Biologically Active Polyketide Metabolites from an Undetermined Fungicolous Hyphomycete Resembling *Cladosporium*

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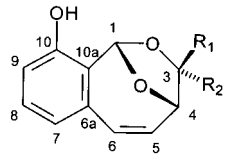
Eight new polyketide-derived metabolites [cladoacetals A and B (**1** and **2**), 3-(2-formyl-3-hydroxyphenyl)-propionic acid (**3**), 3-deoxyisoochracinic acid (**4**), isoochracinol (**5**), 7-hydroxy-3-(2,3-dihydroxybutyl)-1(3*H*)-isobenzofuranone (**6**), (+)-cyclosordariolone (**10**), and altersolanol J (**11**)] and six known metabolites [two isomeric 1-(1,3-dihydro-4-hydroxy-1-isobenzofuranyl)butan-2,3-diols (**7a/b**), 7-hydroxy-1(3*H*)-isobenzofuranone (**8**), isoochracinic acid (**9**), altersolanol A (**12**), and macrosporin (**13**)] have been isolated from solid-substrate fermentation cultures of an undetermined fungicolous isolate (NRRL 29097) that resembles *Cladosporium* sp. All structures were assigned primarily by analysis of 1D and/or 2D NMR data. Five of the compounds showed antibacterial activity.

Fungi inhabiting certain ecological niches (e.g., coprophilous fungi) have proven to be promising sources of new and biologically active natural products.[1] Fungicolous (or mycophilic) fungi represent a specialized group that grow on other fungal species. While it seems likely that fungal toxins are involved in many reported interspecies interactions, fungicolous fungi attracted our attention because they have rarely been studied chemically. Our initial work in this field involved chemical investigations of sclerotium-colonizing fungi.[2−4] In the current study, a fungus resembling the genus *Cladosporium* (Hyphomycetes; mitosporic fungi) was isolated from a resupinate polypore (Polyporaceae). An organic extract of fermented rice cultures of this unusual and undetermined isolate (NRRL 29097) exhibited antimicrobial activity in standard disk assays and was subjected to chemical investigation.

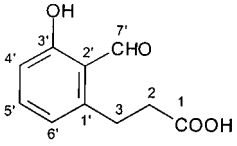
Results and Discussion

The undetermined isolate (Hyphomycetales) was grown in solid-substrate fermentation on rice, and the mature cultures were extracted with EtOAc. The resulting extract was fractionated by Sephadex LH-20 column chromatography, followed by normal (Si-60 and Diol) and/or reversed-phase (C_{18}) HPLC to yield eight new metabolites: cladoacetals A and B (**1** and **2**), 3-(2-formyl-3-hydroxyphenyl)-propionic acid (**3**), 3-deoxyisoochracinic acid (**4**), isoochracinol (**5**), 7-hydroxy-3-(2,3-dihydroxybutyl)-1(3*H*)-isobenzofuranone (**6**), (+)-cyclosordariolone (**10**), and altersolanol J (**11**). Six known metabolites [diastereomers of 1-(1,3-dihydro-4-hydroxy-1-isobenzofuranyl)butane-2,3-diol (**7a/b**), 7-hydroxy-1(3*H*)-isobenzofuranone (**8**), isoochracinic acid (**9**), altersolanol A (**12**), and macrosporin (**13**)] were also isolated from this extract.

The molecular formula for the first new metabolite, cladoacetal A (**1**), was determined to be $C_{12}H_{12}O_3$ (seven degrees of unsaturation) by HRCIMS analysis, as neither EIMS nor ESIMS gave parent ions intense enough for HRMS measurements. The ^{13}C and DEPT NMR spectra (Table 1) contained signals for eight sp^2-hybridized carbons



	R_1	R_2
1	H	CH_3
2	CH_3	H



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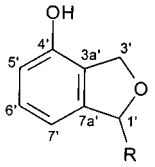
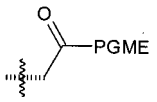
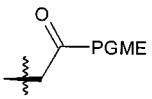


	R
4	CH_2COOH
4a/d	
4b/c	
7a/b	$CH_2CHOHCHOHCH_3$

(five protonated), which were assigned to an olefin unit and an oxygen-substituted aromatic ring. These units accounted for all but two degrees of unsaturation, and since no other sp^2 carbon signals were present, the molecule must be tricyclic. The remaining ^{13}C NMR signals were consistent with the presence of a methyl group (δ 13.6) and three

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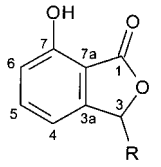
Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Cladoacetals A (**1**) and B (**2**) in $CDCl_3$

	cladoacetal A (**1**)			cladoacetal B (**2**)	
position	δ_H (mult.; *J* in Hz)	δ_C	HMBC correlations (H → C #)	δ_H (mult.; *J* in Hz)	δ_C
1	6.75 (s)	98.7	3, 4, 6a, 10, 10a[a]	6.86 (s)	99.4
3	4.41 (dq; 4.5, 6.3)	84.7	4, 5	4.56 (dq; 1.5, 6.3)	85.2
4	4.63 (dd; 4.8, 4.5)	77.9	1, 5, 6, 6a[b]	4.43 (dd; 4.9, 1.5)	80.0
5	5.81 (dd; 12, 4.8)	129.8	3, 4, 6, 6a, 7,[a,b] 8,[c] 10a[a,b]	6.07 (dd; 12, 4.9)	132.5
6	6.47 (d; 12)	132.4	3,[b] 4, 5, 6a, 7,[a] 10,[b] 10a[a]	6.34 (d; 12)	130.9
6a	-	136.0			136.2
7	6.80 (br d; 7.5)	125.8	1,[b] 6, 6a, 8, 9, 10,[b] 10a	6.80 (br d; 7.6)	125.5
8	7.05 (dd; 8.3, 7.5)	129.4	6a, 7,[a] 9, 10	7.06 (dd; 8.1, 7.6)	129.3
9	6.56 (dd; 8.3, 1.0)	115.9	1,[b] 6a,[b] 7,[a] 10, 10a[a]	6.59 (dd; 8.1, 1.0)	115.7
10-OH	5.03 (br s)	153.0	10a[a]	5.07 (br s)	153.1
10a		125.7			126.1
CH_3-3	1.33 (d; 6.3)	13.6	3, 4	1.30 (d; 6.3)	20.2

[a] Correlation to C-7 and/or C-10a. [b,c] Four-bond (b) and weak five-bond (c) correlations. Occurrence of such correlations is consistent with the presence of both the π-system and a relatively rigid tricyclic structure.

oxygenated methine carbons, one of which must be dioxygenated (δ 98.7). The ^{1}H NMR spectrum displayed signals for a hydroxy group (δ 5.03, br s; exchangeable), an isolated acetal or hemiacetal methine proton (δ 6.75, s), and spin systems corresponding to a 1,2,3-trisubstituted benzenoid ring and to the CH_3−C3−C6 subunit of **1**.



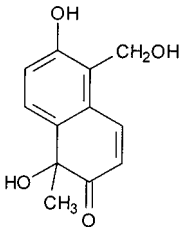
R

5 CH_2CH_2OH

6 $CH_2CHOHCHOHCH_3$

8 H

9 CH_2COOH



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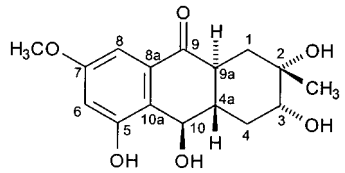
The structure for cladoacetal A (**1**) was proposed on the basis of HMBC and NOE results. Strong HMBC correlations from olefinic proton H-5 to nonprotonated aromatic carbon C-6a and from H-7 to olefinic carbon C-6 supported attachment of the CH_3−C-3−C-6 unit to the aromatic ring at position C-6a. However, the observation of several four- and five-bond correlations prevented an unambiguous assignment solely on the basis of HMBC results. In an NOE experiment, irradiation at the resonance frequency of H-6 (δ 6.47) resulted in enhancement of the signals corresponding to H-5 and H-7. Therefore, CH-6 and H-7 must be adjacent substituents on the aromatic ring, confirming the attachment of C-6 to C-6a.

HMBC correlations of the dioxygenated methine proton signal (H-1) to the three contiguous nonprotonated aryl carbons (C-6a, C-10a, and C-10) secured the connection of C-1 to the aromatic ring at C-10a between C-6a and oxygenated carbon C-10. Strong correlations of H-1 with



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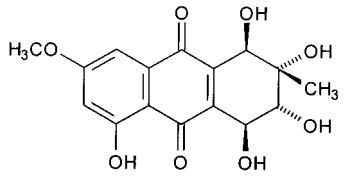

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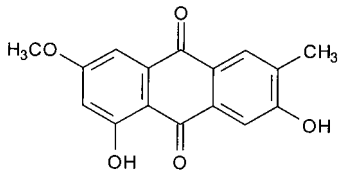

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both oxygenated sp^3 methines (C-3 and C-4) required that these carbons be attached to C-1 through ether linkages to form a 1,3-dioxolane ring. The remaining OH group must therefore be a phenolic OH located at C-10. The resulting rigid tricyclic ring system required the relative configuration at C-1 and C-4 to be 1*R**, 4*S**. Assignment of the relative configuration at C-3 was proposed on the basis of NOE difference spectroscopy. Irradiation at the resonance frequency of the methyl group protons (δ 1.33) enhanced the signal for olefinic proton H-5. Analysis of Dreiding molecular models indicated that this is likely only if the methyl group is endo. Thus, the relative configuration at C-3 was assigned as *R**. The structure of cladoacetal A was therefore established as shown in **1** and represents a new ring system. Several natural products are known that contain a similar octahydrogenated 1,3-epoxy-2(1*H*)-benzoxocin moiety as part of more complex ring systems and include sesterterpenes from the marine sponges *Dactylospongia* sp. and *Petrosaspongia nigra*.[5,6]

Cladoacetal B (**2**) was revealed to be an isomer of **1** by ^{13}C NMR, DEPT, and HRCIMS analysis. The ^{1}H and ^{13}C NMR data (Table 1), UV and IR properties, and HMBC correlations observed for **2** were very similar to those of **1**. These results suggested the same planar structure for the

two metabolites. The only significant differences in the NMR spectra were observed in the signals for the CH_3−C-3−C-6 portion of the molecule. A downfield shift of the methyl signal from δ 13.6 to 20.0 was observed in the ^{13}C NMR spectrum of **2**, and the $^1H-^1H$ coupling constant between H-3 and H-4 was reduced to 1.5 Hz, compared to 4.5 Hz for **1**. These differences suggested that compounds **1** and **2** differed only in the relative configuration at C-3, given that the relative configurations of C-1 and C-4 are fixed by the rigid tricyclic ring system. Support for this conclusion was provided by an NOE experiment. In this case, NOE irradiation of the methyl signal (δ 1.30) for **2** resulted in enhancements of the signals for H-3 and H-4 only; the signal for H-5 was unaffected. These results led to assignment of structure **2** for cladoacetal B.

HRESIMS analysis of compound **3** revealed a molecular formula of $C_{10}H_{10}O_4$ (six unsaturations). The ^1H, ^{13}C, and DEPT NMR spectra contained signals for a monooxygenated 1,2,3-trisubstituted benzene ring, a phenolic OH group (δ_H 8.0), an isolated CH_2CH_2 unit, an isolated aldehyde moiety (δ_H 10.34, s; δ_C 194.7), and a carboxylic acid group (δ_C 177.6; δ_H 11.9). The only possible arrangement of these functional groups is an aromatic ring bearing OH, CHO, and CH_2CH_2COOH substituents. The regiochemistry of **3** was established by NOE difference measurements. Irradiation at the H_2-3 resonance frequency (δ 3.26) enhanced the signals for one aryl proton (H-6′) and the aldehyde proton. Thus, the structure of **3** was assigned as 3-(2-formyl-3-hydroxyphenyl)propionic acid. Although the 3-methoxy derivative of **3** is known as a synthetic product,[7] compound **3** has not been previously reported to our knowledge. The closest analogues reported from fungal sources differ from **3** in the substituent at the 1′-position of the aromatic ring. For example, pyriculol and epipyriculol, isolated from *Pyricularia oryzae*, possess a 3,4-dihydroxy-1,5-heptadienyl moiety at that position.[8]

Compound **4** was the most abundant metabolite and also had a molecular formula of $C_{10}H_{10}O_4$ as deduced by HREIMS. The ^1H, ^{13}C, and DEPT NMR spectra again revealed the presence of a monooxygenated 1,2,3-trisubstituted aromatic ring, a phenolic hydroxy group (δ 8.55), and a carboxylic acid moiety (δ_C 172.1; δ_H 10.79), as well as isolated CH_2O and $OCHCH_2$ units. In this case, an additional ring must be present to account for the remaining degree of unsaturation. Significant homobenzylic coupling observed between one of the CH_2O protons and the methine proton (H-1′) of the $OCHCH_2$ unit suggested that C-1′ and C-3′ are adjacent substituents on the aromatic ring. This arrangement is consistent with the ^{13}C NMR shifts of the aromatic ring carbon signals and led to the assignment of a 1,3-dihydroisobenzofuran system with an acetic acid side chain at C-1′. The regiochemistry of the isobenzofuran system was established by NOE difference measurements. Irradiation at the resonance frequencies of aromatic protons H-5′ and H-7′ (δ 6.78, overlapping signals) enhanced the signals corresponding to H-1′ and H_2-2. Thus, the hydroxy group must be positioned at C-4′. Compound **4** was therefore identified as (1′,3′-dihydro-4′-hydroxy-1′-isobenzofuranyl)acetic acid, the 3′-deoxy derivative of the known fungal metabolite isoochracinic acid (**9**).[9,10]

Determination of the absolute configuration of **4** was attempted using a recently published modification of Mosher's method developed for chiral β,β-disubstituted propionic acid derivatives.[11] Treatment of compound **4** with (*R*)- and (*S*)-phenylglycine methyl ester (PGME) reagents in separate reactions produced the desired amide derivatives. In each reaction, however, two separable epimers were formed in a ratio of approximately 1.5:1, indicating that the natural product was not enantiomerically pure. The four epimeric amide derivatives, labeled **4a**−**d**, were isolated by normal-phase HPLC and analyzed by ^1H NMR. As expected, two sets of identical NMR data corresponding to the two pairs of enantiomeric amides (**4a/4b** and **4c/4d**) were obtained, and optical rotation measurements confirmed the NMR results. The ^1H NMR shift differences ($\Delta\delta = \delta_{(R)} - \delta_{(S)}$) between the diastereomeric (*R*)- and (*S*)-PGME amide derivatives arising from the major and minor enantiomers of **4** were calculated, and the data for one set (**4a** and **4d**) are provided here. Negative $\Delta\delta$ values were expected for protons that reside on the same side of the molecule as the phenyl group in a given PGME derivative, and positive $\Delta\delta$ values were expected for protons on the opposite side.[11] Molecular models of the PGME derivatives suggested that all of the relevant protons of the isobenzofuran moiety for a given isomer lie on the same side of the PGME plane. Thus, while it was possible to assign the stereochemistry for **4** on the basis of the signs of the $\Delta\delta$ values, the absence of protons expected to lie on the opposite side did not allow a cross-check for the applicability of the method in this case. As predicted, the signs of the calculated $\Delta\delta$ values ($\delta_{(\mathbf{4a})} - \delta_{(\mathbf{4d})}$) for H_2-3′ (+0.06 and +0.02), H-5′ (+0.02), H-6′ (+0.06), and H-7′ (+0.08) were the same and, in this case, positive. According to the formula presented in the literature, these results indicated that **4a** and **4d** arose from (*R*)-**4**. Therefore, **4b** and **4c** must be derived from (*S*)-**4**.

Acid hydrolysis of the diastereomeric amides produced the (+)-enantiomer of **4** ($[\alpha]_D$ +8.5°) from a combined sample of **4a** and **4d** and the (−)-enantiomer ($[\alpha]_D$ −13°) from **4b** and **4c**. Although the difference in magnitudes of the measured values could be attributed to experimental error, this result is reasonably consistent with the ratio of 1.5:1 found for the corresponding PGME derivatives. The major enantiomer was therefore assigned as *S*-(−)-**4** and the minor enantiomer as *R*-(+)-**4**.

Another metabolite (**5**)[12] also displayed a molecular formula of $C_{10}H_{10}O_4$ based on HRESIMS analysis and was clearly similar in structure to compound **4**. The ^1H, ^{13}C, and DEPT NMR data (CD_3OD) for **5** revealed the presence of the same general features found in **4**, except that the $OCHCH_2$ unit was extended to an $OCHCH_2CH_2O$ moiety and the C-3′ oxymethylene proton signals were absent. These results suggested that the C-3′ group in **4** was oxidized to a carbonyl unit in **5** and that the carboxylic acid group in **4** was reduced to a primary alcohol moiety in **5**. A ^{13}C NMR resonance at δ 171.7 and an absorption in the IR spectrum at 1733 cm^{-1} supported the assignment of a lactone carbonyl group at C-3′. The structure of **5** was therefore assigned as 7-hydroxy-3-(2-hydroxyethyl)-1(3*H*)-isobenzofuranone. Compound **5** was assigned the name isoochracinol, as it is a reduced form of isoochracinic acid (**9**), a metabolite previously reported from *Alternaria kikuchiana*[9] and *Mycosphaerella fijiensis*[10] and also encountered in this study (see below).

Compound **6** was determined to have the molecular formula $C_{12}H_{14}O_5$ by HRESIMS. The ^1H, ^{13}C, and DEPT NMR data for **6** revealed the presence of an isobenzofuranone moiety identical to that in **5** and indicated that the difference is in the nature of the side chain. The new signals in the ^{13}C and DEPT NMR data were consistent with a methyl group (δ 18.9), a methylene unit (δ 38.8), and two oxygenated methine moieties (δ 73.1 and 71.9). Analysis of the corresponding signals in the ^1H NMR spectrum (CD_3OD) of **6** led to the assignment of a $-CH_2$-

$CH(O-)CH(O-)CH_3$ moiety. The remaining two (exchangeable) protons must be attributed to two hydroxy groups in the side chain. The structure of compound **6** was therefore assigned as 7-hydroxy-3-(2,3-dihydroxybutyl)-1(3*H*)-isobenzofuranone and represents a new natural product.

The ^{1}H NMR chemical shift and coupling constant values for two further compounds (**7a** and **7b**) matched closely with those of two stereoisomeric 1-(1,3-dihydro-4-hydroxy-1-isobenzofuranyl)butane-2,3-diols previously reported from *Sordaria macrospora*.[13,14] An *erythro*-configuration for the diol moiety of each of these metabolites was originally proposed on the basis of ^{1}H NMR analysis of the corresponding acetonides.[14] However, the stereochemistry relative to the third chiral center was not assigned. In any event, optical rotations, melting points, ^{13}C NMR, and IR data were not provided in the literature reports, making it impossible to thoroughly compare the stereochemistry of **7a** and **7b** with that of the *Sordaria* metabolites. On the basis of ^{1}H NMR comparisons, **7a** appears to possess the same relative stereochemistry as one of these compounds, while the relative stereochemistry of **7b** differs in some respect from the other. Because compounds **7a** and **7b** were inactive (see below) and because the presence of epimeric mixtures was already shown to be common among the metabolites in this organism (i.e., for **1** and **2**; **4a** and **4b**), in-depth studies directed toward assignment of the stereochemistry of these compounds (or of closely related compound **6**) were not undertaken.

Two additional isobenzofuranone metabolites were determined to be the known compounds 7-hydroxy-1(3*H*)-isobenzofuranone (**8**), previously reported as a synthetic product,[15,16] and isoochracinic acid (**9**).[9,10] To our knowledge, isoocracinic acid has only been isolated and synthesized[17,18] as a racemate, and no absolute configuration has been reported for the two enantiomers. The sample of isoochracinic acid obtained in this study produced a specific rotation of −5.0°, suggesting that a single enantiomer or an excess of one enantiomer was present, as in the case of **4**. However, determination of the absolute stereochemistry of compound **9** was not pursued in this investigation.

Compound **10** was found to have the same gross structure as the fungal metabolite cyclosordariolone by comparison of NMR and MS data with literature values.[14] However, a specific rotation of +168° for a sample of **10** suggested that it is the enantiomer of the previously reported metabolite, which produced a value of −77.8°.[14] The large difference in magnitude of these measurements could be ascribed to the occurrence of scalemic mixtures of varying composition (in one or both samples), which would be consistent with the observation of stereoisomeric mixtures as noted among other compounds above. No definitive conclusion about this issue was drawn. Cyclosordariolone could arise from an intramolecular aldol-like condensation of an oxidized (i.e., quinone) analogue of the co-occurring compound sordariolone [1-(3-hydroxy-9-(1-hydroxymethylphenyl)-4-hydroxypent-1-en-3-one], and one could envision different stereochemical results for the newly formed chiral center. Cyclosordariolone was originally reported from the same species (*Sordaria macrospora*) that produced the 1-(1,3-dihydro-4-hydroxy-1-isobenzofuranyl)butane-2,3-diols related to **7a** and **7b** listed above. Interestingly, compound **3** is a truncated version of a compound called sordarial also obtained from this organism, and yet another related compound called heptacyclosordariolone reported from *S. macrospora* contains a [5.4.0] oxabicyclic system analogous to that contained within the tricyclic cladoacetals A (**1**) and B (**2**). All of these compounds appear to be closely related from a biogenetic standpoint. Despite these chemical similarities and despite the difficulty in identifying the *Cladosporium*-like organism in this report, it is clear that this fungus is taxonomically unrelated to *Sordaria*.

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Altersolanol J (**11**; DMSO-d_6)

position	δ_H (mult.; J in Hz)	δ_C	HMBC correlations (H → C #)
1_{ax}	1.27 (dd, 14, 12)	37.8	2, 3, 4a, 9, 9a, CH_3-2
1_{eq}	2.07 (dd, 14, 3.8)		2, 3, 4a, 9, 9a, CH_3-2
2-OH	3.93 (s)	69.6	1, 2, 3, CH_3-2
3_{ax}	3.16 (ddd, 12, 6.6, 4.5)	72.7	2, 4, 4a, CH_3-2
3_{eq}-OH	4.38 (d; 6.6)		2, 3, 4
4_{ax}	1.57 (br ddd, 12, 12, 12)	33.1	2, 3, 4a, 9a, 10
4_{eq}	2.10 (ov. m)		2, 3, 4a, 9a, 10
4a	1.84 (m)	45.2	1, 4, 9, 9a, 10, 10a
5-OH	10.1 (s)	157.7	5, 6, 10a
6	6.59 (d, 2.5)	107.4	5, 7, 8, 10,[a] 10a
7		159.4	
8	6.83 (d, 2.5)	100.9	6, 7, 8a, 9, 10,[a] 10a
8a		132.7	
9		198.3	
9a	2.51 (ddd; 13, 12, 3.8)	43.6	1, 2, 4, 4a, 9, 10
10_{ax}	4.78 (dd, 9.6, 6.6)	71.4	4, 4a, 5, 6,[a] 7,[b] 8,[a] 8a, 9,[a] 10a
10_{eq}-OH	6.38 (d, 6.6)		4a, 10, 10a
10a		123.3	
CH_3-2	1.17 (s)	26.9	1, 2, 3, 9a[a]
OCH_3-7	3.73 (s)	55.2	7, 8[a]

[a,b] Relatively weak four-bond (a) and five-bond (b) correlations.

The final three metabolites isolated in this study are anthracenone derivatives. The first, compound **11**, was assigned a molecular formula of $C_{16}H_{20}O_6$ on the basis of HRESIMS and ^{13}C NMR data. The ^{1}H, ^{13}C, and DEPT NMR spectra for **11** (DMSO-d_6) revealed the presence of a meta-dioxygenated 1,2,3,5-tetrasubstituted benzene ring, four OH groups, a ketone moiety, a methoxy group, a methyl group, two methylene units, one oxygenated quaternary carbon, and four sp^3 methines, two of which are oxygenated. The planar structure of **11** was determined primarily by interpretation of COSY and HMBC data. Observation of residual ^{1}H−^{13}C one-bond correlations in the HMBC spectrum permitted the assignment of signals for all proton-bearing carbons. An interconnected spin system [$HO^{10}CH-^{4a}CH-(^4CH_2-^3CHOH)-^{9a}CH-^1CH_2$] was assembled on the basis of COSY data. HMBC correlations (Table 2) enabled assignment of the aromatic ring carbons and defined the regiochemistry of the hydroxy and methoxy substituents. Correlations from both H-9a and H-8 to ketone carbon C-9 linked C-9a to C-8a via the keto moiety. Linkage of C-10 to the remaining substituted aromatic carbon C-10a was established by observation of NOE correlations between OH-5 (δ 10.1) and H-10 and supported by HMBC correlations of H-4a, H-10, and OH-5 with C-10a. HMBC correlations from the CH_3-2 and OH-2 groups to C-1, C-2, and C-3 placed these groups at C-2 and required both C-1 and C-3 to be linked to C-2, leading to the assignment of partially reduced anthraquinone structure **11**.

The large values of J_{4a-9a} (13 Hz) and J_{4a-10} (9.6 Hz) indicated that H-4a is *trans* to H-9a and to H-10, and that all of these hydrogens are axially positioned. An 11 Hz value for J_{3-4ax} indicated an axial position for H-3. The equatorial orientation of the methyl group at C-2 was suggested by the downfield chemical shift of its carbon atom (δ 26.9),[19] and this assignment was supported by an identical chemical shift reported for the analogous methyl group in the similar compound tetrahydroaltersolanol B. Tetrahydroaltersolanol B is an isomer of **11** that has been

reported from *Alternaria solani*.[20] It differs from **11** in having a keto moiety at position 10, while the keto moiety C-9 is reduced to an OH group. The trivial name altersolanol J is proposed for compound **11**.

Two related, known metabolites, altersolanol A (**12**)[21,22] and macrosporin (**13**),[23,24] were also isolated from the fermentation extract of NRRL 29097 and were identified by comparison of their spectral data to literature values. The absolute stereochemistry of **12** was originally suggested on the basis of its production by the same fungal strain that produced altersolanol B, the 1,4-dideoxy derivative of **12** with known absolute stereochemistry.[25] In our case, the production of **11** together with **12** suggested that these two compounds may possess the same configurations at C-2 and C-3. Thus, the absolute configuration of **11** is proposed to be 2*S*, 3*R*, 4a*S*, 9a*S*, 10*R*.

The most abundant compounds (**1**, **3**, **4**, **6**, **7a**, and **9**−**13**) were tested in standard agar diffusion assays for inhibition of *Candida albicans* (ATCC 90029), *Bacillus subtilis* (ATCC 6051), and *Staphylococcus aureus* (ATCC 29213) at 200 μg/disk. Compounds **1**, **3**, **4**, **6**, **7a**, **7b**, and **9**−**13** were further investigated in agar diffusion assays against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) at 250 μg/disk.[3] Antibacterial activity was observed for five compounds. Compounds **3**, **4**, **12**, and **13** inhibited the growth of *B. subtilis*, producing zones of inhibition of 22, 8, 33, and 23 mm, respectively, and compounds **1**, **12**, and **13** inhibited *S. aureus*, causing inhibition zones of 13, 31, and 20 mm. While the antibacterial activity of **12** and its analogues is well established,[22] no activity appears to have been reported for **13**. None of the compounds tested showed antifungal activity in our assays.

All of these compounds appear to be of polyketide origin, and compounds **1**−**10** are presumably closely related tetra- (**8**), penta- (**3**−**5**, **9**), and hexaketide (**1**, **2**, **6**−**7b**, **10**) metabolites. None of the known compounds described here have been reported from a *Cladosporium* sp. Similar families of metabolites have been reported from *Sordaria macrospora* (hexaketides)[13,14] and *Pyricularia oryzae* (heptaketides).[8] The structures of **11**−**13** are consistent with an octaketide biogenesis. Examples of metabolites in this family have been reported from various *Alternaria* spp., such as *A. solani*[20−22] and *A. porri*.[24]

Experimental Section

General Experimental Procedures. ^{1}H, ^{13}C, DEPT, and difference NOE NMR spectra were recorded on a Bruker AC-300 or DRX-400 spectrometer. ^{1}H−^{1}H COSY NMR spectra for compounds **1** and **2** were recorded on the DRX-400. HMBC NMR spectra were recorded on a Bruker AMX-600 spectrometer. All spectra were referenced to the corresponding residual solvent signals: acetone-d_6, δ_H 2.04/δ_C 29.8; $CDCl_3$, δ_H 7.24/δ_C 77.0; DMSO-d_6, δ_H 2.49/δ_C 39.5; CD_3OD, δ_H 3.30/δ_C 49.0. EIMS (70 eV) data were obtained using a Finnigan Voyager instrument, FABMS and HREIMS data were recorded on a VG ZAB-HF spectrometer, and HRESIMS data were obtained on a Fisons Autospec instrument. UV spectra were recorded on a HP 8452A diode array spectrophotometer, and IR data were obtained on a Mattson Cygnus 25 FT spectrophotometer. Optical rotations were determined on a JASCO DIP-1000 polarimeter. Melting points were determined on a Fisher-Johns micro melting point apparatus and are not corrected. HPLC separations were carried out on a Beckman System Gold instrument, equipped with a 127P Solvent Module, a 166P UV detector, a Rheodyne 1295 injector, and an Alltech Linear recorder. All columns used (Varian Dynamax-100 Å RP-18, Rainin Microsorb Si-60-110-C5, or Phenomenex LiChrosorb Diol 5-μm) were semipreparative (250 × 10 mm).

Cultures and Fermentation Conditions. A basidiocarp of an unidentified resupinate polypore that had formed on bark of a dead conifer log was collected from Malette Meadow (elevation 8700 ft) near Red River, New Mexico, by Dr. Harry D. Thiers, on September 4, 1996. This specimen (H.D. Thiers #55639), including portions of the woody substrate on which the resupinate basidiocarps had formed, was placed in a plastic bag and stored in the freezer (−7 °C) for 8 months until it could be processed. To isolate microfungal colonists, basidiocarp surfaces were gently abraded with a sterilized fingernail file to produce "filings" from the fungal tissues. These tissue filings were collected on a sheet of white paper (8.5 × 11 in.), which was folded into an envelope and placed in the freezer. Direct plating of basidiocarp tissue filings was accomplished by sprinkling a small portion (~100−200 mg) of the filings over the surface of each of two plates of dextrose-peptone-yeast extract agar (DPYA) containing streptomycin (25 mg/L) and tetracycline (1.25 mg/L). Plates were incubated in the dark at 25 °C for 5 days, and representative cultures were isolated from each colony type showing a distinctive morphology on DPYA. After 7−12 days incubation, the tube cultures isolated from filings of basidiocarp tissues were segregated into groups of presumptive species and maintained for identification and rice fermentation (25 °C). One of these cultures, MYC-391 (= NRRL 29097), produced conidia sympodially in long chains reminiscent of the genus *Cladosporium*. However, NRRL 29097 is readily distinguished from any previously described species of *Cladosporium* including those recorded from fungal basidiocarps.[26] Dr. John C. David of the Centre for Agriculture and Biosciences International (CABI), Bioscience UK Centre, Egham, Surrey, UK, examined a culture of the fungus and concluded that it is not a *Cladosporium sensu stricto*. The unnamed culture has been deposited with CABI as Hyphomycete gen. indet. (IMI 386054).

The fungus was grown on several slants of potato dextrose agar (PDA) for 14 days (25 °C). A hyphal fragment-spore suspension (propagule density 10^3/mL of sterile distilled water) prepared from the PDA slants served as the inoculum. Fermentations were carried out in eight 500 mL Erlenmeyer flasks, each containing 50 g of rice (Botan Brand; J.F.C. International). Distilled water (50 mL) was added to each flask, and the contents were soaked overnight before being autoclaved at 15 lb/in.^{2} for 30 min. After the flasks had cooled to room temperature, they were inoculated with 1.0 mL of the hyphal fragment-spore suspension and incubated for 40 days at 25 °C.

Extraction and Purification. Following incubation, the fermented rice substrate in each flask was first fragmented with a spatula and then extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were filtered and concentrated, yielding 2.4 g of an orange-brown oil (MYC-391 L-3 extract), which was stored at −20 °C. The extract was dissolved in a minimum amount of acetone, and the resulting solution was applied to a Sephadex LH-20 column (35 × 2.5 cm, preconditioned with CH_2Cl_2/hexanes, 4:1). The column was eluted sequentially with 4:1 CH_2Cl_2/hexanes, 4:1 CH_2Cl_2/acetone, 3:2 CH_2Cl_2/acetone, 1:4 CH_2Cl_2/acetone, and pure acetone (300 mL each). Fractions (50 mL each) were collected and pooled according to TLC analysis, resulting in a total of 10 fractions. Fraction 3 (92 mg) was separated by normal-phase HPLC (Si-60, UV detection at 254 nm, 50/50 EtOAc/hexanes, 3 mL/min) to yield six subfractions (31−36). Subfraction 31 (18 mg) was further purified by reversed-phase HPLC (RP-18, UV detection at 220 nm, 40/60 CH_3CN/0.1% aqueous HCOOH for 20 min, then a linear gradient to 50/50 over 5 min, 1.5 mL/min) to yield **3** (9.1 mg) and a mixture containing **1** and **2** (3.9 mg). This mixture was separated by normal-phase HPLC (Diol, UV detection at 254 nm, 90/10 hexanes/EtOAc for 10 min, then a linear gradient to 50/50 over 45 min, 3 mL/min) to yield **1** (2.7 mg) and **2** (0.9 mg). Subfraction 36 (5.0 mg) was further purified by reversed-phase HPLC (RP-18, UV detection at 220 nm, 20/80 CH_3CN/0.1% aqueous HCOOH, 1.5 mL/min) to yield **5** (2.5 mg).

Fraction 4 (127 mg) was triturated with cold acetone to yield a yellow solution and compound **13** as an orange residue (95

mg). A portion of fraction 5 (110 of 335 mg) was separated by normal-phase HPLC (Si-60, UV detection at 254 nm, 70/30 EtOAc/hexanes, 3 mL/min) to afford **6** (24 mg) and another fraction (25 mg), which yielded **8** (1.1 mg) and **9** (13 mg) upon further purification by reversed-phase HPLC (RP-18, UV detection at 254 nm, 20/80 CH_3CN/H_2O, 1.5 mL/min). Fraction 6 (108 mg) was separated by normal-phase HPLC (Si-60, UV detection at 254 nm, 75/25 EtOAc/hexanes, 3 mL/min) to yield 10 additional subfractions (61−70). Subfraction 64 (6.7 mg) was further purified by reversed-phase HPLC (RP-18, UV detection at 254 nm, 20/80 CH_3CN/H_2O, 1.5 mL/min) to yield **10** (2.3 mg). Subfraction 67 (27 mg) was further purified by reversed-phase HPLC (RP-18, UV detection at 220 nm, 20/80 CH_3CN/H_2O for 20 min, then a linear gradient to 50/50 over 30 min, 1.5 mL/min) to yield **5** (1.8 mg), **7a** (9.7 mg), and **7b** (3.4 mg). Subfraction 68 (14 mg) was further purified by normal-phase HPLC (Si-60, UV detection at 254 nm, 80/20 EtOAc/hexanes, 3 mL/min) to yield 8.7 mg of **11**. A portion of fraction 7 (125 of 381 mg) was separated by normal-phase HPLC (Si-60, UV detection at 254 nm, 80/20 EtOAc/hexanes, 3 mL/min) to give **4** (80 mg), **11** (7.1 mg), and **12** (6.3 mg).

Preparation of PGME Derivatives of 4. The PGME derivatives were prepared according to the method of Yabuuchi and Kusumi,[11] using identical reagent concentrations and reaction conditions for the preparation of both sets of derivatives. A sample of **4** (5.0 mg) and 6.3 mg of (*R* or *S*)-PGME hydrochloride (Aldrich) were dissolved in 1 mL of dry DMF. The solution was cooled in an ice−water bath, and 16.3 mg of PyBOP, 4.2 mg of HOBT, and 135 μL of *N*-methylmorpholine were added. The mixture was stirred at room temperature for 3.5 h. EtOAc (25 mL) was added, and the organic layer was successively extracted with 6% HCl (2 × 25 mL), saturated $NaHCO_3$ solution (2 × 25 mL), and 3.5% aqueous NaCl (2 × 25 mL) and dried over $NaSO_4$. After removal of the solvent, 13 mg of the crude (*R*)-PGME derivatives and 15 mg of the crude (*S*)-PGME derivatives were obtained. Purification by normal-phase HPLC (Si-60, UV detection at 254 nm, 60/40 EtOAc/hexanes, 3 mL/min) afforded **4a** (3.6 mg), **4b** (5.4 mg), **4c** (5.2 mg), and **4d** (3.6 mg).

Hydrolysis of the PGME Derivatives of 4. Samples of **4a** and **4d** were combined (1.5 mg each), as were samples of **4b** and **4c** (2.8 mg each). The two resulting mixtures were separately hydrolyzed with 1 mL of 6 N HCl at 110 °C for 24 h. The reaction mixtures were dried under N_2, redissolved in 100 μL of MeOH each, and separately purified by reversed-phase HPLC (RP-18, UV detection at 220 nm, 22/78 CH_3CN/0.1% aqueous HCOOH, 1.5 mL/min) to give (*R*)-**4** (1.4 mg) and (*S*)-**4** (0.9 mg).

Cladoacetal A (1): white solid (MeOH); mp 149−150 °C; $[\alpha]_D^{22}$ +266° (*c* 1.4 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.3), 268 (3.9), 280 (sh, 3.7), 310 (3.7), 320 (sh, 3.6) nm; IR (film, NaCl) ν_{max} 3314, 2983, 2930, 2907, 1580, 1461, 1288, 1095, 956 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 204 $[M]^+$ (3), 161 (24), 160 (100), 132 (89), 131 (90), 103 (40), 77 (46); HRCIMS (NH_3) *m*/*z* 205.0868 $[M + H]^+$, calcd for $C_{12}H_{13}O_3$, 205.0865.

Cladoacetal B (2): white solid (MeOH); mp 161−162 °C; $[\alpha]_D^{22}$ −135° (*c* 0.6 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.2), 266 (3.8), 277 (sh, 3.6), 308 (3.5), 318 (sh, 3.5) nm; IR (film, NaCl) ν_{max} 3292, 2967, 2924, 2852, 1574, 1461, 1288, 1115, 1052, 960 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 204 $[M]^+$ (2), 161 (10), 160 (100), 132 (81), 131 (71), 103 (21), 77 (23); HRCIMS (NH_3) *m*/*z* 205.0867 $[M + H]^+$, calcd for $C_{12}H_{13}O_3$, 205.0865.

3-(2-Formyl-3-hydroxyphenyl)propionic acid (3): white solid (MeOH); mp 78−79 °C; UV (MeOH) λ_{max} (log ϵ) 218 (4.1), 266 (4.0), 339 (3.5) nm; IR (film, NaCl) ν_{max} 3050, 2972, 2929, 1705, 1616, 1453, 1280, 1203, 785 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.9 (1H, br s, COOH), 10.34 (1H, s, CHO), 8.0 (1H, br s, O*H*-3′), 7.41 (1H, dd, *J* = 8.4, 7.4 Hz, H-5′), 6.85 (1H, d, *J* = 8.4 Hz, H-4′), 6.75 (1H, d, *J* = 7.4 Hz, H-6′), 3.26 (2H, t, *J* = 7.4 Hz, H_2-3), 2.72 (2H, t, *J* = 7.4 Hz, H_2-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.7 (s, CHO), 177.6 (s, COOH), 163.5 (s, C-3′), 144.2 (s, C-1′), 137.6 (d, C-5′), 120.7 (d, C-6′), 117.7 (s, C-2′), 116.9 (d, C-4′), 36.0 (t, C-2), 25.9 (t, C-3); EIMS *m*/*z* 194 $[M]^+$ (44), 177 (35), 176 (42), 166 (41), 151 (46), 149 (44), 148 (71), 147 (73), 135 (49), 134 (50), 131 (43), 121 (85), 120 (80), 107 (59), 103 (58), 91 (87), 77 (100); HRESIMS (negative ion mode) *m*/*z* 193.0514 $[M - H]^-$, calcd for $C_{10}H_9O_4$, 193.0501.

3-Deoxyisoochracinic acid (4); (1,3-dihydro-4-hydroxy-1-isobenzofuranyl)acetic acid: white solid (acetone); mp 166−167 °C; naturally occurring scalemic mixture $[\alpha]_D^{22}$ −3.5° (*c* 0.7 mg/mL, MeOH), (*S*)-**4** $[\alpha]_D^{22}$ −13° (*c* 0.8 mg/mL, MeOH), (*R*)-**4** $[\alpha]_D^{22}$ +8.5° (*c* 1.2 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.1), 271 (3.2) nm; IR (film, NaCl) ν_{max} 3329, 1711, 1599, 1469, 1290, 1175, 1066, 1004, 776 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 10.8 (1H, br s, COOH), 8.55 (br s, OH-4′), 7.13 (1H, ddd, *J* = 8.0, 7.6, 0.8 Hz, H-6′), 6.81 (1H, br d, *J* = 7.6 Hz, H-7′), 6.75 (1H, dd, *J* = 8.0, 0.8 Hz, H-5′), 5.56 (1H, m, H-1′), 5.06 (1H, dd, *J* = 12, 2.8 Hz, H-3′a), 4.95 (1H, br d, *J* = 12 Hz, H-3′b), 2.81 (1H, dd, *J* = 16, 4.7 Hz, H-2a), 2.62 (1H dd, *J* = 16, 8.1 Hz, H-2b); ^{13}C NMR (acetone-d_6, 100 MHz) δ 172.1 (s, C-1), 152.5 (s, C-4′), 144.5 (s, C-7a′), 130.1 (d, C-6′), 126.3 (s, C-3a′), 115.1 (d, C-5′), 113.3 (d, C-7′), 81.7 (d, C-1′), 71.2 (t, C-3′), 42.0 (t, C-2); EIMS *m*/*z* 194 $[M]^+$ (35), 176 (3), 151 (3), 148 (7), 135 (100), 134 (63), 107 (33), 91 (10), 77 (28); HREIMS *m*/*z* 194.0574, calcd for $C_{10}H_{10}O_4$, 194.0579.

(*R*)-(1,3-Dihydro-4-hydroxy-1-isobenzofuranyl)acetic acid-(*R*)-PGME-amide (4a): colorless oil; $[\alpha]_D^{22}$ −32° (*c* 1.8 mg/mL, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.43 (1H, d, *J* = 7.2 Hz, NH), 7.37−7.29 (5H, m, phenyl-group protons), 7.12 (1H, dd, *J* = 8.0, 7.5 Hz, H-6′), 6.71 (1H, d, *J* = 7.5 Hz, H-7′), 6.65 (1H, d, *J* = 8.0 Hz, H-5′), 5.56 (1H, d, *J* = 7.2 Hz, C*H*NH), 5.51 (1H, m, H-1′), 5.18 (1H, dd, *J* = 12, 2.8 Hz, H-3′a), 5.07 (1H, dd, *J* = 12, 1.4 Hz, H-3′b), 3.68 (3H, s, OCH_3), 2.76 (1H, dd, *J* = 15, 3.6 Hz, H-2a), 2.64 (1H, dd, *J* = 15, 8.2 Hz, H-2b); EIMS *m*/*z* 342 $[M + H]^+$ (20), 341 $[M]^+$ (18), 282 (48), 166 (42), 164 (27), 148 (42), 135 (83), 121 (36), 118 (37), 106 (100), 104 (59), 91 (45), 77 (51).

(*S*)-(1,3-Dihydro-4-hydroxy-1-isobenzofuranyl)acetic acid-(*S*)-PGME-amide (4b): colorless oil; $[\alpha]_D^{22}$ +31° (*c* 2.7 mg/mL, MeOH); ^1H NMR and MS data identical with those for **4a**.

(*S*)-(1,3-Dihydro-4-hydroxy-1-isobenzofuranyl)acetic acid-(*R*)-PGME-amide (4c): colorless oil; $[\alpha]_D^{22}$ −80° (*c* 2.6 mg/mL, MeOH); ^1H NMR and MS data identical with those for **4d**.

(*R*)-(1,3-Dihydro-4-hydroxy-1-isobenzofuranyl)acetic acid-(*S*)-PGME amide (4d): colorless oil; $[\alpha]_D^{22}$ +76° (*c* 1.8 mg/mL, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.44 (1H, d, *J* = 7.0 Hz, NH), 7.24−7.18 (5H, m, phenyl-group protons), 7.06 (1H, t, *J* = 7.7 Hz, H-6′), 6.63 (2H, br d, *J* = 7.7 Hz, H-5′, H-6′), 5.56 (1H, m, H-1′), 5.54 (1H, d, *J* = 7.0 Hz, C*H*NH), 5.12 (1H, dd, *J* = 12, 2.6 Hz, H-3′a), 5.05 (1H, dd, *J* = 12, 1.9 Hz, H-3′b), 3.71 (3H, s, OCH_3), 2.80 (1H, dd, *J* = 15, 3.6 Hz, H-2a), 2.67 (1H, dd, *J* = 15, 7.7 Hz, H-2b); EIMS *m*/*z* 342 $[M + H]^+$ (11), 341 $[M]^+$ (10), 282 (51), 166 (42), 164 (24), 148 (55), 135 (100), 121 (40), 118 (22), 106 (95), 104 (47), 91 (33), 77 (47).

Isoochracinol (5); 7-hydroxy-3-(2-hydroxyethyl)-1(3*H*)-isobenzofuranone: white solid (acetone); mp 105−107 °C; $[\alpha]_D^{22}$ +35° (*c* 1.7 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.18), 238 (3.79), 302 (3.62) nm; IR (film, NaCl) ν_{max} 3436, 3090, 2947, 1733, 1604, 1470, 1342, 1299, 1200, 1020 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.54 (1H, app. t, *J* = 7.8 Hz, H-5), 6.98 (1H, d, *J* = 7.8 Hz, H-4), 6.87 (1H, d, *J* = 7.8 Hz, H-6), 5.60 (1H, dd, *J* = 9.3, 3.5 Hz, H-3), 3.80 (1H, ddd, *J* = 11, 8.3, 5.7 Hz, H-2a′), 3.75 (1H, ddd, *J* = 11, 6.7, 4.7 Hz, H-2b′), 2.23 (1H, dddd, *J* = 15, 8.3, 6.7, 3.5 Hz, H-1a′), 1.83 (1H, dddd, *J* = 15, 9.3, 5.7, 4.7 Hz, H-1b′); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.7 (s, C-1), 158.4 (s, C-7), 153.7 (s, C-3a), 137.7 (d, C-5), 116.7 (d, C-4), 113.7 (d, C-6), 112.4 (s, C-7a), 79.7 (d, C-3), 59.0 (t, C-2′), 39.2 (t, C-1′); EIMS *m*/*z* 194 $[M]^+$ (29), 176 (93), 163 (16), 149 (100), 121 (82), 120 (63), 93 (57), 91 (41), 77 (27), 65 (70), 63 (34); HRESIMS (negative ion mode) *m*/*z* 193.0505 $[M - H]^-$, calcd for $C_{10}H_9O_4$, 193.0501.

7-Hydroxy-3-(2,3-dihydroxybutyl)-1(3*H*)-isobenzofuranone (6): white crystals (MeOH); mp 139−140 °C; $[\alpha]_D^{22}$ +67° (*c* 0.67 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.39), 237 (3.08), 302 (3.65) nm; IR (film, NaCl) ν_{max} 3535−2925, 2815,

2684, 1739, 1708, 1623, 1476, 1349, 1300 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.53 (1H, app. t, $J = 7.8$ Hz, H-5), 6.95 (1H, dd, $J = 7.8$ Hz, H-4), 6.86 (1H, dd, $J = 7.8$ Hz, H-6), 5.67 (1H, dd, $J = 11$, 2.5 Hz, H-3), 3.73 (1H, ddd, $J = 11$, 5.7, 2.2 Hz, H-2′), 3.61 (1H, dq, $J = 5.7$, 6.4 Hz, H-3′), 2.00 (1H, ddd, $J = 14$, 11, 2.5 Hz, H-1a′), 1.80 (1H, ddd, $J = 14$, 11, 2.2 Hz, H-1b′), 1.18 (3H, d, $J = 6.4$ Hz, H_3-4′); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.8 (s, C-1), 158.3 (s, C-7), 154.1 (s, C-3a), 137.6 (d, C-5), 116.6 (d, C-4), 113.6 (d, C-6), 112.4 (s, C-7a), 79.8 (d, C-3), 73.1 (d, C-2′), 71.9 (d, C-3′), 39.8 (t, C-1′), 18.9 (q, C-4′); HRESIMS *m/z* 261.0749 $[M + Na]^+$, calcd for $C_{12}H_{14}O_5Na$, 261.0739.

1-(1,3-Dihydro-4-hydroxy-1-isobenzofuranyl)butan-2,3-diol (7a): white solid (MeOH); mp 131−132 °C; $[\alpha]_D^{22}$ +33° (*c* 2.4 mg/mL, MeOH); IR (film, NaCl) ν_{max} 3615−3050, 2960, 2915, 2865, 1600, 1470, 1293, 1056, 993 cm^{-1}; ^{13}C NMR (CD_3OD, 100 MHz) δ 152.9 (s, C-4′), 145.7 (s, C-7a′), 130.1 (d, C-6′), 126.1 (s, C-3a′), 114.8 (d, C-5′), 113.0 (d, C-7′), 82.6 (d, C-1′), 73.5[a] (d, C-2), 72.0[a] (d, C-3), 71.3 (t, C-3′), 40.5 (t, C-1), 18.6 (q, C-4); [a]these assignments may be interchanged; ^1H NMR data as previously published.[13,14]

1-(1,3-Dihydro-4-hydroxy-1-isobenzofuranyl)butan-2,3-diol (7b): colorless oil; $[\alpha]_D^{22}$ −23° (*c* 1.7 mg/mL, MeOH); IR (film, NaCl) ν_{max} 3660−3015, 2971, 2921, 2860, 1598, 1468, 1370, 1294, 1053, 998 cm^{-1}; ^{13}C NMR (CD_3OD, 100 MHz) δ 152.9 (s, C-4′), 145.7 (s, C-7a′), 130.1 (d, C-6′), 126.1 (s, C-3a′), 114.8 (d, C-5′), 113.0 (d, C-7′), 82.6 (d, C-1′), 73.2[a] (d, C-2), 71.7[a] (d, C-3), 71.3 (t, C-3′), 40.8 (t, C-1), 18.9 (q, C-4); [a]these assignments may be interchanged; ^1H NMR data did not match either of the stereoisomers previously reported[13,14] and are included here: ^1H NMR (CD_3OD, 300 MHz) δ 7.14 (1H, dddd, $J = 7.8$, 7.6, 0.7, 0.7 Hz, H-6′), 6.71 (1H, br d, $J = 7.4$ Hz, H-5′), 6.68 (1H, ddd, $J = 8.0$, 0.7, 0.7 Hz, H-7′), 5.44 (1H, br d, $J = 9.8$ Hz, H-1′), 5.09 (1H, dd, $J = 12$, 2.4 Hz, H-3a′), 5.00 (1H, dd, $J = 12$, 1.1 Hz, H-3b′), 3.74 (1H, ddd, $J = 10$, 4.7, 2.3 Hz, H-2), 3.66 (1H, dq, $J = 6.3$, 4.7 Hz, H-3), 1.93 (1H, ddd, $J = 14$, 10, 2.4 Hz, H-1a), 1.73 (1H, ddd, $J = 14$, 10, 2.4 Hz, H-1b), 1.19 (1H, d, $J = 6.2$ Hz, H_3-4).

7-Hydroxy-1(3*H*)-isobenzofuranone (8): white solid (MeOH); mp 135−136 °C; EIMS *m/z* 150 $[M]^+$ (85), 149 (52), 122 (68), 121 (100), 94 (39), 93 (79), 65 (69); ^1H and ^{13}C NMR data have been published previously.[16]

Isoochracinic acid (9): white solid (MeOH); mp 160−161 °C; $[\alpha]_D^{22}$ −5° (*c* 0.7 mg/mL, MeOH); EIMS *m/z* 208 $[M]^+$ (30), 162 (100), 149 (68), 134 (25), 121 (53); ^1H NMR and UV data have been published previously.[9,10]

(+)-Cyclosordariolone (10): pale greenish-yellow solid (MeOH); mp 70−71 °C; $[\alpha]_D^{22}$ +168° (*c* 1.5 mg/mL, MeOH); IR (film, NaCl) ν_{max} 3356, 2971, 2922, 2851, 1667, 1566, 1461, 1395, 1359, 1282, 1161, 1130, 1001, 827 cm^{-1}; EIMS *m/z* 220 $[M]^+$ (28), 192 (100), 189 (32), 187 (49), 177 (39), 174 (81), 159 (83), 131 (75), 103 (56), 91 (24), 77 (44); UV, ^1H NMR, and ^{13}C NMR data for the (−)-enantiomer have been published previously.[14]

Altersolanol J (11); (2*S,3*R**,4a*S**,9a*S**,10*R**)-1,3,4,4a,-9a,10-hexahydro-2,3,5,10-tetrahydroxy-7-methoxy-2-methyl-9(2*H*)-anthracenone:** white crystals (MeOH); mp 240−242 °C (decomp); $[\alpha]_D^{22}$ −46° (*c* 0.7 mg/mL, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (4.3), 271 (3.8), 328 (3.4) nm; IR (film, NaCl) ν_{max} 3458, 3390, 3184, 2967, 2926, 2857, 1684, 1614, 1324, 1151, 1065, 1037, 848, 752 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 331.1153 $[M + Na]^+$, calcd for $C_{16}H_{20}O_6Na$, 331.1158.

Altersolanol A (12): yellow-orange solid (acetone); $[\alpha]_D^{22}$ −149° (*c* 1.2 mg/mL, EtOH); all other spectral data have been published previously.[21,22]

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