **20** was isolated following the procedure for **1**. The compound eluted from the HSCCC column with the mobile phase ($R_t \approx 3$ h) and was further purified by chromatography on Sephadex LH-20 (acetone) to afford pure **20**. Yield 0.5 mg and 2.0 mg from precursors **17** and **18**, respectively. R_f (TLC) = 0.45 (hexanes/EtOAc, 5:1), yellow spot, + NH_3: blue. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.57, 1.58, 1.60 (each s, 3 H, 3 × CH_3), 1.68 (s, 3 H, 16′-H), 1.75 (s, 3 H, 17′-H), 1.92–2.14 (m, 12 H, 6 × CH_2), 2.08 (s, 3 H, 6-CH_3), 3.16 (d, J = 7.1 Hz, 2 H, 1′-H), 5.05–5.15 (m, 4 H, 4 × CH_{vin}), 6.55 (q, $J \approx 1.4$ Hz, 1 H, 5-H), 6.85 (br. s, 1 H, OH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 16.1 (2 × CH_3), 16.3 (CH_3), 16.7 (6-CH_3), 17.8 (C-20′), 22.2 (C-1′), 25.8 (C-16′), 26.60, 26.75, 26.87 (each CH_2, C-5′, C-9′, C-13′), 39.8–40.0 (3 × CH_2, C-4′, C-8′, C-12′), 119.7 (C-2′), 120.7 (C-2), 124.1, 124.3, 124.5 (each CH_{vin}), 128.5 (C-5), 131.3 (C-15′), 135.0, 135.1, 137.3 (each C_{vin}), 149.2 (C-6), 150.7 (C-3), 183.5 (C-4), 187.5 (C-1) ppm. Numbering of the geranylgeranyl chain carbon atoms as in ref.[18] EI-MS: *m*/*z* (%) = 410 (1) [M$^+$], 257 (25), 231 (7), 191 (100), 153 (14), 121 (6), 81 (23) [C_6H_9]$^+$, 69 (80) [C_5H_9]$^+$. HRMS (EI): *m*/*z* = 410.2808 [M$^+$] (calcd. for $C_{27}H_{38}O_3$: 410.2821).

Detection of 4-Hydroxybenzoic Acid (6) and 3,4-Dihydroxybenzoic Acid (7) by GC/MS: Fruit bodies of *S. tridentinus* were frozen with liquid N_2 immediately after collection. The crude acetone extract was treated with ethereal CH_2N_2 and analyzed by GC/EI-MS. Methyl 4-methoxybenzoate: R_t = 12.39 min; methyl 3,4-dimethoxybenzoate: R_t = 15.33 min. The permethylated compounds were not detectable when the methylation of the extract was omitted.

Crystallographic Data for (+)-(1′′*S*)-*O*-Camphanoyl-(14′*R*)-tridentoquinone (4): $C_{36}H_{46}O_7$, M = 590.76, crystal dimensions 0.53 × 0.40 × 0.20 mm, triclinic system, space group $P1$, unit cell

dimensions and volume: a = 9.164, b = 10.330, c = 10.652 , α = 62.24(2)°, β = 69.56(3)°, γ = 69.49(3)°, V = 814.4 Å^3, Z = 1, $D_{calcd.}$ = 1.204 g/cm^3, F (000) 318, μ 0.082 mm^{-1}, radiation: Mo-K_α, wavelength λ = 0.71073 Å, 2.22 ≤ θ ≤ 22.97, h_{min}/h_{max} = –10/10, k_{min}/k_{max} = –11/11, l_{min}/l_{max} = –11/11, No. of measured reflections: 4530, 4530 were independent (R_{int} = 0.0000), No. of parameters 396, R factor 0.0414, $wR(F^2)$ = 0.1005, Goodness of fit: 0.991, $R1$ = 0.0414 and wR_2 = 0.1005 for all reflections, R_1 = 0.0351 and wR_2 = 0.0959 [$I > 2\sigma(I)$], refining: 396 parameters and 3 restraints, no absorption correction, absolute structure parameter: 0.1(9), final electron density between 0.111 and –0.111 e Å^{-3}. Structure solution with SHELXLS-86,[32] refinement by SHELXTL-PLUS.[33] The structure was displayed using ZORTEP.[34]

CCDC-204781 (for **4**) contains supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see also the footnote on the first page of this article): CD spectra of compounds **5** and **13**, ^{1}H and ^{13}C NMR spectra of compounds **1**, **5** and **11** are provided.

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[12] In the relevant cases, either ethereal solutions of 1,2,4-triacetoxy-6-[1′-^{13}C](geranylgeranyl)benzene or of meroterpenoid **14*** (cf. Table 1) had been administered to the fruit bodies (no incorporation into **11**).
[13] All our attempts to convert tridentoquinone (**1**) into deoxy compound **11** were unsuccessful.
[14] The belamcandones, red dioxotetrahydrodibenzofurans from the seeds of *Belamcanda chinensis* and *Iris pallasii* (Iridaceae), may be formed by an analogous mechanism: a) K. Seki, K. Haga, R. Kaneko, *Phytochemistry* **1995**, *38*, 703–709; b) K. Seki, K. Haga, R. Kaneko, *Phytochemistry* **1995**, *38*, 965–973.
[15] The possibility that tridentorubin (**5**) is at least partially an artefact, can not be excluded. It could be formed non-enzymatically from hydroxybenzoquinone **10** and tridentoquinone (**1**) in the fruit bodies or during the work-up procedure, including the polyamide chromatography. The latter is known to catalyze the dimerization of hydroxybenzoquinones (E. Jägers, W. Steglich, *Angew. Chem.* **1981**, *93*, 1105; *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 1016–1017). Hydroxyquinone **10** could be formed from the bolegrevilol (**3**) present by hydrolysis and subsequent oxidation of the resulting triol **8**. The greatly varying yields of **5** in the individual isolations point to this possibility.
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