Unusual polyketides from the wood-decay fungus *Sistotrema raduloides*

Albert K. Amegadzie, William A. Ayer, and Lynne Sigler

Abstract: The metabolites of the wood rot decay fungus *Sistotrema raduloides* (P. Karst) Donk have been investigated and a new type of norpentaketide has been discovered. The compounds sistodiolynne (**1**), sistolynone (**5**), and sistopyrone (**6**) represent new carbon skeletons among natural products. The compounds are very unstable and readily polymerize in the presence of air to give black insoluble material. It has been shown by ^{13}C NMR labelling experiments that they arise from five acetate units, and that a methyl carbon of one of the acetate units is lost during the biosynthesis. The structures were determined by spectroscopy methods, mainly NMR, and the absolute configuration of sistodiolynne was established by circular dichroism methods.

Key words: fungal metabolites, *Sistotrema raduloides*, norpentaketides, biosynthetic studies, polyacetylenes.

Résumé : On a étudié les métabolites du champignon *Sistotrema raduloides* (P. Karst) Donk de la pourriture du bois et on a découvert un nouveau type de norpentacétide. Les composés sistodiolynne (**1**), sistolynone (**5**) et sistopyrone (**6**) correspondent à de nouveaux squellettes carbonés des produits naturels. Les composés sont très instables et ils se polymérisent facilement en présence d'air pour donner des matériaux noirs qui sont insolubles. On a montré par des expériences de marquage en RMN du ^{13}C qu'ils proviennent de cinq unités acétates et qu'un carbone de méthyle de l'une des unités acétates est perdue au cours de la biosynthèse. On a déterminé les structures par des méthodes spectroscopiques, principalement la RMN, et on a déterminé la configuration absolue de la sistodiolynne par des méthodes de dichroïsme circulaire.

Mots clés : métabolites de champignons, *Sistotrema raduloides*, norpentacétides, études biosynthétiques, polyacétylènes.

[Traduit par la rédaction]

Introduction

The wood-decay fungus *Sistotrema raduloides* (P. Karst) Donk was recovered from indoor air during a study to evaluate building air quality of a museum in San Juan, Puerto Rico.[2] A member of the Corticiaceae (Basidiomycotina) having a widespread distribution in temperate areas, *S. raduloides* is known to cause white rot decay of both angiospermous and gymnospermous wood (1). On agar media of various kinds, the Puerto Rican isolate[3] was observed to form flaky carbonaceous deposits especially when the mycelium came into contact with glass surfaces. In this study we examined the metabolites produced when the fungus is grown in liquid still culture on Sabouraud dextrose medium. The metabolites proved to be very sensitive to air, forming an insoluble black polymer when exposed to the atmosphere in the absence of solvent. The structures of three of the air-sensitive metabolites are described, and the biosynthetic pathway by which they may be produced from acetate units is discussed.

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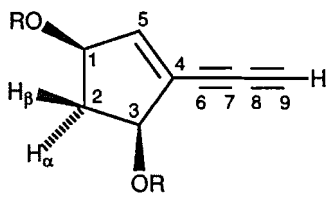
[2] The fungus is further described in the Experimental.

[3] We thank Dr. B. Bolanos, School of Medicine, University of Puerto Rico, San Juan, for bringing the isolate to our attention.

Results and discussion

Sistotrema raduloides was grown in still culture on Sabouraud dextrose broth at room temperature for 5 weeks. The mycelium was then separated from the culture broth by filtration and the filtrate was concentrated and extracted, first with CH_2Cl_2, then with ethyl acetate. In the initial experiment, the extracts were dried (Na_2SO_4) and concentrated to dryness under reduced pressure to give brown oils. On standing open to the atmosphere, the oils rapidly (ca. 2 min) solidified to a black amorphous solid. This material could not be dissolved in organic solvents or water. In subsequent experiments, the crude extracts were not concentrated to dryness but were kept as much as possible in a small volume of the extracting solvent under an inert atmosphere.

Sistodiolynne (**1**) was purified by preparative TLC. To



1 R = H
1a R = Ac

avoid polymerization the compound was eluted directly from the plate with acetone-d_6 and the solution was used for NMR measurements. The HREIMS was obtained by placing a drop

Table 1. ^{1}H NMR and ^{13}C NMR data for **1** and **1***a*.

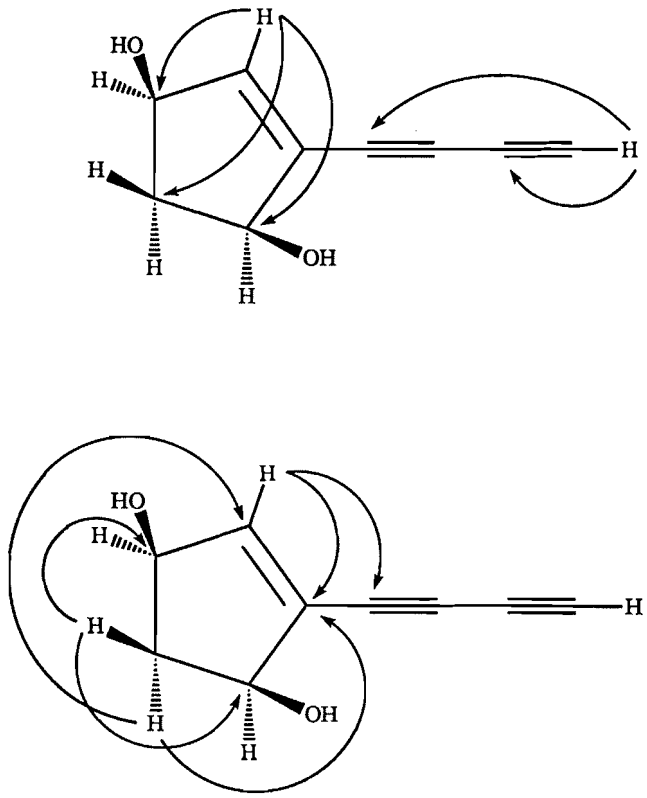
	1	**1***a*	**1**	
Atom	δ, multi., *J*(Hz)	δ, multi., *J*(Hz)	Atom	δ_C, multi.
H-1α	4.62, bdd	5.60, m	C-1	76.1, d
H-2α	2.74, ddd, 13.5, 7.0, 7.0	2.95, ddd, 14.6, 6.7, 6.7	C-2	44.7, t
H-2β	1.50, ddd, 13.5, 6.2, 6.2	1.75, ddd, 14.6, 4.2, 4.2	C-3	73.4, d
H-3α	4.55, m	5.60, m	C-4	129.8, s
H-5	6.30, dd, 2.0, 1.0	6.44, bdd, 2.2	C-5	146.7, d
H-9	3.30, s	2.50, s	C-6	76.9, s
OH-1β	4.70, d, 6.5		C-7	72.0, s
OH-3β	4.48, d, 6.5		C-8	68.4, s
OH-1			C-9	74.1, d
-$COCH_3$		2.08, 2.02 (singlets)		

of the solution directly on the probe and determining the spectrum immediately. The spectrum indicates a molecular formula $C_9H_8O_2$. Peaks at $M^+ - 1$ and $M^+ - 18$ indicate the presence of hydroxyl group(s). The FTIR spectrum (thin film after evaporation of solvent) shows broad hydroxyl absorption centered around 3500 cm^{-1}, with a sharp absorption band protruding at 3287 cm^{-1}. This latter absorption, coupled with absorption at 2230 and 2050 cm^{-1}, is indicative of a terminal and a non-terminal alkyne (2). The ^{13}C NMR spectrum shows four signals in the *sp* carbon region, at 76.9, 74.1, 72.0, and 68.4, consistent with the presence of two triple bonds. A *J*-compensated APT spectrum revealed that the carbon at δ 74.1 carries a hydrogen. The UV spectrum shows absorption bands at 252 and 310 nm, the latter the more intense. This is consistent with the presence of a conjugated endiyne (ref. 2, pp. 3–21).

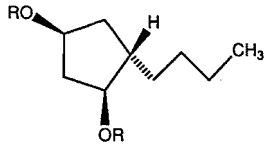
The ^{1}H NMR spectrum shows signals for all eight hydrogens (Table 1) and the ^{13}C NMR shows the nine carbons (Table 1). The sole methylene carbon, located at δ 44.7, was shown in an HETCOR (3) experiment to correlate to the protons at 2.74 (ddd, *J* = 13.5, 7.0, 7.0) and 1.50 (ddd, *J* = 13.5, 6.0, 6.0). These protons are coupled to the protons at δ 4.62 and 4.55, which are attached to the carbons at δ 73.4 and 76.1, respectively. This results in the sequencing of carbons 1, 2, and 3, with the hydroxyl groups assigned as *cis* because of the large chemical shift between the protons of the methylene group. It has been shown that in such *cis* 4-cyclopenten-1,3-diols, the hydrogen *syn* to the hydroxyl groups is upfield of the *anti* proton (4, 5). In confirmation of this, nOe enhancements of H-1α (7.5%) and H-3α (7.5%) were observed when the H-2α signal is irradiated, and no enhancement was seen when H-2β is irradiated. The HMBC spectrum shows that the alkenic hydrogen is correlated with the other alkenic carbon and with the alkynic carbon at δ 76.9, confirming that the trisubstituted double bond is in conjugation with the carbon–carbon triple bonds, as implied by the UV spectrum. Correlated of the alkynic H (δ 3.30) with this same carbon (δ 76.9) as well as with C-8 (δ 68.4) completes the connectivity of the endiyne system. The HMBC correlations are shown in Fig. 1. Since sistodiolynne must be monocyclic, structure **1** is assigned.

Acetylation of sistodiolynne (**1**) provided the diacetyl derivative **1***a* as an unstable oil. The HREIMS confirmed the molecular formula and, as shown in Table 1, the ^{1}H NMR spectrum is in good agreement with structure **1***a*.

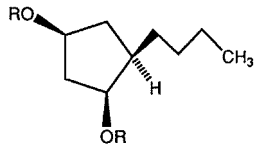
Fig. 1. Significant HMBC correlations of sistodiolynne (**1**).



Catalytic hydrogenation (Pd–C, EtOAc) of sistodiolynne (**1**) provides a mixture of the saturated stereoisomers **2** and **3** that proved to be much more stable than sistodiolynne. However, they could not be separated by chromatography, but the derived di-*p*-bromobenzoates **2***a* and **3***a* were separable by preparative TLC. The isomer **2***a* was the major component



2 R = H
2a R = *p*-BrC_6H_4CO

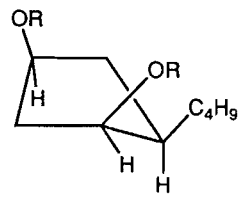


3 R = H
3a R = *p*-BrC_6H_4CO

(perhaps due to coordination of the catalyst with the hydroxyl groups), although **3***a* crystallized more readily. The HREIMS of **2***a* and **3***a* are very similar with each showing a molecular ion at *m/z* 522 ($C_{23}H_{24}O_4Br_2$). Loss of two molecules of *p*-bromobenzoic acid gives rise to a base peak at *m/z* 122 (C_9H_{14}). The ^{1}H NMR spectra of **2***a* and **3***a* are also very similar (see Experimental). The methylene hydrogens at C-2 and C-5 were identified by their different *geminal* couplings and by the ^{1}H–^{1}H COSY spectrum of **3***a*. The ^{13}C NMR (APT) spectra each show three methine carbons, two of which are oxygenated, five methylene carbons, and one methyl carbon. The complete ^{13}C NMR signal assignments were made by comparing the results of an HMQC experiment with the ^{13}C NMR spectra of **2***a* and **3***a* obtained during the ^{13}C-labelling experiments described later.

The stereochemistry of the butyl group in **2***a* and **3***a* is assigned on the basis of the chemical shift difference of H-3 in **2***a* and **3***a*. An alkyl group *vicinal* to the carbinyl proton is expected to make an upfield shift if it is *syn* to the carbinyl proton relative to when it is *anti* (6). In addition, irradiation of H-5α (δ 2.6) in **3***a* causes enhancement of H-1α (12%) and H-4 (14%). Similarly, irradiation of H-4 enhances the signal for H-3α by 15%.

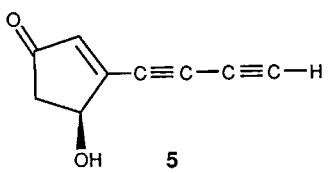
The circular dichroism (CD) spectrum of **3***a* shows the first Cotton effect (negative) at 251 nm and the second (positive) at 231 nm. If we assume that the cyclopentane ring exists predominantly in the envelope conformation with the large butyl group in the equatorial lip position as depicted in **4**, a negative



4 R = *p*-BrC_6H_4CO

chirality (7) exists between the chromophoric groups and the absolute configuration is as depicted in the structural formulae. MM2 calculations indicate that the conformation shown in **4** is the energy minimum (8).

Sistolynone (**5**) was isolated as an unstable oil by preparative TLC. The compound was washed from the adsorbent with deuterated chloroform and was not evaporated to dryness in order to prevent decomposition. This compound was less polar (higher R_f) than sistodiolynne (**1**). The molecular formula



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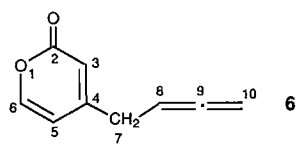
$C_9H_6O_2$ was determined by HREIMS, which indicated a molecular ion peak at *m/z* 146. The peak at *m/z* 118 represents loss of CO, suggesting the presence of a carbonyl group. A strong absorption band at 1710 cm^{-1} in the IR spectrum and a signal in the ^{13}C NMR (APT) spectrum at δ 204.4 confirm the presence of a ketonic carbonyl functionality. The position of this signal suggests an α,β-unsaturated five-membered ring ketone (9). The band at 3389 cm^{-1} is strong evidence for a hydroxyl group, and bands at 3284 and 2204 cm^{-1} indicate a terminal alkyne group.

A singlet at δ 2.9 in the ^{1}H NMR spectrum was easily recognized as the CH of a terminal alkyne. A methylene pair is evident from signals at δ 2.8 and 2.4 (J_{gem} = 18.6 Hz), the chemical shifts and the magnitude of the coupling constant suggesting that they are attached to the carbon α to the carbonyl group. The proton signal at δ 5.0 (dddd, J = 6.5, 5.0, 2.5, 1.0 Hz) is vicinally coupled to this methylene pair by 6.5 and 2.5 Hz and by 5.0 and 1.0 Hz to protons at δ 2.3 and 6.4, respectively. The signal at δ 2.3 disappears when D_2O is added to the sample, indicating that it is a hydroxyl hydrogen. The ^{13}C NMR spectrum is very straightforward and contains nine signals, three of which appear in the region of C≡C at δ 89.8, 68.2, and 66.8. This suggests the presence of a dialkyne system by analogy to the spectra observed for **1**. The terminal alkyne CH at δ 78.3 was distinguished from the rest of the signals by a *J*-compensated APT spectrum.

Based on all the spectral data discussed above, structure **5** is proposed for sistolynone. The small quantity of sistolynone obtained precluded other correlation experiments and derivatization.

Sistopyrone (**6**) also was obtained as an unstable oil by preparative TLC. The HREIMS spectrum displays a molecular ion peak at *m/z* 148, establishing $C_9H_8O_2$ as the molecular formula. The strong absorption at 1725 cm^{-1} in the FTIR spectrum suggests a carbonyl group. The ^{1}H NMR spectrum displays a total of six signals, three of which appear in the olefinic region between δ 7.41 and 6.18. The proton at δ 6.18 (H-3, ddd, J = 2.0, 1.0, 1.0 Hz) is coupled to protons at 7.41 (H-6, dd, J = 5.2, 1.0 Hz), 6.12 (H-5, dd, J = 5.2, 1.0 Hz), and 3.12 (2H, H-7, dtd, J = 7.0, 2.9, 1.0 Hz). The chemical shifts and coupling constants displayed by these olefinic protons, in addition to signals at δ 162.2 (s), 156.8 (s), 150.7 (d), 113.7 (d), and 108.0 (d) in the ^{13}C NMR spectrum, suggest a pyrone ring substituted at the 4-position (10, 11). HMQC correlations confirm the assignments for the partial structure mentioned above.

Infrared bands at 1955 and 853 cm^{-1} are indicative of an allene group (-HC=C=CH_2) (12). Signals at δ 209.7 (s), 85.5 (d), and 76.3 (t) are consistent with this functionality (12). The remaining signals in the ^{1}H NMR spectrum are a one-proton signal at δ 5.15 (qn, J = 7.0 Hz) and a two-proton signal at δ 4.79 (dt, J = 6.7, 2.9 Hz). Decoupling experiments provide confir-



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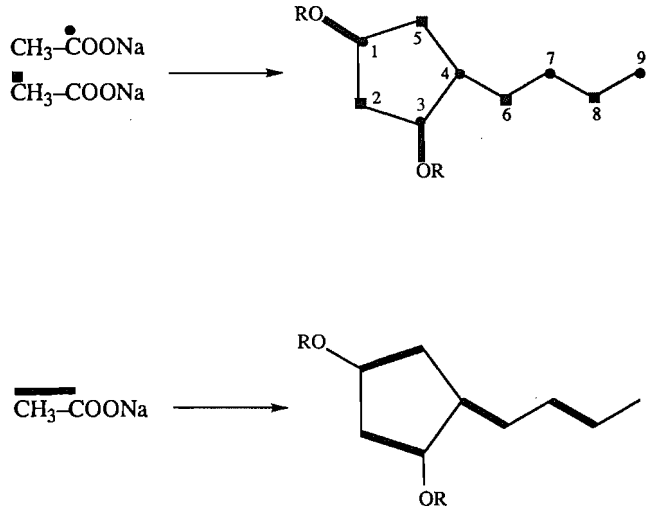
matory information concerning the allene group. In particular, the irradiation of the signal at δ 3.12 (dtd, 7.0, 2.9, 1.0 Hz) results in the collapse of signals at δ 6.18, 5.15, and 4.79. The signal at δ 5.15 becomes a triplet with J = 6.7 Hz, coupling only to the signal at δ 4.79. It became evident at this point that the signal at δ 5.15 is in fact an overlapping pair of triplets rather than a quintet. Structure **6** is consistent with these data. Lack of material precluded further experimentation.

Biosynthesis

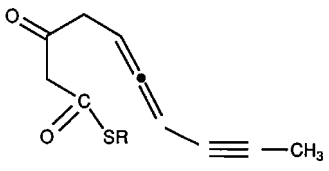
The metabolites of *S. raduloides* represent a new structural

Fig. 2. Acetate labelling patterns in sistodiolynne (**1**).



type of natural product. We have been unable to locate a compound in the literature with this carbon skeleton. The unbranched nature of the carbon skeletons in sistodiolynne (**1**) and sistolynone (**5**), along with the endiyne unit, suggest a polyketide origin. Liquid cultures of the fungus were grown in the presence of [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetate. Since the compounds are unstable, the labelled metabolites were reduced and transformed to the *p*-bromobenzoates in order to study the incorporation pattern. In the case of both di-*p*-bromoacetates, carbons 1, 3, 4, 7, and 9 were enhanced in the [1-^{13}C]acetate experiment and carbons 2, 5, 6, and 8 in the [2-^{13}C] case (illustrated in Fig. 2 for compound **1**). In the case of the C-1 labelling, the signals for C-3 and C-4 show some coupling, indicating that a significant number of molecules incorporating two labelled acetate units were present. In the case of the [1,2-C_2] acetate, C-9 appeared as an enhanced singlet, while all the other signals appeared as doublets. This implies that the compounds are norpentaketides, in which the methyl carbon of one of the acetate units has been lost (see Fig. 2). A 2D-INADEQUATE experiment on **2***a* obtained from the [1,2-$^{13}C_2$]acetate confirmed the spin-coupled carbons and firmly identified the carbon pairs as: C-1, C-5; C-2, C-3; C-4, C-6; and C-7, C-8 (see Experimental).

Most natural polyacetylenes are acetate derived, many by degradation of oleic acid (14). Loss of the terminal methyl via the sequence -CH_3 → CH_2OH → -CHO → -COOH and decarboxylation to yield the terminal acetylene is well documented (2, 14). One possible pathway is illustrated in Scheme 1 in which we use the carboxyl labelled case for illustrative purposes. The intermediate **7** may be derived from five acetate units or by degradation of oleic acid. Ring closure, adjustment of the oxidation level, and loss of the terminal methyl via oxidation and decarboxylation leads to sistodiolynne (**1**). An alternative scheme



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involving contraction of a six-membered ring derived from the tautomeric (with **7**) allene **8** is discussed in ref. 15.

The ^{13}C-labelling pattern established for sistopyrone is shown in Fig. 3 and suggests that it is derived by oxidative ring expansion of the sistodiolynne skeleton. Scheme 2 depicts a possible biogenetic pathway from sistolynone (**5**) to sistopyrone involving a Baeyer–Villager oxidation, dehydration, adjustment of side-chain oxidation level, and tautomerization to the allene. Again we choose the [1-^{13}C]acetate labelled case for illustrative purposes. As in the case of sistodiolynne, the signals for C-3 and C-4 appear as doublets, flanking a central singlet, indicating that these two carbons arise from acetate carbonyls.

The insoluble black polymeric material resulting when the crude extracts were exposed to oxygen was subjected to combustion analysis and shows an approximate empirical formula C_3H_2O that is not consistent with any of the individual compounds isolated, but suggestive of a general oxidative polymerization. We conclude that the graphite-like particles observed on solid agar media result from polymerization of the polyacetylenes produced by the fungus. The polymer was not investigated further. The instability of the compounds also precluded any investigation of biological activity.

Experimental

General methods

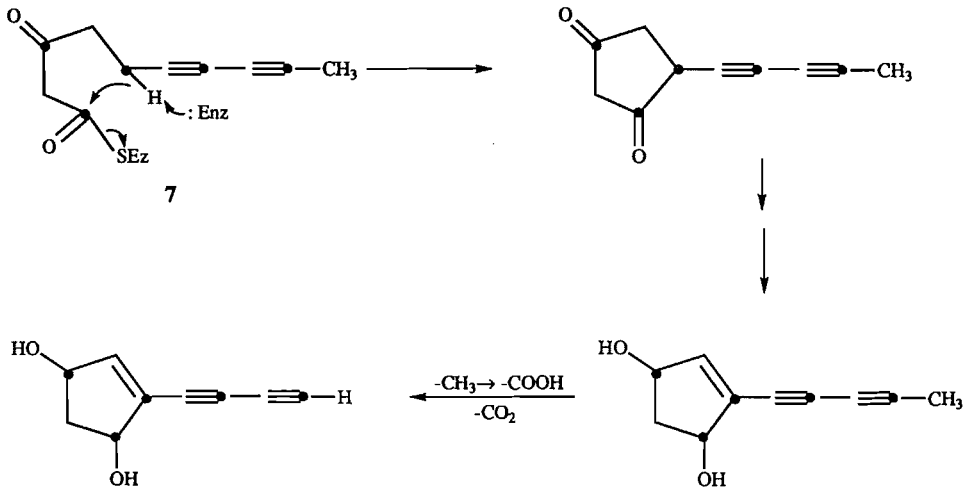
HREIMS were recorded on a Kratos AEI MS-50 mass spectrometer. FTIR spectra were recorded on a Nicolet 7199 FTIR and Nicolet MX-1 FTIR. NMR (^{1}H and ^{13}C) were obtained on a Varian Unity-500 spectrometer and Bruker WH-300, WM-360, and WH-400 spectrometers. NOE experiments were determined in the difference mode. The following 2D experiments, COSY, HMQC, and HMBC, were obtained on a Varian Unity-500 spectrometer. INADEQUATE was performed using a Bruker WH-300 spectromter. UV spectra were determined on a Hewlett Packard 8450A diode array spectrophotometer. Optical rotations were determined using a Perkin Elmer 241 polarimeter. Circular dichroism (CD) spectra were measured using a Jasco SS-20-2 spectropolarimeter. Preparative thin-layer chromatography (PTLC) was performed on E. Merck precoated 20 × 20 glass plates of silica gel 60 F-254. Hexanes refer to light petroleum (bp 60–68°C). All solvents were distilled prior to use.

Description of the fungus

In culture, *Sistotrema raduloides* (UAMH 7326) is moderately slow growing, reaching the edge of the petri dish in 4 or 5 weeks. It forms a dense, moderately thick, felty mat of mycelium. On 2% malt extract agar, UAMH 7326 developed a lattice-like network of reddish brown hyphal tufts that was associated with droplets of clear or amber liquid. On malt and other media such as cornmeal agar, the isolate formed yellowish to reddish brown sclerotia. Microscopic features of the fungus were observed in slide culture preparations in which the fungus is inoculated onto a block of cereal agar placed between two sterile glass coverslips. Microscopic preparations are made when the mycelium of the fungus grows onto the glass surfaces (16). Flaky black deposits were noticed on the glass coverslips and on the surface of colonies grown on various media when growth was examined under a dissecting microscope. These deposits were detected easily in UAMH 7326, but observed sparsely only in two of five other isolates of *S. raduloides*.

Microscopically, the isolate from air samples in Puerto Rico

Scheme 1.



Scheme 2.

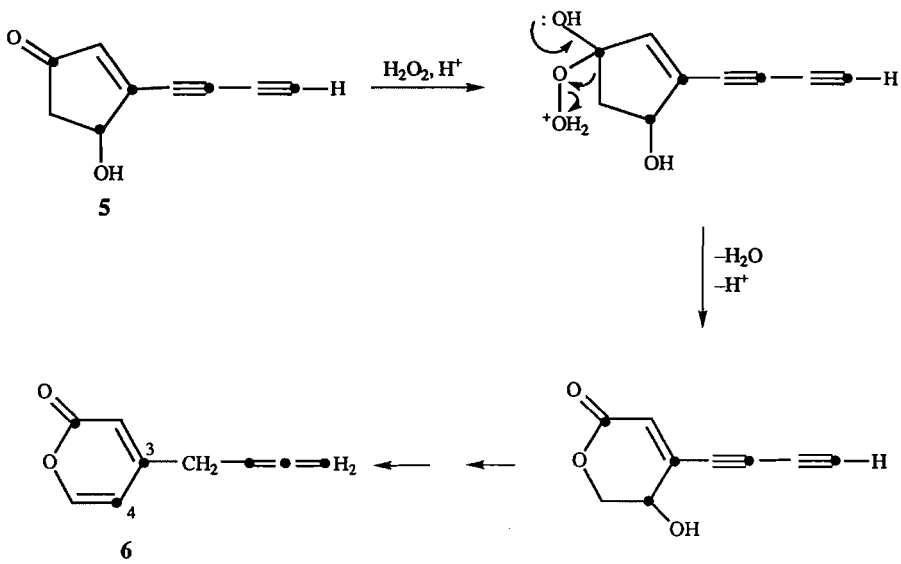
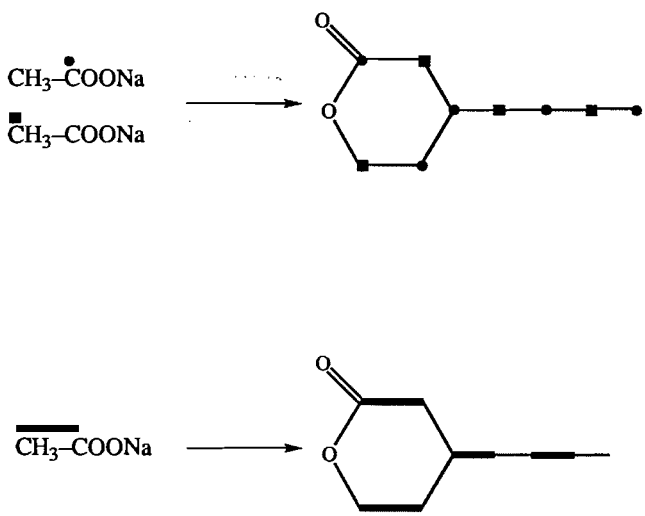


Fig. 3. Acetate labelling patterns in sistopyrone (**6**).



resembled other isolates of *S. raduloides* in forming conidia and yellowish-brown sclerotia having short spinelike appendages, but it lacked clamp connections. A characteristic feature of *S. raduloides*, as noted by others (1, 17, 18), is the formation of hyaline, thin-walled, pyriform (tear-drop) or obovate (egg-shaped) conidia having a flat basal scar. A single conidium forms at the end of a lateral branch and this branch proliferates sympodially to form additional short, blunt, branches, each of which forms a conidium. The entire structure may be simple with two to six conidia or the conidiophore may proliferate to form a small cluster of conidia. Detachment of the mature conidium leaves a truncate scar.

Where a fungus has more than one distinctive stage in its life cycle, it has been common practice to name both states. The solitary blastic conidia borne sympodially on blunt denticles are unusual among the Corticiaceae but this asexual stage has not previously been connected to a genus of Hyphomycetes. *Dexhowardia* Taylor (19) was described for a single species *Dexhowardia tetraspora* Taylor for a fungus suspected to have basidiomycetous affinity, and which lacked

clamp connections, and formed conidia on blunt denticles in the manner of *S. raduloides*. A further report on the biology of *S. raduloides* and its possible connection with *Dexhowardia* will be submitted to a mycological journal by L.S.

Growth of *Sistotrema raduloides* and isolation of the metabolites

Sistotrema raduloides strain UAMH 7326 was grown on Sabouraud dextrose agar (20 g/L) and the mycelial suspension was used to inoculate five 4-L Erlenmeyer flasks, each containing 2 L of Sabouraud dextrose broth (15 g in 1 L of redistilled water). The cultures were grown as still cultures at room temperature for 5 weeks. The culture broth was separated from the mycelium by filtration and the volume reduced under vacuum to ca. 400 mL. The concentrated broth was first extracted with methylene chloride and then with ethyl acetate. The organic layers were dried separately with anhydrous sodium sulphate, filtered, and concentrated to small volume under reduced pressure. Polymerization occurred if the solvent was completely removed. The methylene chloride extract was separated by preparative TLC and gave compounds **1**, **5**, and **6**. The ethyl acetate extract gave mainly compound **1**.

Labelling experiments

Liquid cultures were prepared as described above. A solution of [1-^{13}C] sodium acetate (5 mL, 0.2 mol/L) was added under sterile conditions 3 days after inoculation of the fungus. Four further additions were made at 2 day intervals, and the culture was allowed to grow for 5 weeks. The same procedure was followed using [2-^{13}C] sodium acetate and [1,2-^{13}C] sodium acetate. The isolation procedure was the same as described above.

*Sistodiolynne (**1**)*

(−)1*R*,3*S*-4-Butadiynyl-4-cyclopentene-1,3-diol, **1**, was isolated as an unstable colorless oil; TLC: R_f 0.18 (CH_2Cl_2:CH_3OH, 19:1); UV (MeOH): 310, 252 nm; FTIR ($CDCl_3$, cast): 3544 (br, OH), 3287 (≡C-H), 2230 (C≡C) cm^{-1}; ^{1}H NMR (acetone-d_6) δ: 6.30 (1H, dd, J = 2.0, 1.0 Hz, H-5), 4.70 (1H, d, J = 6.5 Hz, OH-1β), 4.62 (1H, bdd, H-1α), 4.55 (1H, m, H-3α), 4.48 (1H, d, J = 6.5 Hz, OH-3β), 3.30 (1H, s, H-9), 2.74 (1H, ddd, J = 13.4, 7.0, 7.0 Hz, H-2α), 1.50 (1H, ddd, J = 13.5, 6.2, 6.2 Hz, H-2β); ^{13}C NMR (acetone-d_6) δ: 146.7 (C-5, d), 129.8 (C-4, s), 76.9 (C-6, s), 76.1 (C-1, d), 74.1 (C-9, d), 73.4 (C-3, d), 72.0 (C-7, s), 68.4 (C-8, s), 44.7 (C-2, t); HREIMS (probe 100°C), *m/z* calcd. for $C_9H_8O_2$ (M^+): 148.0509; found: 148.0524, 147 ($C_9H_7O_2$, 25%), 130 (C_9H_6O, 10%), 119 (C_8H_7O, 49%), 102 (C_8H_6, 62%), 76 (C_6H_4, 89%), 74 (C_6H_2, 53%).

*O,O-Diacetylsistodiolynne (**1**a)*

Treatment of sistodiolynne (3–4 mg, estimated) in CH_2Cl_2 with acetic anhydride (0.2 mL) and pyridine (1.0 mL) for 12 h, followed by removal of the solvents under vacuum, gave an oil, which was purified by preparative TLC using 5% MeOH in CH_2Cl_2 and was eluted from the plate with deuterated chloroform; TLC: R_f 0.72; FTIR ($CDCl_3$, cast): 3251 (≡C-H), 1737)-COO-) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.44 (1H, bd, J = 2.2 Hz, H-5), 5.60 (2H, m, H-1α3α), 2.95 (1H, ddd, J = 14.6m 6.7, 6.7 Hz, H-2α), 2.50 (1H, s, H-9), 2.08 (3H, s, OAc), 2.02 (3H, s, OAc), 1.75 (1H, ddd, J = 14.6, 4.2, 4.2 Hz, H-2β); HREIMS (probe 100°C) *m/z* calcd. for $C_{13}H_{12}O_4$ (M^+): 232.0736; found: 232.0730, (5%), 172 ($C_{11}H_8O_2$, 11%), 130 (C_9H_6O, 100%), 102 (C_8H_6, 34%).

Reduction and *p*-bromobenzoylation of sistodiolynne (**1**)

Catalytic hydrogenation (Pd–C, EtOAc, 40 psi (1 psi = 6.9 kPa)) of **1** provided a mixture of the saturated isomers (**2***a* and **3***b*), which were not separable by TLC. The mixture was treated with *p*-Br-BzCl (40 mg) and pyridine (2 mL) in CH_2Cl_2 with stirring at room temperature for 24 h. The resulting mixture was concentrated under vacuum to dryness. Separation of the di-*p*-bromobenzoate derivatives (**2***a* and **3***a*) was achieved by preparative TLC (5% EtOAc in hexanes, developed several times).

*Compound **2**a*

(−)1*S*,3*S*-Di-*p*-bromobenzoyloxy-4*S*-butylcyclopentane, **2***a*, was isolated as a yellowish sticky solid that could not be crystallized. TLC: R_f 0.31 (hexane:EtOAc, 9:1); FTIR (CH_2Cl_2, cast), 2957, 2955 (C-H), 1717 (-COO-) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 7.9 (4H, m), 7.5 (4H, m), 5.4 (1H, m), 5.1 (1H, ddd, J = 7.2, 4.4, 3.8 Hz), 2.6 (1H, ddd, J = 15.5, 7.5, 7.2 Hz), 2.5 (1H, m), 2.3 (1H, dddd, J = 14.0, 7.6, 2.1, 2.1 Hz), 2.1 (1H, bd, J = 15.5 Hz), 1.7 (1H, ddd, J = 14.0, 9.3, 5.8 Hz), 1.6–1.3 (6H, m), 0.9 (3H, t, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ: 165.6 (2C, s), 131.7 (4C, d), 131.1 (4C, d), 129.3 (2C, s), 128.1 (2C, s), 80.5 (C-3, d), 75.6 (C-1, d), 44.0 (C-4, d), 38.9 (C-2, t), 37.5 (C-5, t), 33.1 (C-6, t), 30.1 (C-7, t), 22.8 (C-8, t), 14.0 (C-9, q); HMQC (125, 500 MHz) δ: 80.5 ⇔ 5.1; 75.6 ⇔ 5.4; 44.0 ⇔ 2.5; 38.9 ⇔ 2.6, 2.1; 37.5 ⇔ 2.3, 1.7; 33.1 ⇔ 1.6, 1.3; 30.1 ⇔ 1.3; 22.8 ⇔ 1.3; 14.0 ⇔ 0.9; HREIMS (probe 150°), *m/z* calcd. for $C_{23}H_{24}O_4{}^{79}Br^{81}Br$ (M^+ + 2): 524.0021; found: 524.0001 (2%), 522 (M^+, $C_{23}H_{24}O_4{}^{79}Br_2$, 1%), 322 ($C_{16}H_{19}O_2{}^{79}Br$, 3%), 122 ($C_9H_{14}$, 100%), 80 ($C_6H_8$, 48%).

*Compound **3**a*

(−)1*S*,3*S*-Di-*p*-bromobenzoyloxy-4*R*-butylcyclopentane, **3***a*, was recrystallized from hexane to give fine white needles, mp 110–111°C; TLC: R_f 0.27 (hexane:EtOAc, 9:1); $[\alpha]_D{}^{24}$ −14 (*c* 0.004, hexanes); CD, $\Delta\epsilon_{241}$: −3.6 (*c* 8.8 × 10^{-4}, hexanes); FTIR (CH_2Cl_2 cast) similar to **2***a*; ^{1}H NMR ($CDCl_3$) δ: 7.9 (2H, J = 9.0 Hz), 7.8 (2H, d, J = 9.0 Hz), 7.6 (2H, J = 9.0 Hz), 7.5 (2H, J = 9.0 Hz), 5.5 (1H, dddd, J = 10.0, 7.5, 5.0, 2.5 Hz), 5.4 (1H, bdd, J = 5.0, 4.1 Hz), 2.6 (1H, ddd, J = 13.7, 7.5, 7.0 Hz), 2.4 (1H, ddd, J = 15.5, 8.0, 5.0 Hz), 2.2 (1H, bd, J = 15.5 Hz), 2.0–2.1 (1H, m), 1.8 (1H, ddd, J = 13.7, 10.0, 5.2 Hz), 1.6–1.3 (6H, m), 0.9 (3H, m); ^{1}H–^{1}H COSY ($CDCl_3$) δ: 5.5 (H-1α) ⇒ 2.6 (H-5α), 2.4 (H-2α), 2.2 (H-2β), 1.8 (H-5β); 5.4 (H-3α) ⇒ 2.4 (H-2α), 2.2 (H-2β), 2.1 (H-4α); 2.6 (H-5α) ⇒ 5.5 (H-1α), 2.1 (H-4α), 1.8 (H-5β); 2.4 (H-2α) ⇒ 5.5 (H-1α), 5.4 (H-3α), 2.2 (H-2β); 2.2 (H-2β) ⇒ 5.5 (H-1α), 5.4 (H-3α), 2.4 (H-2α), 1.8 (H-5β); 2.1 (H-4α) ⇒ 5.4 (H-3O), 2.6 (H-5α), 1.8 (H-5β), 1.6–1.3 (H-6); 1.8 (H-5β) ⇒ 5.5 (H-1α), 2.6 (H-5α), 2.1 (H-4α); 1.6 (H-6α) ⇒ 2.1 (H-4α), 1.3 (H-6β), 1.5–1.3 (H-6, 7); differential nOe: H-4α to H-3α 15%, H-2α 10%, H-5α 7%, H-6α 7%; H-5α to H-5β 34%, H-4α 14%, H-1α 12%; ^{13}C NMR ($CDCl_3$) δ: 165.5 (1C, s), 165.4 (1C, s), 131.8 (2C, d), 131.6 (2C, d), 131.1 (2C, d), 131.0 (2C, d), 129.5 (1C, s), 129.3 (1C, s), 128.1 (2C, s), 76.8 (C-3, d), 75.0 (C-1, d), 43.6 (C-4, d), 39.8 (C-2, t), 37.7 (C-5, t), 30.4 (C-7, t), 29.1 (C-6, t), 22.8 (C-8, t), 14.0 (C-9, q), HMQC (125, 500 MHz) δ: 76.8 ⇔ 5.4; 75.0 ⇔ 5.5; 43.6 ⇔ 2.1; 39.8 ⇔ 2.4, 2.2; 37.7 ⇔

2.6, 1.8; 29.1 ⇔ 1.5; 22.8 ⇔ 1.3; 14.0 ⇔ 0.9; HREIMS (probe 150°C) similar to **3***a*.

*Sistolynone (**4**)*

Butadiynyl-4-hydroxyl-2-cyclopenten-1-one, **4**, was isolated as an unstable oil. TLC: R_f 0.33 (CH_2Cl_2:CH_3OH, 19:1); FTIR ($CDCl_3$ cast): 3387 (O-H), 3284 (≡C-H), 2204 (C≡C), 1710, 1683 (α,β-unsaturated carbonyl) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.45 (1H, d, J = 1.0 Hz, H-2), 5.00 (1H, dddd, J = 6.5, 5.0, 2.5, 1.0 Hz, H-4), 2.90 (1H, s, H-10), 2.85 (1H, dd, J = 18.6, 6.5 Hz, H-5α), 2.40 (1H, dd, J = 18.6, 2.5 Hz, H-5β), 2.30 (OH, d, J = 5.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 204.4 (C-1, s), 154.7 (C-3, s), 139.3 (C-2, d), 89.9 (C-7, s), 78.3 (C-9, d), 72.1 (C-4, d), 68.2 (C-6, s), 66.8 (C-8, s), 44.0 (C-5, t); HREIMS (probe 100°C), *m/z* calcd. for $C_9H_6O_2$ (M^+): 146.0368; found: 146.0363 (51%), 118 (C_8H_6O, C_8H_6O, 31%), 74 (C_6H_2, 100%).

*Sistopyrone (**5**)*

(2,3-Butadienyl)pyran-2-one, **5**, was obtained in deuterated chloroform as an oil after preparative TLC, R_f 0.73 (CH_3OH, 19:1); FTIR, $CDCl_3$, cast): 1955 (C=C=C), 1725, 1651, 1637, 853 (=CH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.41 (1H, dd, J = 5.2, 1.0 Hz, H-6), 6.18 (1H, ddd, J = 2.0, 1.0, 1.0 Hz, H-3), 6.12 (1H, dd, J = 5.2, 2.0 Hz, H-5), 5.15 (1H, tt, J = 7.0, 6.7 Hz, H-8), 4.79 (2H, dt, J = 6.7, 2.9 Hz, H-10)), 3.12 (2H, dtd, J = 7.0, 2.9, 1.0 Hz, H-7); homonuclear decoupling (irradiation ⇒ effect): δ 6.18 ⇒ 7.41 (dd to d, J = 5.2 Hz), 6.12 (dd to d, J = 5.2 Hz), 3.12 (dtd to dt, J = 7.0, 2.9 Hz); δ 4.79 ⇒ 5.15 (tt to t, J = 7.0 Hz), 3.12 (dtd to dd, J = 7.0, 1.0 Hz); δ 3.12 ⇒ 6.18 (ddd to dd, J = 2.0, 1.0 Hz), 5.15 (tt to t, J = 6.7 Hz), 4.79 (dt to d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ: 209.7 (C-9, s), 162.2 (C-2, s), 156.8 (C-4, s), 150.7 (C-6, d), 113.7 (C-3, d), 108.0 (C-5, d), 85.5 (C-8, d), 76.3 (C-10, t), 34.3 (C-7, t); HMQC (125, 500 MHz) δ: 113.7 ⇔ 6.18; 108.0 ⇔ 6.12; 76.3 ⇔ 4.79; 34.3 ⇔ 3.12; HREIMS (probe 100°C), *m/z* calcd. for $C_9H_8O_2$: 148.0524 (M^+); found: 148.0522 (8%), 120 (C_8H_8O, 75%), 91 (C_7H_7, 100%), 65 (C_5H_5, 23%), 39 (C_3H_3, 34%).

^{13}C-Labelled metabolites

[1,2-$^{13}C_2$]-Acetate labelled metabolites

*[1,2-$^{13}C_2$]-Di-p-bromobenzoate derivative (**2**a)*: ^{13}C NMR (CD_2Cl_2, 100 MHz) spin-coupled carbons: δ 80.9 (C-3, d, $J_{3,2}$ = 37.6 Hz), 76.1 (C-1, d, $J_{1,5}$ = 36.0 Hz), 44.3 (C-4, D, $J_{4,6}$ = 35.6 Hz), 39.3 (C-2, d, $J_{2,3}$ = 37.6 Hz), 37.8 (C-5, d, $J_{5,1}$ = 36.0 Hz), 33.5 (C-6, d, $J_{6,4}$ = 35.7 Hz), 30.5 (C-7, d, $J_{7,8}$ = 34.5 Hz), 23.1 (C-8, d, $J_{8,7}$ = 34.5 Hz); singlet carbon: 14.0 (C-9); INADEQUATE ($CDCl_2$, 75 MHz) cross peaks (two double quantum); δ: 80.9 (C-3) ⇔ 39.3 (C-2); 76.1 (C-1) ⇔ 37.8 (C-5); 44.3 (C-4) ⇔ 33.5 (C-6); 30.5 (C-7) ⇔ 23.1 (C-8).

*[1,2-$^{13}C_2$]-Sistopyrone (**5**)*: ^{13}C NMR ($CDCl_3$) spin-coupled carbons: δ209.7 (C-9, d, $J_{8,7}$ = 102.1 Hz), 162.2 (C-2, d, $J_{1,2}$ = 74.1 Hz), 156.8 (C-4, d, $J_{3,6}$ = 42.1 Hz), 150.7 (C-6, d, $J_{5,4}$ = 70.4 Hz), 113.7 (C-3, d, $J_{2,1}$ = 74.1 Hz), 108.0 (C-5, d, $J_{4,5}$ = 70.5 Hz), 85.5 (C-8, d, $J_{7,8}$ = 102.1 Hz), 34.3 (C-7, d, $J_{6,3}$ = 42.1 Hz); singlet carbon: 76.3 (C-10).

[1-^{13}C]-Acetate labelled metabolites

*[1-^{13}C]-Di-p-bromobenzoate derivative (**2**a)*: ^{13}C NMR (CD_2Cl_2) enhanced signals: δ 80.5 (C-3), 76.7 (C-1), 44.0 (C-4), 30.1 (C-7), 14.0 (C-9); natural abundance signals: δ 38.9 (C-2), 37.5 (C-5), 33.1 (C-6), 22.8 (C-8).

*[1-^{13}C]-Sistopyrone (**5**)*: ^{13}C NMR ($CDCl_3$) enhanced signals: δ 162.2 (C-1), 156.8 (C-3), 108.8 (C-4), 85.5 (C-7), 76.3 (C-9); natural abundance signals: δ 209.7 (C-8), 150.7 (C-5), 113.7 (C-2), 34.3 (C-6).

[2-^{13}C]-acetate labelled metabolites

*[2,^{13}C]-di-p-Bromobenzoate derivative (**2**a)*: ^{13}C NMR (CD_2Cl_2) enhanced signals: δ 38.9 (C-2), 37.5 (C-5), 33.1 (C-6), 22.8 (C-8); natural abundance signals: δ 80.5 (C-3), 76.7 (C-1), 44.0 (C-4), 30.1 (C-7), 14.0 (C-9).

*[2-^{13}C]-Sistopyrone (**5**)*: ^{13}C NMR ($CDCl_3$) enhanced signals: δ 209.7 (C-9), 150.7 (C-6), 113.7 (C-3), 34.3 (C-7); natural abundance signals: δ 162.2 (C-2), 156.8 (C-4), 108.8 (C-5), 85.5 (C-8), 76.3 (C-10).

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