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Retipolides – Unusual Spiromacrolactones from the Mushrooms *Retiboletus retipes* and *R. ornatipes*

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Dedicated to Professor Wolfgang Beck on the occasion of his 75th birthday

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Mushrooms of the genus *Retiboletus* contain the retipolides A–D (**1**, **6**–**8**), unusual 14-membered spiromacrolides with a biphenyl ether linkage. The structures of these metabolites suggested a biogenetic sequence starting from retipolide E (**16**), which could furnish the unique 2(3*H*)-oxepinone unit of retipolide C (**7**) via an oxidative enlargement of the 4-hydroxyphenyl ring. A subsequent *O*/*C*-acyl shift would then lead to the cyclopenta[*c*]pyran system of retipolide A (**1**). The proposed precursor **16** was synthesized and subsequently detected in the fungal extract in addition to the probable biosynthetic intermediates butyrolactone II (**13**), tyrosol (**14**), and secoretipolide E (**15**). The structural elucidation of retipolide A (**1**) was accomplished after transformation into a methoxyfulvene derivative **2a**, and the absolute (*R*) configuration of **1** was assigned by a single-crystal X-ray analysis of the corresponding (*S*)-*sec*-butoxyfulvene analogue **2b**. Some samples of *R. retipes*/*ornatipes* contained isoretipolide A (**9**), an isomer of **1** with a highly strained 12-membered lactone ring incorporating two C,C-coupled 4-hydroxyphenyl units. A comparison of the CD spectrum of retipolide A (**1**) with those of the other retipolides and isoretipolide A (**9**) indicates that all of these macrolides possess *R* configuration.

Introduction

Retiboletus retipes (Berkeley & Curtis) Binder & Bresinsky is a showy mushroom with yellow pores and flesh, easily recognized by a conspicuous reticulated stalk. It occurs together with a twin species, *R. ornatipes* (Peck) Binder & Bresinsky, the Ornate-stalked Bolete,[1] in mixed or deciduous woods in eastern North America, Japan, and Yunnan (China). The mushrooms are unpalatable because of their bitter taste and stain the fingers yellow on handling. On cutting or bruising the affected areas develop yellow-orange to rusty brown colors. We were attracted by the bright color of these remarkable fungi and now report the structural elucidation of a biosynthetically and structurally unique set of fungal metabolites, named retipolides A–E.

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Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

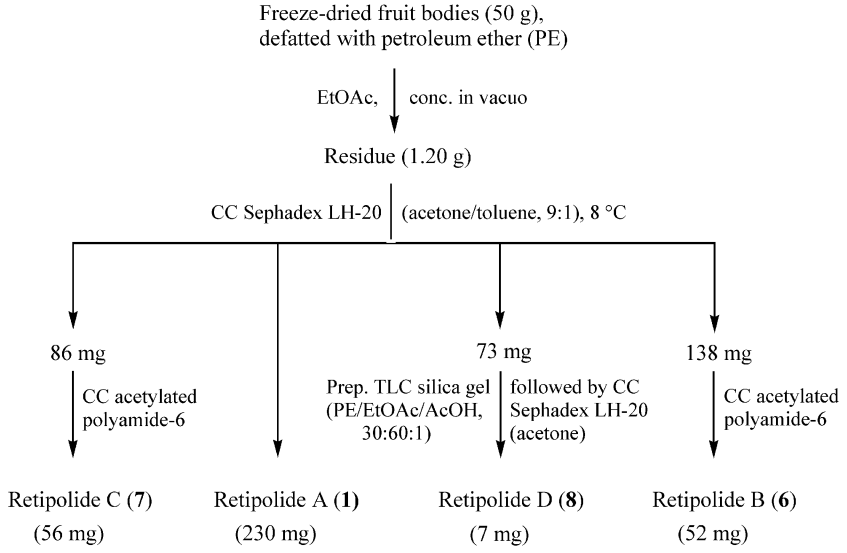
Results and Discussion

Isolation of the Retipolides A–D

Our first attempts to isolate the pigments from MeOH extracts of *R. retipes*/*ornatipes*[1] were unsuccessful, due to rapid degradation of the metabolites in this solvent resulting in the formation of dark brown to black materials. This problem could be bypassed by column chromatography of an EtOAc extract from freeze-dried fruit bodies of *R. retipes*/*ornatipes* on Sephadex LH-20 with acetone/toluene at 8 °C (Scheme 1). This procedure yielded four fractions, which on further chromatographic purification afforded the retipolides A–D.

Retipolides A (1) and B (6)

Retipolide A was obtained as a yellowish, amorphous powder, which appears on TLC plates as a pale yellow spot. On exposure to HCl (gaseous) the spot turns colorless and changes to yellow-orange with ammonia. Retipolide A is optically active, $[\alpha]_D^{20}$ = +160, and exhibits UV maxima at λ_{max} = 208, 316, 390, and 428 nm. Its rather simple EI-MS shows a molecular peak at *m*/*z* = 476, corresponding to



Scheme 1. Procedures outlining the isolation of retipolides A–D.

$C_{26}H_{20}O_9$ on high resolution. In addition to peaks indicating the loss of OH, H_2O, and CO_2 from the molecular ion, only the base peak at m/z = 225 ($C_{15}H_{13}O_2$) is remarkable.

In the ^{1}H NMR spectrum of retipolide A all 20 protons including those of two exchangeable OH groups are clearly discernible. Characteristic is an aromatic ABCD system at δ_H = 7.00, 7.24, 7.35, and 7.67 ppm, whose signals are each split into a doublet of doublets with J = 8.5 and 2.2 Hz. This is in accord with a sterically fixed 1,4-disubstituted benzene ring with strongly hindered rotation around the *para*-axis. In addition, a 1,2,4-trisubstituted benzene ring can be recognized with signals at δ_H = 4.83, 6.51, and 6.70 ppm. The first signal occurs at unusually high field, suggesting a highly shielded position in a macrocycle. In the aliphatic region signals for 10 additional protons are present, which belong to an isolated methylene group (δ_H = 3.32 and 3.89, AB-q, J = 13.8 Hz) and two -CH_2CH_2- units. According to the HMBC spectra, one of these units (δ_H = 2.62/2.85 and 4.00/4.12 ppm) connects the trisubstituted benzene ring with an oxygen atom carrying the lactone carbonyl group at δ_C = 167.8 ppm. Noticeable are the very different vicinal couplings of the individual protons indicating a fixed conformation. The two isolated methylene protons at C-13′ exhibit HMBC correlations with a neighboring quaternary carbon atom at δ_C = 86.8, three C atoms of the *p*-phenylene unit at δ_C = 131.6, 132.4, and 133.1, and the lactone carbon at δ = 167.8 ppm. From these results partial structure **A** containing a 14-membered macrolide ring incorporating a biphenyl ether moiety can be postulated for retipolide A. Structure **A** explains the formation of the base peak at m/z = 225 ($C_{15}H_{13}O_2$) in the mass spectrum (Figure 1) and the strong shielding of the aromatic 19′-hydrogen atom located under the *para*-disubstituted benzene ring.

The remaining part of the structure shows NMR signals for a -CH_2CH_2CO- unit (δ_{CO} = 203.5), a hemiacetal group at δ_H = 6.49 (δ_C = 93.9), and five quaternary carbon atoms

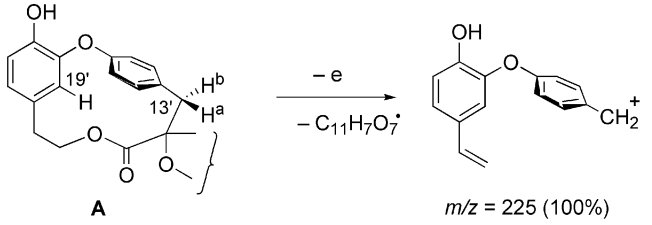
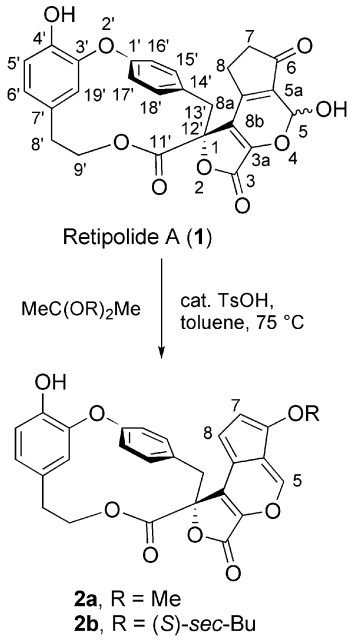


Figure 1. Partial structure **A** of retipolide A and formation of the base ion m/z = 225 under electron impact.

with δ_C = 127.0, 131.7, 143.9, 157.6, and 163.4 ppm. At this stage, the completion of the structural determination was prevented by strong line broadening of several ^{13}C NMR signals,[2] missing connectivities for the quaternary carbons in the 2D spectra, and the propensity of retipolide A to undergo decomposition.

These problems could be solved by conversion of retipolide A (**1**) into a red, stable methoxyfulvene, anhydroretipolide A 6-*O*-methyl ether (**2a**), by heating with 2,2-dimethoxypropane/*p*-TsOH in toluene at 75 °C (Scheme 2). In contrast to the natural product, the new compound exhibited sharp NMR signals for all hydrogen and carbon atoms and meaningful cross peaks in the COLOC and HMBC spectra (Figure 2, a). In addition to the unchanged NMR signals of the macrolide ring, signals for a cyclopenta[*c*]pyran[3] unit carrying a methoxy substituent were clearly discernible. Of importance is the signal for 5-H at δ_H = 8.51 (d, J = 1.1 Hz, δ_C = 146.0) exhibiting a long-range coupling to 8-H at δ_H = 7.12 (dd, J = 3.1, 1.1 Hz, δ_C = 116.9), itself coupled with the vicinal 7-H proton at δ_H = 6.57 (d, J = 3.1 Hz, δ_C = 110.5 ppm). The NMR spectroscopic data and chemical shifts are in accordance with those reported in the literature.[3]

Combining the two partial structures with the remaining lactone carbonyl at δ_C = 163.0 leads to structure **2a** for the red methoxy derivative. The assignment of the individual



Scheme 2. Conversion of retipolide A into anhydroretipolide A 6-*O*-alkyl ethers **2**.

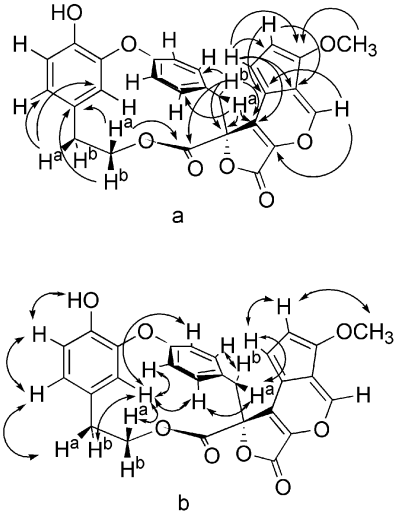
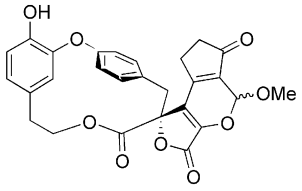


Figure 2. Selected HMBC (a) and NOE (b) relationships for anhydroretipolide A 6-*O*-methyl ether (**2a**).

protons was secured by NOE experiments (Figure 2, b). Of special importance were the NOE effects observed for 19′-H, which relate this H atom to all protons of the second benzene ring and to 8′-H^b and 9′-H^a of the neighboring dimethylene unit, thereby confirming the conformation given in Figure 2. An NOE between 13′-H^a and 8-H at the cyclopenta[*c*]pyran system establishes the relative stereochemistry, and NOE correlations between 13′-H^a and 18′-H as well as between 13′-H^b and 15′-H allow the assignment of these protons in the sterically fixed 1,4-disubstituted benzene ring.

During the transformation of retipolide A (**1**) into anhydro derivative **2a**, the signals of the hemiacetal group as well as of the -CH_2CH_2- unit and the carbonyl group at δ_C = 203.5 ppm disappear, and new signals for olefinic hydrogen and carbon atoms are visible. From these spectral changes and the appearance of a new methoxy group, structure **1** can be proposed for retipolide A.

The first intermediate in the conversion of retipolide A (**1**) into methoxyfulvene **2a** is methyl acetal **3**, obtained in high yield by treatment of **1** with 2,2-dimethoxypropane and catalytic amounts of *p*-TsOH at room temperature. On heating this mixture to 75 °C, acetal **3** is converted into the stable methoxyfulvene **2a**. In addition to retipolide A, methyl acetal **3** could also be obtained as an extraction artefact from the methanol extract of *R. retipes*/*ornatipes*. According to the NMR spectra, acetal **3** exists as a 2:1 mixture of the two epimers.



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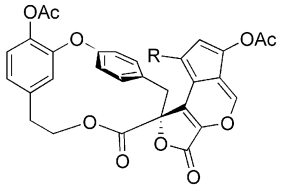


4, R = H
5, R = Ac

Treatment of retipolide A (**1**) with acetic anhydride in the presence of catalytic amounts of concd. H_2SO_4 led to the formation of a complex mixture of acetylation products, from which a yellow diacetate and a triacetyl compound could be separated by preparative TLC. The similarity of the NMR spectroscopic data of these compounds to those of anhydroretipolide A 6-*O*-methyl ether (**2a**) allowed assignment of the fulvene structures **4** and **5** to these derivatives. In the triacetyl compound **5** an additional *C*-acylation at C-8 had occurred, the position of which follows from the small ^5J coupling of 0.3 Hz between protons 5-H and 7-H.[3] The acetyl residue causes a remarkable down field shift of the 13′-H^a signal from δ_H = 3.93 in diacetate **4** to 5.40 ppm in the case of **5**. Molecular models indicate that proton 13′-H^a is strongly exposed to the deshielding region of the acetyl carbonyl group attached at C-8.

The high tendency to form anhydro derivatives could be used to determine the absolute configuration of retipolide A (**1**). For this purpose, the latter was heated with 2,2-bis[(*S*)-*sec*-butoxy]propane,[4] preformed by stirring acetone and (*S*)-*sec*-butanol in the presence of *p*-TsOH and 5-Å molecular sieves. The resulting (*S*)-*sec*-butoxy derivative **2b** crystallized from an acetone/MeOH mixture as orange-red needles suitable for a single-crystal X-ray structural analysis (Figure 3). From the known *S* configuration of the introduced ether residue, the absolute configuration of fulvene **2b** could be unambiguously determined as 1*R*. Since the conversion of retipolide A (**1**) into **2b** does not affect the stereogenic center at C-1, retipolide A (**1**) must possess the same stereochemistry.

The molecular structure of fulvene **2b** in the crystal (Figure 3) shows that the highly strained macrolide ring adopts the same conformation as deduced from the ^{1}H NMR parameters in solution. Thus, the ^3J coupling of 11.0 Hz be-

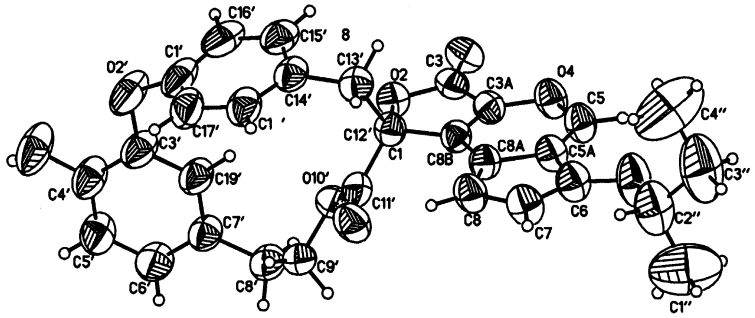
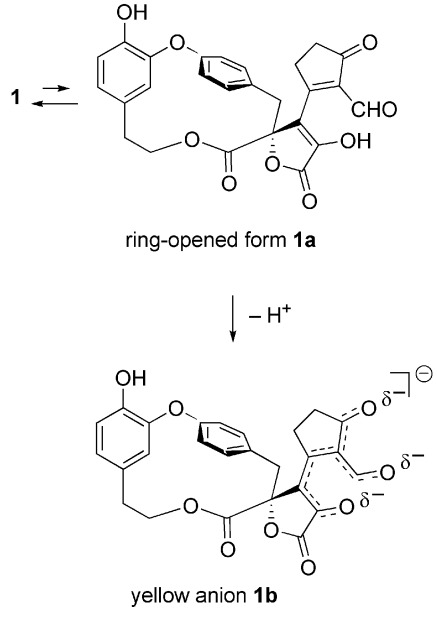


Figure 3. ORTEP plot derived from a single-crystal X-ray analysis of anhydroretipolide A 6-*O*-(*S*)-*sec*-butyl ether (**2b**).

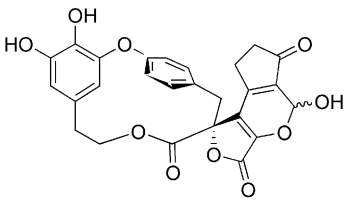
tween 8′-H^b and 9′-H^a is in accord with an *antiplanar* position of these protons, whereas 8′-H^a and 9′-H^a exhibit no visible coupling, suggesting a dihedral angel near 90°. Due to the ring strain of the macrocycle the *p*-phenylene ring is considerably bent.[5] The conformation shown in Figure 3 also explains the strong shielding of aromatic proton 19′-H by the second benzene ring.

The structure of retipolide A (**1**) accounts for the development of the orange-yellow color upon treatment with bases. Opening of the cyclic hemiacetal unit yields enol **1a**, which forms the highly delocalized yellow anion **1b** on deprotonation (Scheme 3).[6] Even solutions of retipolide A (**1**) in acetic acid remain yellow due to an equilibrium concentration of anion **1b**. The color disappears on addition of one drop of concd. aqueous HCl, thus showing that the acidity of enol **1a** is comparable to that of AcOH. An equilibrium concentration of anion **1b** in the cells of *R. retipes*/*ornatipes* seems therefore responsible for the yellow color of the fruit bodies.

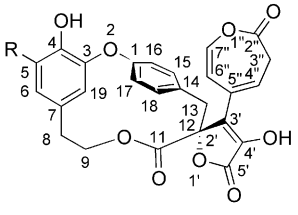


Scheme 3. Formation of the delocalized yellow anion **1b** from retipolide A (**1**).

Retipolide B (**6**) exhibits the same yellow color as retipolide A (**1**) upon treatment with bases and shows nearly identical NMR spectra. The high resolution EI-MS indicates the molecular formula $C_{26}H_{20}O_{10}$, containing one more oxygen atom than retipolide A. The position of the additional oxygen could be located as a phenolic OH group at C-5′, accounting for the expected NMR changes in the benzene ring. The highly shielded proton 19′-H gives rise to a doublet of doublets at δ_H = 4.35 (J = 2.1, 1.0 Hz), coupling with the *meta*-proton 6′-H, which occurs at δ_H = 6.20 ppm (dd, J = 2.1, 0.5 Hz). The small splittings of 1.0 and 0.5 Hz are due to ^4J couplings with the benzylic protons 8′-H^b and 8′-H^a, respectively. The additional OH group in the biaryl ether portion of retipolide B (**6**) accounts for the strong MS fragment at m/z = 241 ($C_{15}H_{13}O_3$), containing one more oxygen than that formed from retipolide A (Figure 1). From the optical rotation of $[a]_D^{20}$ = +61 and the close agreement of the CD spectrum with that of retipolide A (**1**), the (1*R*) configuration can be assigned to retipolide B (**6**).



Retipolide B (**6**)



Retipolide C (**7**), R = H
Retipolide D (**8**), R = OH

Retipolides C (7) and D (8)

Retipolide C is a colorless, amorphous solid with a specific optical rotation of $[a]_D^{20}$ = +112 and UV absorptions at λ_{max} = 233, 240, and 275 nm. The IR spectrum (KBr) shows strong bands at 3410 (OH) and 1255 (C–O), and two intense bands at 1760 and 1725 cm^{-1} in the carbonyl region. The molecular formula was determined as $C_{26}H_{20}O_9$ by high-resolution electron ionization (EI) mass spectrometry. The molecular ion appeared at m/z = 476, and the characteristic fragment ion at m/z = 225 ($C_{15}H_{13}O_2$) implied the

presence of the same partial structure **A** as in retipolide A (**1**) (Figure 1). The ^{1}H NMR spectrum of retipolide C exhibits only 18 of the expected 20 proton signals, suggesting the presence of two exchangeable protons as OH groups. In the ^{13}C NMR spectrum all 26 carbon signals are visible, which can be assigned to 4 methylene, 10 methine, and 12 quaternary carbons, three of the latter representing lactone carbons at δ_C = 166.8, 167.1, and 168.5 ppm. Comparison with the ^{1}H and ^{13}C NMR spectra of the isomeric retipolide A (**1**) confirmed the presence of partial structure A (Figure 1). The unknown portion of the structure contains a disubstituted double bond, whose H atoms at δ_H = 6.54 and 6.83 ppm are coupled with ^3J = 6.9 Hz, indicating their position on a ring system. In addition, signals for a fragment –CH_2–CH=C(*R*)– were visible, whose olefinic proton is coupled with ^3J = 7.0 Hz to the protons of the allylic methylene group (δ_H = 3.28 and 3.29 ppm, ^2J = 12 Hz). In addition, signals due to a tetrasubstituted double bond and two ester groups can be recognized. HMBC experiments (Figure 4) allowed the determination of the connectivities of these fragments, leading to structure **7** for retipolide C.

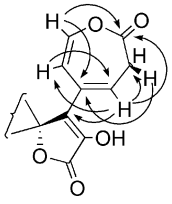


Figure 4. Selected HMBC relationships for the oxepinone moiety of retipolide C (**7**).

Retipolide D (**8**), $[a]_D^{20}$ = +128, exhibits a molecular ion signal at *m*/*z* = 492, corresponding to the molecular formula $C_{26}H_{20}O_{10}$. Comparison of the NMR spectra of **8** with those of retipolide C (**7**) revealed the presence of an additional OH group at C-5. All other spectroscopic data were in excellent agreement with those of retipolide C, which allowed the assignment of structure **8** to retipolide D. The absolute configuration of retipolides C (**7**) and D (**8**) was assumed to be (*R*), as per retipolide A (**1**), based on the observation that a strong positive exciton couplet is present in the short-wavelength region of the CD spectra (Figure 5). In support of this, all of these compounds are dextrorotatory.

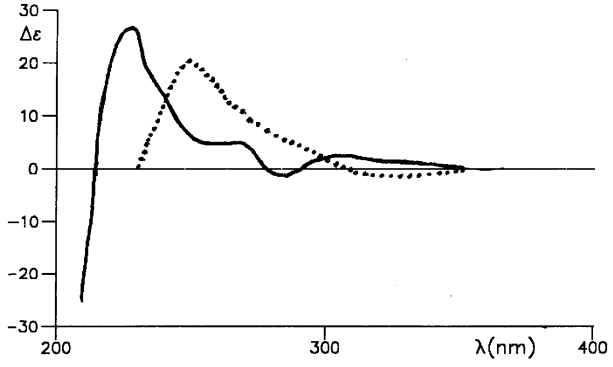


Figure 5. CD spectra of retipolides A (**1**) (––) and C (**7**) (···) (MeCN).

Surprisingly, the retipolides C and D contain a simple 2(3*H*)-oxepinone residue, a heterocycle, found for the first time in a natural product. The basic ring system has only recently been synthesized,[7] and the 2-oxepinoxy radical has aroused interest as a possible intermediate in the combustion of benzene.[7]

Isoretipolide A (9)

Several collections of *R. retipes*/*ornatipes* contain in addition to the retipolides a constituent,[8] which undergoes rapid decomposition in solution with the formation of black material. Extraction of the air-dried mushrooms with acetone followed by preparative HPLC on RP 18 yields the new compound as a pale yellow powder. This new metabolite is optically active, $[a]_D^{20}$ = +144, and exhibits UV/Vis maxima resembling those of retipolide A (**1**). In the NMR spectra a set of signals can be observed, which is nearly identical to that of the cyclopenta[*c*]pyran moiety of retipolide A (**1**). In contrast, the signals for the macrolide portion exhibit characteristic differences. Again, two substituted benzene rings are present, which, however, are both 1,2,4-trisubstituted. A closer inspection of the HMBC spectra reveals that instead of the biphenyl ether group, present in all the other retipolides, the molecule contains a 2,2′-dihydroxybiphenyl moiety embedded in the macrolide ring. Most important for this assignment are the correlation signals between 17′-H and C-2′ as well as between 18′-H and C-1′. This leads to structure **9** for the new metabolite, which is appropriately named isoretipolide A. The assignment of the H atoms was established by the NOE relationships depicted in Figure 6.

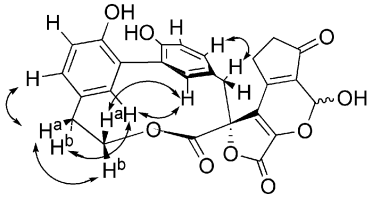
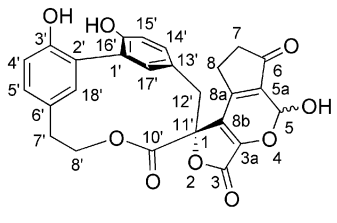


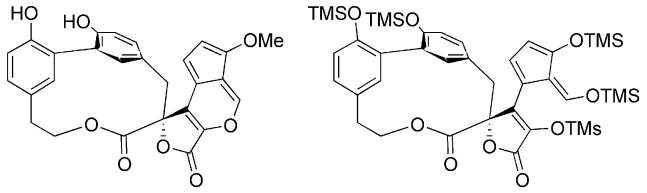
Figure 6. Selected NOE relationships for isoretipolide A (**9**).



Isoretipolide A (**9**)

Structure **9** is in accordance with the formation of an orange anhydroisoretipolide A 6-*O*-methyl ether (**10**) under similar conditions used for the preparation of methoxyfulvene **2a**. Treatment of isoretipolide A with *N*-methyl(tri-

methylsilyl)trifluoroacetamide (MSTFA) yields a fulvene derivative **11** containing five TMS residues. The molecular composition $C_{41}H_{60}O_9Si_5$ was determined by high-resolution EI-MS. Under identical conditions, retipolide A forms an analogous tetrakis(trimethylsilyl) derivative, $C_{38}H_{52}O_9Si_4$, indicating the different kind of coupling of the 4-hydroxyphenyl residues.



10 **11**

According to a MacroModel[9] calculation, the 12-membered ring system of isoretipolide A (**9**) adopts a conformation in which the two benzene rings are less twisted than in retipolide A (**1**) (Figure 7). This causes only a weak shielding effect of the neighboring benzene ring upon proton 18′-H (δ_H = 7.31 ppm). The positive optical rotation, biosynthetic considerations and the similarity of the CD spectra of isoretipolide A and retipolide A indicate that compound **9** also possesses the *R* configuration.

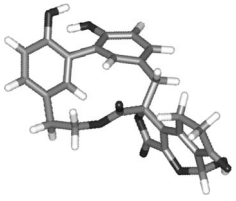


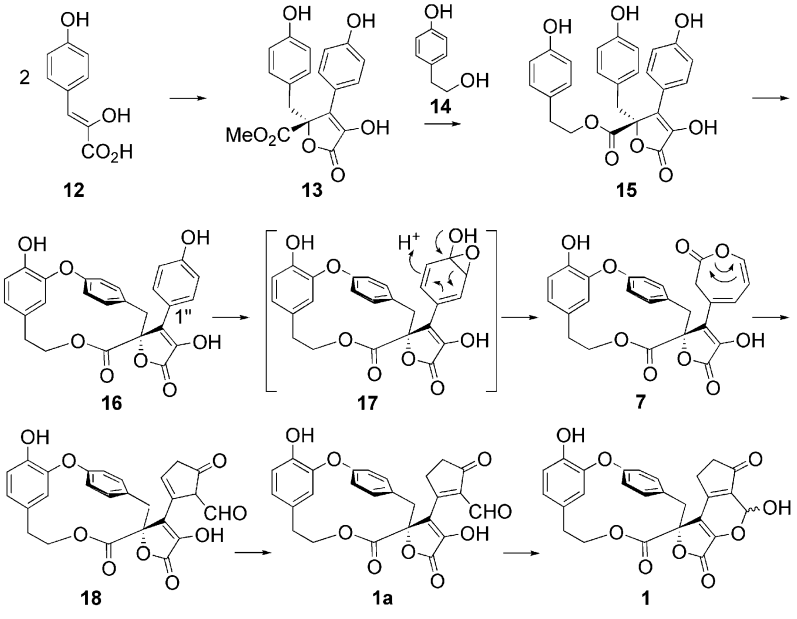
Figure 7. Conformation of isoretipolide A (**9**) according to MacroModel calculations.

Biosynthetic Considerations

Comparison of the structures of the different retipolides leads to the suggestion, that all of these compounds might be formed by a biosynthetic sequence starting from the hypothetical 4-hydroxyphenyl derivative **16**, named retipolide E (Scheme 4). Formally, a mechanism can be proposed, in which this phenol is converted into an epoxide **17**, which then undergoes ring enlargement to yield the 2(3*H*)-oxepinone derivative retipolide C (**7**). Ring contraction of the oxepinone ring by an *O*/*C*-acyl shift could afford formylcyclopentenone **18**, which should rearrange into the conjugated isomer **1a**, the ring-opened form of retipolide A (**1**). The suggested precursor **16** could be formed by oxidative phenol coupling[10] of secoretipolide E (**15**), which could in turn be derived from butyrolactone II (**13**)[11] by transesterification with tyrosol (**14**). There is ample evidence that lactone **13** is formed in *Aspergillus terreus* from two units of 3-(4-hydroxyphenyl)pyruvic acid (**12**), a reaction that could be mimicked by treatment of methyl 3-(4-hydroxyphenyl)-pyruvate with aqueous Na_2CO_3[11b] or triethylamine.[12]

In order to test this proposal, we set out to isolate retipolide E (**16**) from the mushroom extract. For this purpose we synthesized retipolide E in both racemic and optically pure form[13] and studied its behavior on column chromatography and HPLC. Careful chromatography of the extract from *R. retipes*/*ornatipes* and collection of all fractions with the same R_f value as the synthetic compound finally yielded a small amount of retipolide E (**16**), in every respect identical with the synthetic product, including optical rotation $[\alpha]_D^{20}$ = +83 and CD spectrum. In addition to **16**, a considerable amount of tyrosol (**14**) could be isolated.

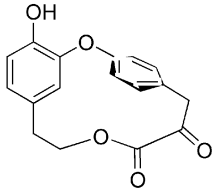
The presence of the precursors butyrolactone II (**13**) and secoretipolide E (**15**) in the crude mushroom extract was confirmed using synthetic racemic mixtures. After chroma-



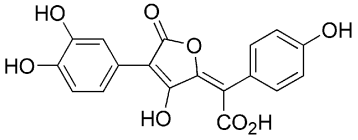
Scheme 4. Proposal for the biosynthesis of butyrolactone II (**13**), secoretipolide E (**15**), retipolide E (**16**), retipolide C (**7**), and retipolide A (**1**).

tographic enrichment of the mushroom extract, compounds **13** and **15** could be identified by analytical HPLC and showed identical R_t values and UV spectra with the synthetic references as well as no differences upon co-chromatography. This supports the biosynthetic sequence depicted in Scheme 3.

Interestingly, Shibata et al.[14] isolated the macrolactone ornatipolide (**19**) from a Japanese collection of *R. ornatipes*. This metabolite may be considered as an alternative precursor of retipolide E (**16**), with formation of this spiro compound by condensation with an equivalent of 3-(4-hydroxyphenyl)pyruvic acid (**12**), a reaction that has ample in vitro precedence.[13] Experiments to detect ornatipolide (**19**) in the North American collections of *R. retipes*/*ornatipes* by HPLC comparison with a synthetic reference compound[13] were, however, unsuccessful.



19



20

From a specimen of *R. retipes*/*ornatipes* with an unusually dark brown cap skin, we were able to identify xerocomic acid (**20**)[15] by HPLC comparison. Pulvinic acids such as **20** are characteristic pigments of the genus *Boletus*,[16] and their occurrence in *Retiboletus* signals the close relationship of both genera. More detailed chemotaxonomic investigations of the genus *Retiboletus* and the detection of retipolides in *Retiboletus flavoniger* (Halling) Binder & Bresinsky from Costa Rica will be reported in a separate publication.

Conclusions

The chemical investigation of *Retiboletus retipes*/*ornatipes* revealed the presence of several spiromacrolides, thus underlining the unique position of this genus in Boletales taxonomy. All of these compounds are biosynthetically closely related, including an unprecedented example for the biochemical conversion of a 4-hydroxyphenyl ring into a 2(3*H*)-oxepinone moiety. The proposed biosynthetic precursor retipolide E (**16**) and its congeners **13**–**15** were identified in the fungal extract in small amounts by comparison with synthetic samples. This technique for establishing biosynthetic relationships might also be useful in other cases.[17]

Experimental Section

General: Melting points (uncorrected): Reichert Thermovar hotstage. Optical rotations: Perkin–Elmer 241. IR: Perkin–Elmer 1420 Ratio Recording Infrared Spectrometer. Intensity of the bands: ss (very strong), s (strong), m (medium), and w (weak). UV/Vis spectra: Varian Cary 17 spectrophotometer. CD spectra: Jobin Yvon Instruments S.A. CD-6-Dichrograph. NMR: Bruker instruments AMX 600, WM 400, ARX 300, AC 200 and WH 90, in $CDCl_3$ and [D_6]acetone with solvent peak as internal standard. Multiplets due to ^1J(C,H) couplings are indicated by capital letters, ^3J and other couplings in small letters. MS: A.M.E. Kratos MS 50 with data system DS 50, Finnigan MAT 90 and 95 Q (direct inlet, 70 eV). X-ray diffraction: Enraf–Nonius CAD4 diffractometer at 293(2) K using Mo-K_α (λ = 0.71069 Å) radiation. All solvents were distilled before use. Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Analytical TLC: Silica gel 60 F_{254} aluminium foils (Merck); solvent system A (v/v): toluene/HCO_2Et/HCO_2H, 10:5:3; B: hexanes/EtOAc, 1:1; C: hexanes/EtOAc, 2:1; D: hexanes/EtOAc, 3:1; E: hexanes/EtOAc/AcOH, 60:40:1; F: hexanes/EtOAc/AcOH, 50:50:1; G: $CHCl_3$/MeOH, 10:1. Preparative TLC: TLC glass plates with silica gel 60 F_{254} 2 mm (Merck). Column chromatography: Silica gel 60, 40–63 μm (Merck), Sephadex LH-20 (Pharmacia), acetylated polyamide-6 (MN Polyamide SC 6-AC, Macherey–Nagel). Analytical HPLC: Waters 600 E Pump and System Controller with Photodiode Array Detector 990+. Preparative HPLC separations: Waters-Millipore with gradient controller M 680, two M 590 EF pumps and U 6 K injector equipped with a Knauer variable-wavelength monitor with a super-preparative flow cell. Pre-filtration of the solutions over SepPak RP18 cartridges (Waters). A Nucleosil 100 C18 (7 μm) pre-packed HPLC column 250 × 20 mm with a pre-column 30 × 20 mm (Macherey–Nagel) and gradient systems with MeOH/H_2O mixtures were used.

Mushrooms: *R. retipes*/*ornatipes* was collected in September 1986 and in August/September 1990 and 1991 around the "Ball Creek area", Coweeta Hydrologic Laboratory, Macon County, NC, USA (leg. et det. W. Steglich). For the isolation of isoretipolide A, collections from the White Mountains National Park, NH, were used (leg. et det. N. Arnold, 1999).

Isolation Procedure for the Retipolides A–D: Air- and freeze-dried fruit bodies of *R. retipes*/*ornatipes* (50 g) were pulverized and defatted with 3 × 400 mL of petroleum ether (60–80 °C). Extraction of the material with EtOAc (3 × 400 mL) yielded a deep orange solution. The volume of the combined extracts was reduced to 200 mL with a rotary evaporator (30 °C, 20 Torr) and the resulting solution washed with water (100 mL), dried ($MgSO_4$), and concentrated to yield a brown residue, which was triturated with hexanes (50 mL). The crude extract (1.20 g) was prepurified by gel chromatography on Sephadex LH-20 at 8 °C with acetone/toluene (9:1) as eluent to yield 4 fractions. Fraction 1 (86 mg) contained crude retipolide C as a brown solid; purification by column chromatography at 8 °C on acetylated polyamide with acetone gave retipolide C (**7**) (56 mg, 0.11% relative to dry weight). Fraction 2 (230 mg) yielded retipolide A (**1**) as a microcrystalline powder (0.46%). Fraction 3 (73 mg) contained a mixture of retipolide D with retipolide A, which was separated by preparative TLC on silica gel (hexanes/EtOAc/AcOH, 30:60:1) to give retipolide D as a yellow glass (22 mg). Rechromatography of the latter on a Sephadex LH-20 column at 8 °C with acetone yielded pure retipolide D (**8**) (7 mg, 0.014%). Fraction 4 (138 mg) contained mainly retipolide B (**6**), which was obtained in pure form (52 mg, 0.10%) by rechromatography on acetylated polyamide with acetone at 8 °C.

The retention times and UV data for an HPLC analysis of a retipolide mixture under standardized conditions are given in Table 1 [Nucleosil 100 C18, 5 μm, 250 × 4.6 mm (Merck); solvent A: H_2O/MeCN, 9:1 + 0.5% TFA; solvent B: H_2O/MeCN, 1:9; gradient:

start 90% A + 10% B, 55 min: 10% A + 90% B, 65 min: 10% A and 90% B, 75 min: 90% A and 10% B; flow rate 1 mL/min; diode array detection, 210–500 nm].

Table 1. HPLC analysis of a retipolide mixture.

Retipolide	UV/Vis spectrum λ_{max} (% rel. int.)	Retention time t_R [min]
Retipolide B (**6**)	219 (100), 327 (33)	20.79
Retipolide D (**8**)	213 (100), 276 (27), 305 (sh, 5)	22.99
Retipolide A (**1**)	216 (100), 327 (35)	25.14
Retipolide E (**16**)	213 (100), 229 (sh, 81), 289 (sh, 35), 306 (36)	26.74
Retipolide C (**7**)	213 (100), 238 (sh, 64), 278 (29)	27.24

Retipolide A, (1*R*,5*RS*)-7,8-Dihydro-4′,5-dihydroxyspiro[cyclopenta-[*d*]furo[3,4-*b*]pyran-1,12′(5*H*)-[2,10]dioxatricyclo[12.2.2.1^{3,7}]nonadeca[1(16),3,5,7(19),14,17]hexaene]-3,6,11′-trione (1): White to yellowish, amorphous powder; easily soluble in acetone, sparingly soluble in $CHCl_3$, decomposes in MeOH; m.p. > 360 °C (the sample darkens above 200 °C). R_f (TLC) = 0.47 (solvent system A), colorless spot, + NH_3 orange-yellow. $[\alpha]_D^{20}$ = +160 (c = 0.1, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 208 sh (4.20), 238 sh (3.71), 288 (3.44), 316 (3.59), 390 (3.04), 428 (3.13) nm; (MeCN + NH_3): 244 (3.96), 284 (3.50), 312 (3.40), 392 (3.62), 429 (3.71) nm; (MeOH + HCl): 208 sh (4.21), 237 sh (3.86), 270 (3.52), 284 (3.49), 322 (3.59) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 215 (0), 227 (+25.90), 268 (+4.70), 280 (0), 285 (–1.03), 291 (0), 302 (+1.47), 385 (0) nm. IR (KBr): $\tilde{\nu}$ = 3400 (m, br), 2920 (m), 2860 (w), 1785 (ss), 1730 (s), 1700 (s), 1650 (m), 1590 (m), 1520 (m), 1500 (m), 1430 (m), 1380 (m), 1370 (w), 1340 (w), 1270 (s), 1210 (m), 1180 (w), 1160 (m), 1140 (m), 1100 (m), 1030 (m), 1000 (w), 980 (m), 930 (w), 920 (w), 880 (w), 840 (w), 820 (w), 780 (w), 750 (w), 740 (w), 610 (w) cm^{-1}. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 2.58 (m, 2 H, 7-H), 2.62 (ddd, J = 17.1, 5.2, 0.8 Hz, 1 H, 8′-H^a), 2.85 (dddd, J = 17.1, 11.0, 1.8, 0.9 Hz, 1 H, 8′-H^b), 3.29 (dddd, J = 18.5, 4.5, 4.5, 1.0 Hz, 1 H, 8-H^a), 3.32 (d, J = 13.8 Hz, 1 H, 13′-H^b), 3.38 (ddd, J = 18.5, 4.5, 4.5 Hz, 1 H, 8-H^b), 3.89 (d, J = 13.8 Hz, 1 H, 13′-H^a), 4.00 (ddd, J = 11.1, 5.2, 1.8 Hz, 1 H, 9′-H^b), 4.12 (dd, J = 11.1, 11.0 Hz, 1 H, 9′-H^a), 4.83 (dd, J = 2.1, 0.9 Hz, 1 H, 19′-H), 6.49 (br. s, 1 H, 5-H), 6.51 (ddd, J = 8.0, 2.1, 0.8 Hz, 1 H, 6′-H), 6.70 (d, J = 8.0 Hz, 1 H, 5′-H), 7.00 (dd, J = 8.5, 2.2 Hz, 1 H, 17′-H), 7.19 (br. s, 1 H, 4′- or 5-OH), 7.24 (dd, J = 8.5, 2.2 Hz, 1 H, 16′-H), 7.35 (dd, J = 8.5, 2.2 Hz, 1 H, 18′-H), 7.67 (dd, J = 8.5, 2.2 Hz, 1 H, 15′-H), 7.97 (br. s, 1 H, 4′- or 5-OH) ppm. ^{1}H-coupled ^{13}C NMR (100.6 MHz, [D_6]DMSO/[D_6]acetone, 3:2): δ = 25.3 (br. Tm, J = 135 Hz, C-8), 30.9 (Tm, J = 126 Hz, C-8′), 35.2 (Tm, J = 133 Hz, C-7), 42.7 (br. Tm, J = 132 Hz, C-13′), 69.9 (Td, J = 150, 9 Hz, C-9′), 86.8 (d, J = 8 Hz, C-1), 93.9 (br. Dm, J = 180 Hz, C-5), 115.2 (Ddd, J = 158, 7, 6 Hz, C-19′), 115.9 (Dd, J = 155, 4 Hz, C-5′), 121.3 (Ddm, J = 158, 6 Hz, C-6′), 124.4 (Dd, J = 162, 5 Hz, C-16′), 124.5 (Dd, J = 162, 6 Hz, C-17′), 127.0 (br. m, C-8b), 130.6 (m, C-7′), 131.6 (m, C-14′), 131.7 (br. m, C-3a), 132.4 (Dq, J = 162, 6 Hz, C-18′), 133.1 (Dtd, J = 163, 8, 4 Hz, C-15′), 143.5 (ddd, J = 7, 6, 2 Hz, C-4′), 143.9 (m, C-5a), 151.5 (dd, J = 8, 4 Hz, C-3′), 157.6 (br. m, C-8a), 158.4 (tt, J = 10, 4 Hz, C-1′), 163.4 (s, C-3), 167.8 (br. d, J = 8 Hz, C-11′), 203.5 (m, C-6) ppm. EI-MS (DI, 200 °C): m/z (%) = 477 (4) [M + 1]$^+$, 476 (15) [M$^+$], 459 (14), 458 (43), 432 (13), 430 (5), 414 (6), 386 (5), 227 (50), 226 (80), 225 (100) [$C_{15}H_{13}O_2$], 212 (11), 120 (45) [C_8H_8O], 107 (37) [C_7H_7O], 91 (42) C_7H_7], 84 (48), 82 (63). $C_{26}H_{20}O_9$ [M$^+$]: calcd. 476.1107; found 476.1111. $C_{26}H_{20}O_9$ (476.4): calcd. C 65.55, H 4.32; found C 65.65, H 4.45.

Retipolide B, (1*R*,5*RS*)-7,8-Dihydro-4′,5,5′-trihydroxyspiro[cyclopenta[*d*]furo[3,4-*b*]pyran-1,12′(5*H*)-[2,10]dioxatricyclo[12.2.2.1^{3,7}]nonadeca[1(16),3,5,7(19),14,17]hexaene]-3,6,11′-trione (6): Yellowish, amorphous powder; easily soluble in acetone, sparingly soluble in $CHCl_3$, decomposes in MeOH; m.p. > 360 °C (the sample darkens above 190 °C). R_f (TLC) = 0.39 (solvent system A), colorless spot, + NH_3 red-orange. $[\alpha]_D^{20}$ = +61 (c = 0.1, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 195 (4.71), 215 sh (4.53), 317 (3.91), 436 (3.19) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 218 (0), 228 (+26.49), 270 (+4.42), 380 (0) nm. IR (KBr): $\tilde{\nu}$ = 3400 (s, br), 2980 (m), 2940 (m), 2880 (w), 1790 (ss), 1740 (s), 1700 (s), 1650 (m), 1620 (m), 1600 (m), 1540 (m), 1530 (m), 1510 (m), 1450 (m), 1370 (m), 1340 (m), 1320 (m), 1270 (s), 1220 (s), 1190 (s), 1170 (s), 1150 (s), 1110 (s), 1030 (s), 970 (w), 950 (w), 920 (w), 900 (w), 880 (w), 840 (w), 820 (w), 790 (w), 760 (w), 740 (w), 720 (w), 710 (w), 690 (w), 680 (w), 640 (w), 610 (w) cm^{-1}. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 2.49 (dddd, J = 17.3, 5.1, 0.7, 0.5 Hz, 1 H, 8′-H^a), 2.58 (m, 2 H, 7-H), 2.80 (dddd, J = 17.3, 10.9, 1.8, 1.0 Hz, 1 H, 8′-H^b), 3.26$^+$ (ddd, J = 18.5, 4.5, 4.5 Hz, 1 H, 8-H^a), 3.30 (d, J = 13.9 Hz, 1 H, 13′-H^b), 3.38$^+$ (br. m, 1 H, 8-H^b), 3.87 (d, J = 13.9 Hz, 1 H, 13′-H^a), 3.96 (ddd, J = 11.4, 5.1, 1.8 Hz, 1 H, 9′-H^b), 4.13 (ddd, J = 11.4, 10.9, 0.7 Hz, 1 H, 9′-H^a), 4.35 (dd, J = 2.1, 1.0 Hz, 1 H, 19′-H), 6.20 (dd, J = 2.1, 0.5 Hz, 1 H, 6′-H), 6.50 (br. s, 1 H, 5-H), 7.00 (dd, J = 8.5, 2.4 Hz, 1 H, 17′-H), 7.21 (dd, J = 8.5, 2.4 Hz, 1 H, 16′-H), 7.31 (dd, J = 8.5, 2.2 Hz, 1 H, 18′-H), 7.63 (dd, J = 8.5, 2.2 Hz, 1 H, 15′-H) ppm ($^+$ assignments are interchangeable). ^{1}H-coupled ^{13}C NMR (100.6 MHz, [D_6]DMSO/[D_6]acetone, 3:2): δ = 26.1 (br. Tm, J = 136 Hz, C-8), 32.1 (Tm, J = 127 Hz, C-8′), 35.8 (Tm, J = 132 Hz, C-7), 43.9 (br. Tm, J = 134 Hz, C-13′), 69.7 (Td, J = 150, 9 Hz, C-9′), 87.6 (d, J = 7 Hz, C-1), 94.7 (br. Dm, J = 180 Hz, C-5), 107.7 (Dt, J = 160, 7 Hz, C-19′), 109.6 (Dm, J = 158 Hz, C-6′), 125.2 (Dd, J = 162, 5 Hz, C-16′), 125.4 (Dd, J = 162, 5 Hz, C-17′), 128.0 (br. m, C-8b), 131.5 (m, C-7′), 132.4§ (br. m, C-4′), 132.5§ (br. m, C-3a), 132.5 (m, C-14′), 133.1 (Dq, J = 161, 6 Hz, C-18′), 133.8 (Dtd, J = 163, 8, 4 Hz, C-15′), 144.6 (d, J = 6 Hz, C-5′), 146.6 (m, C-5a), 152.9 (d, J = 3 Hz, C-3′), 158.6 (br. m, C-8a), 159.9 (tt, J = 10, 5 Hz, C-1′), 163.9 (s, C-3), 168.7 (br. m, C-11′), 203.8 (m, C-6) ppm (§ assignments are exchangeable). EI-MS (DI, 180 °C): m/z (%) = 492 (1) [M$^+$], 242 (11), 241 (17, $C_{15}H_{13}O_3$), 121 (12), 120 (20), 118 (8), 107 (31), 85 (17), 83 (26), 44 (100, CO_2). $C_{26}H_{20}O_{10}$ [M$^+$]: calcd. 492.1056; found 492.1039.

Retipolide C, (*R*)-3′-(2,3-Dihydro-2-oxo-5-oxepinyl)-4,4′-dihydroxyspiro[2,10-dioxatricyclo[12.2.2.1^{3,7}]nonadeca-1(16),3,5,7(19),14,17-hexaene-12,2′(5′*H*)-furan]-11,5′-dione (7): Colorless amorphous solid; easily soluble in acetone, sparingly soluble in $CHCl_3$; m.p. 100–105 °C. R_f (TLC) = 0.51 (solvent system A), colorless spot, + NH_3 pink. $[\alpha]_D^{20}$ = +112 (c = 0.1, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 233 sh (4.31), 240 sh (4.23), 275 (4.03) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 250 (+21.04), 313 (0), 330 (–0.61), 355 (–0.37), 390 (–0.72), 460 (0) nm. IR (KBr): $\tilde{\nu}$ = 3410 (s, br), 2460 (w), 2420 (w), 1760 (s, br), 1725 (s), 1670 (w), 1630 (w), 1590 (w), 1515 (m), 1500 (m), 1455 (w), 1430 (w), 1415 (w, sh), 1390 (w), 1345 (w), 1310 (w, sh), 1255 (s), 1205 (m), 1160 (m), 1105 (m), 1090 (m, sh), 1060 (w, sh), 1030 (w), 1005 (w, sh), 975 (w), 945 (w), 920 (w), 875 (w), 840 (w), 770 (w), 720 (w), 615 (w) cm^{-1}. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 2.58 (dd, J = 17.2, 5.1 Hz, 1 H, 8-H^a), 2.82 (dddd, J = 17.2, 11.0, 1.8, 0.8 Hz, 1 H, 8-H^b), 3.16 (d, J = 13.8 Hz, 1 H, 13-H^b), 3.28/3.29 (each dd, J = 12.0, 7.0 Hz, 1 H, 3′′-H^a and 3′′-H^b), 3.90 (d, J = 13.8 Hz, 1 H, 13-H^a), 3.92 (ddd, J = 11.1, 5.1, 1.8 Hz, 1 H, 9-H^b), 4.15 (dd, J = 11.1, 11.0 Hz, 1 H, 9-H^a), 4.77 (dd, J = 2.0, 0.8 Hz, 1 H, 19-H), 6.49 (dd, J = 8.0, 2.0 Hz, 1 H, 6-H), 6.54 (d, J = 6.9 Hz, 1 H, 6′′-H), 6.64 (t, J = 7.0 Hz, 1 H, 4′′-H), 6.70 (d, J = 8.0 Hz, 1 H, 5-H), 6.83 (d, J = 6.9 Hz, 1 H, 7′′-H), 6.95 (dd, J

= 8.4, 2.5 Hz, 1 H, 17-H), 7.19 (dd, *J* = 8.4, 2.5 Hz, 1 H, 16-H), 7.30 (dd, *J* = 8.4, 2.4 Hz, 1 H, 18-H), 7.61 (dd, *J* = 8.4, 2.4 Hz, 1 H, 15-H) ppm. ^{1}H-coupled ^{13}C NMR (100.6 MHz, [D$_6$]DMSO/[D$_6$]acetone, 3:2): δ = 32.1 (Tm, *J* = 127 Hz, C-8), 36.9 (Td, *J* = 136, 5 Hz, C-3′′), 43.9 (Tt, *J* = 134, 4 Hz, C-13), 69.1 (Td, *J* = 150, 8 Hz, C-9), 88.8 (d, *J* = 8 Hz, C-12), 114.4 (Dt, *J* = 166, 10 Hz, C-6′′), 115.9 (Ddd, *J* = 159, 7, 6 Hz, C-19), 116.3 (Dd, *J* = 156, 3 Hz, C-5), 122.3 (Dddd, *J* = 156, 10, 6, 4 Hz, C-6), 124.8 (Dd, *J* = 163, 5 Hz, C-16), 124.9 (Dd, *J* = 162, 5 Hz, C-17), 126.9 (Dm, *J* = 170 Hz, C-4′′), 127.0 (m, C-3′), 130.0 (m, C-5′′), 132.4 (m, C-7), 132.9 (m, C-14), 133.7 (Dq, *J* = 162, 6 Hz, C-18), 134.4 (Dtd, *J* = 162, 8, 4 Hz, C-15), 141.8 (m, C-4), 142.0 (Dd, *J* = 198, 6 Hz, C-7′′), 144.5 (m, C-4), 152.3 (m, C-3), 159.3 (tt, *J* = 10, 4 Hz, C-1), 166.8 (m, C-2′′), 167.1 (br. s, C-5′), 168.5 (dd, *J* = 10, 3 Hz, C-11) ppm. EI-MS (DI, 230 °C): *m*/*z* (%) = 477 (0.2) [M + 1]$^+$, 476 (0.4) [M$^+$], 300 (8), 226 (12), 225 (20), 122 (5), 121 (10), 120 (10), 107 (14), 91 (14), 44 (100). $C_{26}H_{20}O_9$ [M$^+$]: calcd. 476.1107; found 476.1107.

Retipolide D, (*R*)-3′-(2,3-Dihydro-2-oxo-5-oxepinyl)-4,4′,5-trihydroxyspiro[2,10-dioxatricyclo[12.2.2.1^{3,7}]nonadeca-1(16),3,5,7(19),14,17-hexaene-12,2′(5′*H*)-furan]-11,5′-dione (8): Colorless amorphous solid; easily soluble in acetone, sparingly soluble in $CHCl_3$; m.p. 135–140 °C. R_f (TLC) = 0.46 (solvent system A), 0.28 (solvent system F), colorless spot, + NH_3 pink. $[\alpha]_D^{20}$ = +128 (*c* = 0.14, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 272 (3.92), 280 sh (3.89), 295 sh (3.69) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 255 (+16.21), 356 (0), 385 (–0.08), 470 (0) nm. IR (KBr): $\tilde{\nu}$ = 3430 (s, br), 3100 (w, sh), 2940 (w), 1760 (s, br), 1730 (s), 1610 (w), 1590 (m), 1535 (w, sh), 1520 (w), 1500 (m), 1440 (w), 1390 (w), 1250 (m), 1200 (m), 1160 (m), 1100 (m), 1075 (w, sh), 1010 (m), 970 (w), 875 (w), 835 (w), 830 (w, sh), 810 (w), 785 (w), 770 (w), 760 (w, sh), 740 (w), 650 (w) cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 2.48 (dd, *J* = 17.2, 5.3 Hz, 1 H, 8-H^a), 2.78 (ddd, *J* = 17.2, 11.2, 1.5 Hz, 1 H, 8-H^b), 3.13 (d, *J* = 13.5 Hz, 1 H, 13-H^b), 3.25/3.29 (each dd, *J* = 12.1, 7.3 Hz, 1 H, 3′′-H^a and 3′′-H^b), 3.89 (d, *J* = 13.5 Hz, 1 H, 13-H^a), 3.88 (ddd, *J* = 11.3, 5.3, 1.5 Hz, 1 H, 9-H^b), 4.17 (dd, *J* = 11.3, 11.2 Hz, 1 H, 9-H^a), 4.30 (d, *J* = 2.0 Hz, 1 H, 19-H), 6.20 (d, *J* = 2.0 Hz, 1 H, 6-H), 6.55 (d, *J* = 7.1 Hz, 1 H, 6′′-H), 6.64 (t, *J* = 7.3 Hz, 1 H, 4′′-H), 6.83 (d, *J* = 7.1 Hz, 1 H, 7′′-H), 6.95 (dd, *J* = 8.5, 2.5 Hz, 1 H, 17-H), 7.16 (dd, *J* = 8.5, 2.5 Hz, 1 H, 16-H), 7.27 (dd, *J* = 8.5, 2.5 Hz, 1 H, 18-H), 7.58 (dd, *J* = 8.5, 2.5 Hz, 1 H, 15-H) ppm. ^{1}H-coupled ^{13}C NMR (100.6 MHz, [D$_6$]acetone): δ = 32.3 (Tm, *J* = 128 Hz, C-8), 36.9 (Td, *J* = 135, 5 Hz, C-3′′), 43.9 (Tt, *J* = 134, 5 Hz, C-13), 69.0 (Td, *J* = 150, 8 Hz, C-9), 88.7 (d, *J* = 7 Hz, C-12), 107.5 (Dtd, *J* = 160, 8, 2 Hz, C-19), 109.6 (Dm, *J* = 157 Hz, C-6), 114.4 (Dt, *J* = 164, 10 Hz, C-6′′), 124.8 (Dd, *J* = 162, 6 Hz, C-16), 125.0 (Dd, *J* = 162, 5 Hz, C-17), 126.5 (Dm, *J* = 164 Hz, C-4′′), 126.5 (m, C-3′), 130.2 (m, C-5′′), 131.7 (m, C-7), 132.5 (m, C-4), 132.8 (m, C-14), 133.5 (Dq, *J* = 162, 6 Hz, C-18), 134.2 (Dtd, *J* = 163, 8, 3 Hz, C-15), 141.9 (Dd, *J* = 198, 6 Hz, C-7′′), 142.4 (m, C-4′), 146.6 (m, C-5), 152.9 (m, C-3), 159.6 (tt, *J* = 10, 4 Hz, C-1), 166.8 (m, C-2′′), 167.3 (s, C-5′), 168.5 (d, *J* = 8 Hz, C-11) ppm. EI-MS (DI, 230 °C): *m*/*z* (%) = 492 (0.9) [M$^+$], 107 (8), 44 (100). $C_{26}H_{20}O_{10}$ [M$^+$]: calcd. 492.1056; found 492.1060.

Synthesis of Anhydroretipolide A 6-*O*-Methyl Ether (2a): A stirred solution of **1** (86 mg, 0.181 mmol) in MeOH (0.5 mL) was treated with a 1% solution of TsOH·H_2O in 2,2-dimethoxypropane (2.5 mL) and heated for 20 min at 75 °C (bath temperature). After cooling to room temperature, the dark red solution was immediately flash chromatographed (hexanes/EtOAc, 1:1) to yield a red oil (97 mg). Purification by chromatography on Sephadex LH-20 with acetone afforded **2a** (52 mg, 61%) as an orange-red crystalline solid, which was easily soluble in acetone or chloroform; m.p. 170–175 °C. R_f (TLC) = 0.61 (solvent system A), 0.52 (solvent system B), 0.38 (solvent system C), orange spot. t_R (HPLC) = 38.03 min, conditions as given for Table 1. $[\alpha]_D^{20}$ = –196 (*c* = 0.14, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 250 (4.44), 272 sh (4.06), 374 sh (3.95), 387 (4.00) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 228 (+8.43), 234 (0), 242 (–14.37), 261 (0), 283 (+3.08), 309 (0), 385 (–2.20), 450 (0) nm. IR (KBr): $\tilde{\nu}$ = 3420 (s, br), 1770 (ss), 1720 (s), 1620 (w), 1590 (s), 1550 (m), 1510 (m, sh), 1500 (s), 1495 (m, sh), 1420 (m), 1400 (w), 1370 (w), 1350 (w), 1260 (s), 1200 (m), 1100 (m), 1070 (m), 1040 (w), 1010 (s), 970 (m), 870 (m), 795 (m) cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]-acetone): δ = 2.59 (dddd, *J* = 17.1, 5.1, 0.8, 0.7 Hz, 1 H, 8′-H^a), 2.82 (dddd, *J* = 17.1, 11.2, 1.8, 1.0 Hz, 1 H, 8′-H^b), 3.32 (d, *J* = 13.8 Hz, 1 H, 13′-H^b), 3.96 (s, 3 H, OMe), 3.93 (ddd, *J* = 11.5, 5.1, 1.8 Hz, 1 H, 9′-H^b), 3.99 (d, *J* = 13.8 Hz, 1 H, 13′-H^a), 4.17 (ddd, *J* = 11.5, 11.2, 0.7 Hz, 1 H, 9′-H^a), 4.87 (dd, *J* = 2.1, 1.0 Hz, 1 H, 19′-H), 6.51 (ddd, *J* = 8.0, 2.1, 0.8 Hz, 1 H, 6′-H), 6.57 (d, *J* = 3.1 Hz, 1 H, 7-H), 6.71 (d, *J* = 8.0 Hz, 1 H, 5′-H), 7.04 (dd, *J* = 8.5, 2.5 Hz, 1 H, 17′-H), 7.12 (dd, *J* = 3.1, 1.1 Hz, 1 H, 8-H), 7.26 (dd, *J* = 8.5, 2.5 Hz, 1 H, 16′-H), 7.45 (dd, *J* = 8.5, 2.5 Hz, 1 H, 18′-H), 7.71 (dd, *J* = 8.5, 2.5 Hz, 1 H, 15′-H), 8.02 (br. s, 4′-OH), 8.51 (d, *J* = 1.1 Hz, 1 H, 5-H) ppm. ^{1}H-coupled ^{13}C NMR (100.6 MHz, [D$_6$]acetone): δ = 31.9 (Tm, *J* = 127 Hz, C-8′), 44.8 (Tt, *J* = 134, 4 Hz, C-13′), 58.1 (Q, *J* = 145 Hz, OMe), 69.5 (Td, *J* = 150, 8 Hz, C-9′), 87.5 (d, *J* = 8 Hz, C-1), 110.5 (Dd, *J* = 168, 6 Hz, C-7), 110.5 (ddd, *J* = 10, 6, 3 Hz, C-8a), 116.1 (Dt, *J* = 158, 8 Hz, C-19′), 116.4 (Dd, *J* = 156, 3 Hz, C-5′), 116.9 (Dd, *J* = 174, 5 Hz, C-8), 119.7 (q, *J* = 8 Hz, C-5a), 122.2 (Dm, *J* = 156 Hz, C-6′), 125.1 (Dd, *J* = 163, 5 Hz, C-16′), 125.2 (Dd, *J* = 163, 5 Hz, C-17′), 131.9 (d, *J* = 7 Hz, C-3a), 132.3 (m, C-7′), 132.8 (m, C-14′), 133.5 (Dq, *J* = 161, 6 Hz, C-18′), 134.0 (Dtd, *J* = 163, 8, 3 Hz, C-15′), 137.0 (d, *J* = 2 Hz, C-8b), 144.6 (m, C-4′), 146.0 (Dd, *J* = 203, 2 Hz, C-5), 152.4 (m, C-3′), 154.4 (m, C-6), 159.6 (tt, *J* = 10, 4 Hz, C-1′), 163.0 (s, C-3), 168.0 (d, *J* = 8 Hz, C-11′) ppm. EI-MS (DI, 200 °C): *m*/*z* (%) = 474 (6) [M + 2]$^+$, 473 (32) [M + 1]$^+$, 472 (100) [M$^+$], 445 (13), 444 (47), 429 (7), 401 (8), 249 (11), 236 (6), 226 (10), 225 (32), 173 (8), 120 (28), 119 (7), 107 (11), 91 (26), 44 (13), 32 (64). $C_{27}H_{20}O_8$ [M$^+$]: calcd. 472.1158; found 472.1153.

Preparation of Methoxyfulvene 2a from the Crude Mushroom Extract: The crude extract from *Retiboletus retipes*/*ornatipes* (600 mg) was dissolved in MeOH (2 mL), treated with a 1% solution of TsOH·H_2O in 2,2-dimethoxypropane (2.5 mL), and heated with stirring for 20 min to 75 °C (bath temperature). After cooling to room temperature, the solution was immediately flash chromatographed on silica gel (hexanes/EtOAc, 1:1) to yield a red oil. Repeated flash chromatography (hexanes/EtOAc, 2:1) afforded **2a** (118 mg).

Synthesis of Anhydroretipolide A 6-*O*-(*S*)-*sec*-Butyl Ether (2b): A mixture of acetone (167 mg, 210 μL, 2.88 mmol), (*S*)-2-butanol (850 mg, 1050 μL, 11.5 mmol), cyclohexane (4.3 mL), *p*-toluenesulfonic acid mono hydrate (109 mg, 0.57 mmol), and activated pulverized molecular sieves (5 Å) (1.1 g) was stirred for 2 h at 0 °C. After removal of the molecular sieves by filtration and concentration with a rotary evaporator (20 °C, 20 Torr), the remaining dark-violet solution was treated with **1** (100 mg, 0.210 mmol) and stirred for 1 h at 80 °C (bath temperature). The mixture was cooled to room temperature and flash chromatographed (hexanes/EtOAc, 1:1) to yield a red oil (100 mg), which was adsorbed on silica gel (0.40 g). Flash chromatography on silica gel (hexanes/EtOAc, 3:1) afforded **2b** (22 mg, 20%) as orange red crystals, easily soluble in acetone and $CHCl_3$; m.p. 305 °C. Crystals suitable for the X-ray crystal structure determination were obtained by dissolving **2b** (22 mg) in acetone (2 mL), adding MeOH (2 mL), and keeping the probe for 4 weeks at room temperature, which allowed the acetone

to slowly evaporate. R_f (TLC) = 0.72 (solvent system A), 0.77 (solvent system B), 0.39 (solvent system D), orange spot. $[\alpha]_D^{20}$ = –160 (c = 0.43, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 241 (4.26), 273 (4.00), 391 (3.97) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 236 (+25.31), 244 (0), 253 (–21.11), 263 (0), 283 (+3.81), 324 (0), 392 (–2.14), 465 (–0.04), 490 (–0.16), 550 (0) nm. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 1.00 (t, J = 7.5 Hz, 3 H, 4′′-H), 1.37 (d, J = 6.2 Hz, 3 H, 1′′-H), 1.70/1.80 [each dqd, J = 14.3, 7.5, 6.0 (6.4) Hz, 2 H, 3′′-H], 2.59 (dd, J = 17.3, 5.2 Hz, 1 H, 8′-H^a), 2.87 (dddd, J = 17.3, 11.0, 1.7, 0.8 Hz, 1 H, 8′-H^b), 3.30 (d, J = 13.8 Hz, 1 H, 13′-H^b), 3.94 (ddd, J = 11.1, 5.2, 1.7 Hz, 1 H, 9′-H^b), 3.99 (d, J = 13.8 Hz, 1 H, 13′-H^a), 4.18 (dd, J = 11.1, 11.0 Hz, 1 H, 9′-H^a), 4.44 (dqd, J = 6.4, 6.2, 6.0 Hz, 1 H, 2′′-H), 4.87 (dd, J = 2.1, 0.8 Hz, 1 H, 19′-H), 6.51 (dd, J = 8.0, 2.1 Hz, 1 H, 6′-H), 6.55 (d, J = 3.2 Hz, 1 H, 7-H), 6.71 (d, J = 8.0 Hz, 1 H, 5′-H), 7.04 (dd, J = 8.4, 2.5 Hz, 1 H, 17′-H), 7.12 (dd, J = 3.2, 1.1 Hz, 1 H, 8-H), 7.26 (dd, J = 8.4, 2.5 Hz, 1 H, 16′-H), 7.44 (dd, J = 8.4, 2.3 Hz, 1 H, 18′-H), 7.71 (dd, J = 8.4, 2.3 Hz, 1 H, 15′-H), 8.02 (br. s, 4′-OH), 8.50 (d, J = 1.1 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100.6 MHz, [D$_6$]acetone): δ = 9.9 (C-4′′), 19.2 (C-1′′), 29.7 (C-3′′), 32.0 (C-8′), 44.8 (C-13′), 69.5 (C-9′), 78.7 (C-2′′), 87.5 (C-1), 110.0 (C-8a), 111.5 (C-7), 116.1 (C-19′), 116.4 (C-5′), 117.2 (C-8), 120.7 (C-5a), 122.2 (C-6′), 125.1 (C-16′), 125.2 (C-17′), 131.8 (C-3a), 132.3 (C-7′), 132.9 (C-14′), 133.5 (C-18′), 134.1 (C-15′), 137.1 (C-8b), 144.6 (C-4′), 146.0 (C-5), 152.4 (C-3′), 152.7 (C-6), 159.6 (C-1′), 163.1 (C-3), 168.1 (C-11′) ppm. EI-MS (DI, 200 °C): m/z (%) = 515 (18) [M + 1]$^+$, 514 (70) [M$^+$], 459 (28), 458 (100), 431 (16), 430 (74), 250 (6), 249 (11), 229 (7), 227 (5), 226 (17), 225 (42), 232 (11), 120 (20), 107 (12), 91 (11). $C_{30}H_{26}O_8$ [M$^+$]: calcd. 514.1628; found 514.1640.

Synthesis of Retipolide A 5-*O*-Methyl Ether (3): To a solution of **1** (2.0 mg, 4.2 μmol) in acetone (50 μL) was added a 1% solution of TsOH in 2,2-dimethoxypropane (50 μL). The mixture was kept for 24 h at room temperature and then separated by preparative TLC on silica gel (solvent system C) to yield **3** (1.4 mg, 68%) as a colorless, amorphous solid, and less than 0.1 mg of **2a**.

3 (Mixture of Epimers): M.p. 110–112 °C. R_f (TLC) = 0.56 (solvent system A), 0.24 (solvent system B), 0.31 (solvent system F). t_R (HPLC) = 29.84 min, conditions as given for Table 1. UV/Vis (MeCN): λ_{max} (log ε) = 212 (4.12), 290 sh (3.39), 318 (3.58), 438 (2.09) nm. ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 3.54 (s, 1.95 H, OMe), 3.57 (s, 1.05 H, OMe), 6.18 (d, J = 1.2 Hz, 0.65 H, 5-H), 6.19 (d, J = 1.2 Hz, 0.35 H, 5-H) ppm. EI-MS (DI, 200 °C): m/z (%) = 491 (28) [M + 1]$^+$, 490 (84) [M$^+$], 462 (8), 459 (14), 458 (5), 257 (12), 256 (5), 228 (12), 227 (74), 226 (100), 225 (44), 212 (5), 211 (10), 120 (51), 119 (10), 107 (36), 92 (10), 91 (58), 85 (10), 57 (28), 45 (22), 44 (62), 32 (74), 31 (44). $C_{27}H_{22}O_9$ [M$^+$]: calcd. 490.1264; found 490.1272.

Acetylation of Retipolide A (1): A solution of **1** (30 mg, 0.063 mmol) in acetic anhydride (30 mL) was treated with one drop of concd. H_2SO_4 and kept for 65 h at room temperature. The reaction mixture was then stirred for 1 h with water (200 mL) and the aqueous phase subsequently extracted with EtOAc (2 × 200 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The complex reaction mixture was chromatographed on a silica gel column (hexanes/EtOAc, 2:1), and the two acetates were purified by preparative TLC on silica gel with the same solvent mixture to yield the diacetyl and triacetyl derivatives **4** (15 mg) and **5** (5 mg), respectively. Both compounds are rather sensitive and decompose on standing in solution.

Anhydroretipolide A 4′,6-Di-*O*-acetate (4): Yellow glass, m.p. 130 °C (decomp.). R_f (TLC) = 0.50 (solvent system C), yellow spot. IR (KBr): $\tilde{\nu}$ = 2920 (m), 1780 (s), 1730 (m), 1200 (s) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.37, 2.40 (each s, 3 H, CH_3CO), 2.58 (ddm, J = 17.1, 5.2 Hz, 1 H, 8′-H^a), 3.04 (ddm, J = 17.1, 10.6 Hz, 1 H, 8′-H^b), 3.30 (d, J = 13.8 Hz, 1 H, 13′-H^b), 3.88 (ddd, J = 11.5, 5.2, 1.5 Hz, 1 H, 9′-H^b), 3.93 (d, J = 13.8 Hz, 1 H, 13′-H^a), 4.28 (dd, J = 11.5, 10.6 Hz, 1 H, 9′-H^a), 4.97 (dd, J = 2.1, 0.9 Hz, 1 H, 19′-H), 6.60 (ddd, J = 8.1, 2.1, 0.4 Hz, 1 H, 6′-H), 6.89 (d, J = 8.1 Hz, 1 H, 5′-H), 7.04 (dd, J = 8.3, 2.4 Hz, 1 H, 17′-H), 7.21 (dd, J = 3.3, 1.2 Hz, 1 H, 8-H), 7.25 (dd, J = 3.3, 0.4 Hz, 1 H, 7-H), 7.26 (dd, J = 8.5, 2.4 Hz, 1 H, 16′-H), 7.34 (dd, J = 8.3, 2.3 Hz, 1 H, 18′-H), 7.68 (dd, J = 8.5, 2.3 Hz, 1 H, 15′-H), 8.33 (dd, J = 1.2, 0.4 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100.6 MHz, [D$_6$]acetone): δ = 20.8 (CH_3), 21.1 (CH_3), 31.9 (CH_2), 44.9 (CH_2), 68.6 (CH_2), 87.2 (C), 112.9 (C), 115.4 (CH), 116.7 (CH), 119.5 (C), 121.5 (CH), 122.3 (CH), 124.4 (CH), 124.6 (CH), 124.8 (CH), 131.1 (C), 132.4 (C), 132.5 (CH), 133.3 (CH), 136.0 (C), 137.4 (C), 138.4 (C), 140.2 (C), 145.5 (CH), 154.8 (C), 158.9 (C), 162.4 (C), 167.1 (C), 168.2 (C), 169.1 (C) ppm. EI-MS (DI, 240 °C): m/z (%) = 542 (13) [M$^+$], 501 (29), 500 (100, $C_{28}H_{20}O_9$), 472 (17), 459 (17), 458 (64), 431 (8), 430 (33), 249 (10), 226 (16), 225 (37, $C_{15}H_{13}O_2$), 212 (9), 120 (21), 107 (17), 91 (21). $C_{30}H_{22}O_{10}$ [M$^+$]: calcd. 542.1213; found 542.1249.

8-Acetylanhydroretipolide A 4′,6-Di-*O*-acetate (5): Yellow glass, m.p. > 200 °C (decomp.). R_f (TLC) = 0.75 (toluene/HCO_2Et/HCO_2H, 10:5:3), yellow spot. $[\alpha]_D^{20}$ = –169 (c = 0.09, $CHCl_3$). UV/Vis (MeCN): λ_{max} (log ε) = 231 (sh, 4.26), 278 (3.42), 370 (3.11) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 225 (+5.56), 235 (0), 248 (–4.51), 272 (–0.19), 277 (+0.12), 280 (0), 289 (+1.11), 305 (0), 321 (–0.86), 460 (0) nm. IR (KBr): $\tilde{\nu}$ = 2920 (s), 1780 (ss), 1730 (s), 1680 (m), 1200 (ss) cm^{-1}. ^{1}H NMR (200 MHz, [D$_6$]acetone): δ = 2.28, 2.32 (each s, 3 H, CH_3CO), 2.60 (s, 3 H, CH_3CO), 3.13 (d, J = 14.0 Hz, 1 H, 13′-H^b), 3.94 (m, 2 H, 9′-H), 5.03 (dd, J = 2.0, 0.8 Hz, 1 H, 19′-H), 5.40 (d, J = 14.0 Hz, 1 H, 13′-H^a), 6.62 (ddd, J = 8.0, 2.0, 0.9 Hz, 1 H, 6′-H), 6.88 (d, J = 8.0 Hz, 1 H, 5′-H), 7.02 (dd, J = 8.3, 2.5 Hz, 1 H, 17′-H), 7.20 (dd, J = 8.5, 2.5 Hz, 1 H, 16′-H), 7.45 (d, J = 0.3 Hz, 1 H, 7-H), 7.49 (dd, J = 8.3, 2.2 Hz, 1 H, 18′-H), 7.67 (dd, J = 8.5, 2.2 Hz, 1 H, 15′-H), 9.01 (d, J = 0.3 Hz, 1 H, 5-H) ppm; signals for 8′-H obscured. EI-MS (DI, 220 °C): m/z (%) = 585 (5), 584 [M$^+$] (12), 543 (18), 542 (53), 501 (30), 500 (100, $C_{28}H_{20}O_9$), 472 (12), 458 (8), 455 (9), 226 (26), 225 (46, $C_{15}H_{13}O_2$), 212 (10), 107 (14), 91 (28), 77 (8), 71 (9), 69 (8), 57 (13), 44 (12), 43 (44). $C_{32}H_{24}O_{11}$ [M$^+$]: calcd. 584.1318; found 584.1318.

Isolation Procedure for Tyrosol (14) and Retipolide E (16): Air- and freeze-dried fruit bodies of *R. retipes/ornatipes* (102 g) were pulverized and defatted with hexanes (3 × 500 mL). Extraction of the material with EtOAc (3 × 500 mL) yielded a solution, which was used for the production of the retipolides A–D (see above). The residue was treated with MeOH (1 L), 2 N HCl (1 mL), and ascorbic acid (0.5 g), and extracted for one week to yield a black solution. The extract was concentrated under reduced pressure and then dissolved in EtOAc (200 mL). The solution was washed with acidified water (200 mL, adjusted to pH 3 with 2 N HCl), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting black solid was flash chromatographed on silica gel ($CHCl_3$/acetone, 4:1), whereby all fractions with R_f = 0.30–0.40 were combined and concentrated to yield a brown oil (1.04 g), which was purified by a second flash chromatography on silica gel (hexanes/EtOAc/AcOH, 60:40:1). All fractions with R_f = 0.35–0.45 were combined and concentrated to yield a brown solid (102 mg). Preparative HPLC [Nucleosil C18, 7 μm, 20 × 250 mm (Knauer); solvent A: H_2O/MeOH, 9:1; solvent B: H_2O/MeOH, 1:9; gradient: start 70% A + 30% B, 45 min: 10% A + 90% B, 50 min: 0% A + 100% B, 55 min: 70% A + 30% B; flow rate: 3.38 mL/min, probe 1000 μL (MeOH), detection at 225 nm] yielded **14** (64 mg, 0.063%, relative to dry

weight) and **16** (2.2 mg, 2.2×10^{-3}%). Retention times: t_R = 15 min (**14**), t_R = 38 (**16**), t_R = 40 (unidentified compound).

Tyrosol (14): Colorless solid, m.p. 92 °C. R_f (TLC) = 0.40 (solvent system E), 0.44 (solvent system F), 0.35 ($CHCl_3$/acetone, 4:1). The spectroscopic and chromatographic data are in agreement with those of tyrosol purchased from Fluka.

Retipolide E, (*R*)-4,4′-Dihydroxy-3′-(4-hydroxyphenyl)spiro[2,10-dioxatricyclo[12.2.2.1^{3,7}]nonadeca-1(16),3,5,7(19),14,17-hexaene-12,2′(5′*H*)-furan]-11,5′-dione (16): Colorless, amorphous solid; easily soluble in acetone, sparingly soluble in $CHCl_3$; m.p. 195–197 °C. R_f (TLC) = 0.51 (solvent system A), 0.40 (solvent system E). $[\alpha]_D^{20}$ = +83 (*c* = 0.17, MeCN). UV/Vis (MeCN): λ_{max} (log ε) = 223 sh (4.28), 288 (3.97), 305 (3.98), 350 sh (3.10) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 243 (+12.96), 284 (+0.88), 294 (+1.76), 314 (0), 342 (+0.88), 365 (0) nm. IR (KBr): $\tilde{\nu}$ = 3380 (ss, br), 2950 (w), 2920 (w), 1750 (s), 1725 (s), 1600 (m), 1580 (m), 1510 (s), 1495 (m), 1450 (w, sh), 1430 (m), 1380 (m), 1355 (w), 1300 (w, sh), 1250 (s), 1200 (m), 1180 (w, sh), 1155 (m), 1110 (m), 1100 (m), 1070 (w), 1060 (w, sh), 1020 (m), 1000 (w, sh), 970 (m), 915 (m), 870 (m), 840 (m), 820 (m), 770 (w), 760 (w), 740 (w), 620 (m) cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]-acetone): δ = 2.59 (dd, *J* = 17.1, 5.2 Hz, 1 H, 8-H^a), 2.84 (dddd, *J* = 17.1, 11.2, 1.2, 0.9 Hz, 1 H, 8-H^b), 3.11 (d, *J* = 13.8 Hz, 1 H, 13-H^b), 3.88 (ddd, *J* = 11.3, 5.2, 1.2 Hz, 1 H, 9-H^b), 3.97 (d, *J* = 13.8 Hz, 1 H, 13-H^a), 4.22 (dd, *J* = 11.3, 11.2 Hz, 1 H, 9-H^a), 4.80 (dd, *J* = 2.1, 0.9 Hz, 1 H, 19-H), 6.50 (dd, *J* = 8.0, 2.1 Hz, 1 H, 6-H), 6.70 (d, *J* = 8.0 Hz, 1 H, 5-H), 6.93 (3′′/5′′-H, *J* = 8.9 Hz, 2 H, AA′BB′-d), 6.97 (dd, *J* = 8.5, 2.5 Hz, 1 H, 17-H), 7.20 (dd, *J* = 8.5, 2.5 Hz, 1 H, 16-H), 7.36 (dd, *J* = 8.5, 2.5 Hz, 1 H, 18-H), 7.65 (dd, *J* = 8.5, 2.5 Hz, 1 H, 15-H), 8.07 (2′′/6′′-H, *J* = 8.9 Hz, 2 H, AA′BB′-d) ppm. ^{13}C NMR (100.6 MHz, [D$_6$]acetone): δ = 32.2 (C-8), 44.5 (C-13), 68.7 (C-9), 89.1 (C-12), 115.8 (C-19), 115.9 (2 × C-3′′/5′′), 116.3 (C-5), 122.3 (C-6), 123.0 (C-1′′), 124.6 (C-16), 124.7 (C-17), 128.2 (C-3′), 131.7 (2 × C-2′′/6′′), 132.5 (C-7), 133.3 (C-14), 134.0 (C-18), 134.6 (C-15), 141.2 (C-4′), 144.6 (C-4), 152.4 (C-3), 158.4 (C-4′′), 159.2 (C-1), 167.8 (C-5′), 169.0 (C-11) ppm. EI-MS (DI, 220 °C): *m*/*z* (%) = 460 (0.2) [M$^+$], 417 (8), 416 (34), 226 (12), 225 (7), 207 (7), 198 (8), 188 (10), 121 (8), 120 (9), 91 (13), 44 (100). $C_{26}H_{20}O_8$ [M$^+$]: calcd. 460.1158; found 460.1143. In all properties identical with a synthetic sample.[13]

Isolation Procedure for Isoretipolide A (9): Collections of *R. retipes*/*ornatipes* containing **9** in larger quantities (HPLC screening)[8] were cut and extracted in 2-g portions with acetone containing a small amount of HCl under argon atmosphere. The dark yellow to brownish black extracts were concentrated, treated with water and extracted with EtOAc (3×). After removal of the solvent under reduced pressure, the black residue was dissolved in H_2O/MeCN (1:1), filtered through a SepPak RP18 cartridge, and immediately separated by preparative HPLC [Nucleosil 100 C18, 7 µm, 250 × 16 mm (Macherey–Nagel); solvent A: H_2O/MeCN, 9:1 + 0.5% TFA; solvent B: MeCN; gradient: start 80% A + 20% B, 50 min: 45% A + 55% B, 55 min: 100% B, flow rate 6.80 mL/min; detection at 300 nm]. The fractions were stored at –10 °C before further treatment. Concentration under reduced pressure at room temperature and removal of the residual water by lyophilization yielded compounds **9** (15.8 mg, 0.047% of the dry weight) and **1** (78 mg, 0.23%) in pure form. Retention times: t_R = 48.6 min (**9**) and 50.4 min (**1**). Isoretipolide A (**9**) was kept at –10 °C in the freezer.

Isoretipolide A, (1*R*,5*RS*)-7,8-Dihydro-3′,5,16′-trihydroxyspiro[cyclopenta[*d*]furo[3,4-*b*]pyran-1,11′(5*H*)-9-oxatricyclo[11.3.1.1^{2,6}]-octadeca[1(17),2,4,6(18),13,15]hexaene]-3,6,10′-trione (9): Pale yellow powder, m.p. 120 °C (decomp.). R_f (TLC) = 0.55 (solvent system A). $[\alpha]_D^{20}$ = +144 (*c* = 0.1, MeCN). UV/Vis (MeCN): λ_{max} (log ε) = 211 (3.54), 302 (3.14) nm. CD (MeCN): λ_{max} ($\Delta\varepsilon$) = 203 (–2.703), 219 (0), 234 (2.503), 266 (1.464), 296 (2.502) nm. IR (KBr): $\tilde{\nu}$ = 3436 (ss, br), 1779 (m), 1728 (m), 1684 (m), 1637 (m), 1512 (w), 1266 (w), 1209 (w), 1026 (w) cm^{-1}. ^{1}H NMR (600 MHz, [D$_6$]acetone): δ = 2.56 (br., 2 H, 7-H), 2.79 (d, *J* = 16.3 Hz, 1 H, 7′-H), 3.03 (d, *J* = 15.6 Hz, 1 H, 12′-H^b), 3.14 (br. m, 2 H, 8-H), 3.24 (m, 1 H, 7′-H), 4.21 (m, 1 H, 8′-H), 4.24 (d, *J* = 15.6 Hz, 1 H, 12′-H^a), 5.03 (m, 1 H, 8′-H), 6.53 (br. s, 1 H, 5-H), 6.79 (d, *J* = 8.1 Hz, 1 H, 4′-H), 6.83 (d, *J* = 8.1 Hz, 1 H, 15′-H), 7.02 (br., 1 H, 17′-H), 7.05 (dd, *J* = 8.1, 1.8 Hz, 1 H, 14′-H), 7.06 (dd, *J* = 8.1, 1.8 Hz, 1 H, 5′-H), 7.31 (br., 1 H, 18′-H), 8.35 (br., OH) ppm. ^{13}C NMR (150.9 MHz, [D$_6$]acetone): δ = 25.6 (C-8), 32.2 (C-7′), 35.8 (C-7), 41.2 (C-12′), 70.1 (C-8′), 87.7 (C-1), 94.8 (C-5), 115.3 (C-4′), 115.9 (C-15′), 126.8 (C-13′), 127.4 (C-8b), 127.5 (C-2′), 128.0 (C-1′), 129.2 (C-5′), 130.4 (C-14′), 130.8 (m, C-6′), 132.5 (C-3a), 139.2 (C-17′), 140.6 (m, C-18′), 144.1 (C-5a), 153.6 (C-3′, C-16′), 158.0 (C-8a), 164.4 (C-3), 171.4 (C-10′), 203.5 (C-6) ppm. EI-MS of pertrimethylsilyl derivative **11** (DI, 200 °C): *m*/*z* (%) = 477 (4) [M + 1]$^+$, 476 (15) [M$^+$], 459 (14), 458 (43), 432 (13), 430 (5), 414 (6), 386 (5), 227 (50), 226 (80), 225 (100) [$C_{15}H_{13}O_2$], 212 (11), 120 (45), 107 (37), 91 (42), 84 (48), 82 (63).

Synthesis of Anhydroisoretipolide A 6-*O*-Methyl Ether (10): 9 (0.6 mg) was dissolved in 3 drops of MeOH and immediately treated with a solution of *p*-TsOH (1%) in 2,2-dimethoxypropane (1 mL). The mixture was refluxed for 25 min at 75 °C, cooled to room temperature, and concentrated under reduced pressure. The orange-brown residue was separated with EtOAc/hexanes (1:1) using a Pasteur pipette filled with silica gel. The orange-colored fraction was concentrated to yield **10** (0.3 mg) as an orange oil. R_f (TLC) = 0.40 (solvent system B). UV/Vis (MeCN): λ_{max} = 216, 285, 385 nm. EI-MS (DI, 200 °C): *m*/*z* (%) = 472 (3) [M$^+$], 225 (9), 73 (100). $C_{27}H_{20}O_8$ [M$^+$]: calcd. 472.1158; found 472.1137.

***rac*-Butyrolactone II, Methyl (*RS*)-4-Hydroxy-2-(4-hydroxybenzyl)-3-(4-hydroxyphenyl)-5-oxo-2,5-dihydrofuran-2-carboxylate [(*RS*)-13]:** The compound was prepared according to ref.[11b] Colorless powder; m.p. 202–205 °C, ref.[11b] 206–208 °C. R_f (TLC) = 0.25 (solvent system C). ^{1}H NMR (400 MHz, [D$_6$]acetone): δ = 3.50 (s, 2 H, CH_2), 3.79 (s, 3 H, OMe), 6.60, 6.69 (d, *J* = 8.3 Hz, 2 H, 17-H), 6.69 (d, *J* = 8.3 Hz, 2 H, 16-H), 6.98 (d, *J* = 8.7 Hz, 2 H, 8-H), 7.65 (d, *J* = 8.7 Hz, 2 H, 7-H), 8.16, 8.87 (each s, OH), 9.06 (s, OH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 39.2, 53.7, 86.0, 115.5, 116.7, 122.7, 124.9, 128.3, 130.2, 132.3, 139.1, 157.4, 158.9, 168.6, 170.8 ppm.

Synthesis of *rac*-Secoretipolide E, 2-(4-Hydroxyphenyl)ethyl (*RS*)-4-Hydroxy-2-(4-hydroxybenzyl)-3-(4-hydroxyphenyl)-5-oxo-2,5-dihydrofuran-2-carboxylate [(*RS*)-15]: Lactone (*RS*)-**13** (530 mg, 1.49 mmol), tyrosol (415 mg, 3.00 mmol), and 1,3-dichlorotetrabutyldistannoxane (552 mg, 1.00 mmol)[18] were suspended in dry toluene (30 mL) and refluxed for 16 h. The mixture was concentrated and the residue purified by flash chromatography ($CHCl_3$/MeOH, 10:1) to yield (*RS*)-**15** (494 mg, 69%) as a beige solid; m.p. 97 °C. R_f (TLC) = 0.18 (solvent system G). IR (KBr): $\tilde{\nu}$ = 3424 (s, br), 3025 (w), 2975 (w), 2920 (w), 1740 (s), 1700 (w), 1653 (w), 1636 (w), 1611 (m), 1580 (m), 1516 (s), 1444 (m), 1385 (m), 1265 (m, sh), 1242 (m), 1180 (m), 1130 (w), 1106 (w), 1068 (w), 1032 (w), 838 (m) cm^{-1}. ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 2.80 (br. t, *J* = 7.6 Hz, 2 H), 3.46 (s, 2 H), 4.33 (m, 2 H), 6.58 (d, *J* = 8.1 Hz, 2 H, Ar-H), 6.67 (d, *J* = 8.5 Hz, 2 H, Ar-H), 6.71 (d, *J* = 8.1 Hz, 2 H, Ar-H), 6.94 (d, *J* = 8.7 Hz, 2 H, Ar-H), 7.00 (d, *J* = 8.5 Hz, 2 H, Ar-H), 7.62 (d, *J* = 8.7 Hz, 2 H, Ar-H), 8.14, 8.16, 8.87 (each s, OH), 9.05 (br. s, OH) ppm. ^{13}C NMR (75.5 MHz, [D$_6$]acetone): δ

= 34.6, 39.2, 68.1, 86.1, 115.5, 116.1, 116.6, 122.8, 125.0, 128.3, 129.1, 130.2, 130.8, 132.3, 139.2, 157.0, 157.3, 158.9, 168.6, 170.2 ppm. EI-MS (DI, 190 °C): *m/z* (%) = 462 (2) [M$^+$], 280 (5), 147 (9), 138 (18), 121 (10), 120 (8), 108 (9), 107 (100), 91 (8), 77 (15), 44 (72). $C_{26}H_{22}O_8$ (462.5) × MeOH: calcd. C 65.58, H 5.30; found C 65.20, H 5.31.

Identification of Butyrolactone II (13) and Secoretipolide E (15) in the Mushroom Extract: Air- and freeze-dried fruit bodies of *R. retipes/ornatipes* (135 g) were pulverized and defatted with hexanes (1.5 L). The residue was treated with MeOH (1.5 L), 2 N HCl (1.5 mL), and ascorbic acid (1 g), and extracted for 1 day to yield a brown solution. The extract was concentrated under reduced pressure and then dissolved in EtOAc (500 mL). The solution was washed with acidified water (300 mL), adjusted to pH 3 with 2 N HCl, dried ($MgSO_4$), and concentrated under reduced pressure. The resulting black solid (6.8 g) was flash chromatographed on silica gel ($CHCl_3$/acetone, 5:2), whereby all fractions with R_f = 0.25–0.45 were combined and concentrated to yield a yellow-brown solid (0.80 g). Purification by a second flash chromatography on silica gel (hexanes/EtOAc/AcOH, 50:50:1) yielded two fractions. Fraction 1 consisted of impure **13** (77 mg, brown solid), fraction 2 of enriched **15** (96 mg, brown solid). The identity of **13** and **15** with the corresponding synthetic compounds was established by co-HPLC and UV comparison. **13**: Nucleosil 5 C18, 5 µm, 250 × 4.6 mm, (Merck); solvent A: H_2O/MeCN, 9:1; solvent B: H_2O/MeCN, 1:9; gradient: start 100% A, 60 min: 100% B, flow rate 1.0 mL/min; Diode array detection; t_R = 26.99 min. **15**: same column and conditions as above, but MeOH instead of MeCN; t_R = 44.48 min.

Identification of Xerocomic Acid (20): An air-dried, defatted sample (1 g) from a specimen of *R. retipes/ornatipes* with brown cap skin, collected in August 1991 in the 'Ball Creek area', was extracted with MeOH (50 mL) with the addition of 2 N HCl (0.2 mL) and ascorbic acid (0.1 g). The solution was concentrated under reduced pressure, and the resulting residue dissolved in EtOAc (50 mL), washed with H_2O (50 mL, pH 3), dried ($MgSO_4$) and subjected to HPLC analysis (same column as for the separation of **13** and **15**; solvent A: H_2O/MeCN, 9:1 + 0.5% TFA; solvent B: H_2O/MeCN, 1:9; gradient: start 90% A and 10% B, 55 min: 10% A and 90% B, 65 min: 10% A and 90% B, 75 min: 90% A and 10% B, flow rate 1.0 mL/min; Diode array detection): t_R = 17.24 min (**20**), 26.44 (**1**), 28.49 (**7**), 31.19 (**3**). Retention time t_R and UV spectrum agreed with those of authentic **20**.

Crystallographic Data for Anhydroretipolide A 6-*O*-(*S*)-*sec*-Butyl Ether (2b): $C_{30}H_{26}O_8$, M = 514.54, crystal dimensions 0.23 × 0.37 × 0.67 mm, space group $P2_12_12_1$, unit cell dimensions and volume: a = 11.382, b = 14.820, c = 15.231 Å, V = 2569.1 Å^3, Z = 4, $D_{calcd.}$ = 1.329 g/cm^3, $F(000)$ = 1080, μ = 0.097 mm^{-1}, radiation: Mo-K_α, wavelength λ = 0.71073 Å, $2\Theta_{max}$ = 45.9°, h_{min}/h_{max} = –12/12, k_{min}/k_{max} = 0/16, l_{min}/l_{max} = 0/16, No. of measured reflections: 3886, No. of unique reflections: 3485, No. of observed reflections: 3282 [$I > 2\sigma(I)$], No. of parameters 343, R factor 0.0445, $wR(F^2)$ = 0.1207, Goodness of fit: 1.094, structure solution with SHELXLS-86,[19] refinement by SHELXTL-PLUS.[20]

CCDC-167856 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[1] There is a long-standing dispute, if the two species, formerly named *Boletus retipes* and *B. ornatipes*, are autonomous or synonyms for only one quite variable species (see, for example, E. E. Both, *The Boletes of North America*, Buffalo Museum of *Science* Buffalo NY, **1996**). Bender and Bresinsky have recently created the genus *Retiboletus* from molecular biological evidence and the presence of retipolides as chemotaxonomical markers (M. Binder, A. Bresinsky, *Feddes Repertorium* **2002**, *113*, 30–40). The authors state that *R. retipes* and *R. ornatipes* cannot be differentiated by morphological and anatomical characters. As a consequence, our collections probably contain mixtures of both fungi. A HPLC comparison of several individual collections from the Eastern USA indicates the presence of retipolides in all cases. These investigations together with molecular biologic studies will be reported in a separate publication.

[2] The line broadening is most pronounced for the hemiacetal group and atoms in its surrounding. This can be explained by rapid ring opening and re-closure of the hemiacetal in solution, leading to averaged NMR signals for the two epimers.

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[4] D. P. Roelofsen, H. van Bekkum, *Synthesis* **1972**, 419–420.

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[6] A solution of retipolide A (**1**) in acetonitrile exhibits UV/Vis maxima at λ_{max} = 208, 316, 390, and 428 nm. On addition of ammonia, the weak yellow color intensifies, and the intensity of the maxima at λ_{max} = 390 and 428 nm increases considerably. After addition of HCl, the solution becomes colorless, and the maxima at 390 and 428 nm disappear completely.

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