Pergamon

Tetrahedron 54 (1998) 1715–1724

TETRAHEDRON

New Cytotoxic Sesquiterpenoid Nitrobenzoyl Esters from a Marine Isolate of the Fungus *Aspergillus versicolor*

Gilbert N. Belofsky, Paul R. Jensen, Matthew K. Renner, and William Fenical*

Center for Marine Biotechnology and Biomedicine
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA, 92093-0236

Received 15 September 1997; revised 3 November 1997; accepted 10 November 1997

Abstract: Four new sesquiterpenoid nitrobenzoyl esters (**1-4**) have been isolated from organic extracts of the culture broth and mycelia of *Aspergillus versicolor*, a fungus isolated from the surface of the Caribbean green alga *Penicillus capitatus*. The structures of the four compounds were determined through extensive analysis of ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC data. 9α,14-Dihydroxy-6β-*p*-nitrobenzoylcinnamolide (**1**) displayed significant cytotoxicity against HCT-116 human colon carcinoma cells *in vitro* and exhibited moderately selective cytotoxicity toward a panel of renal tumor cell lines.

Marine microorganisms have recently gained prominence as an important new source of novel, biologically active secondary metabolites.[1-4] As judged by the publication record, most research to date in this field has targeted marine bacteria and in particular marine actinomycetes. Although marine bacteria will undoubtedly continue to be a productive source of new metabolites, recent studies of marine fungi indicate that they too are an excellent source of antimicrobial activity[5] and unusual secondary metabolites such as dendryphiellin A,[6] trichoharzin,[7] helicascolides A and B,[8] fumiquinazoles A-C,[9] and chloriolons A-C.[10]

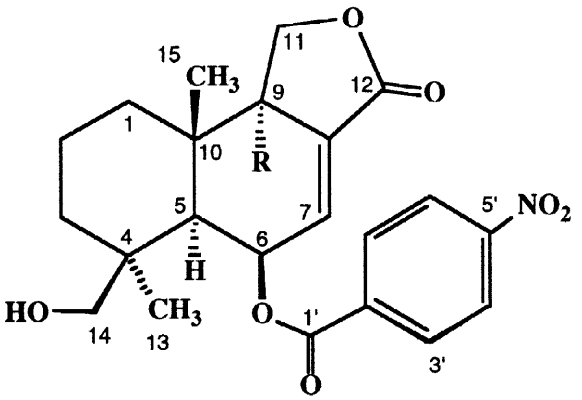
Among marine fungi, of particular promise as a source of novel secondary metabolites are those living in association with marine algae. These associations appear common based on the fact that as of 1971, nearly one third of all higher marine fungi described were so called algicolous or algal associated.[11] Algicolous fungi have recently yielded a number of interesting metabolites, including communesins A and B from a *Penicillium* sp.,[12] halymecins A-D from an *Acremonium* sp.,[13] penostatins A-D from a *Penicillium* sp.,[14] penochalasins A-C from a *Penicillium* sp.,[15] and the leptosins from a *Leptosphaeria* sp.[16] It is interesting to note that all of these fungi, with the arguable exception being *Leptosphaeria*, belong to genera that are best known from terrestrial environments. Although the ecology of these facultatively marine genera remains undescribed, there is mounting evidence that they play important roles in the sea, for example as pathogens of marine invertebrates.[17]

*E-mail: wfenical@ucsd.edu

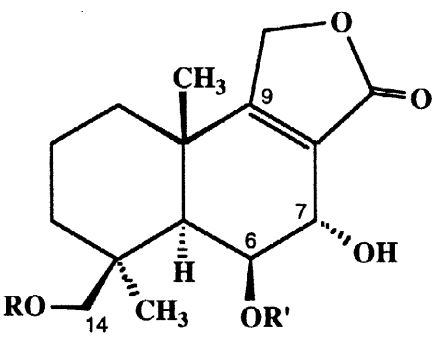
0040-4020/98/$19.00
PII: S0040-4020(97)10396-9

We report here the isolation, structure elucidation, and biological activity of four new sesquiterpenoid nitrobenzoyl esters produced by a marine strain of the fungus *Aspergillus versicolor* isolated from the surface of the Caribbean green alga *Penicillus capitatus.*

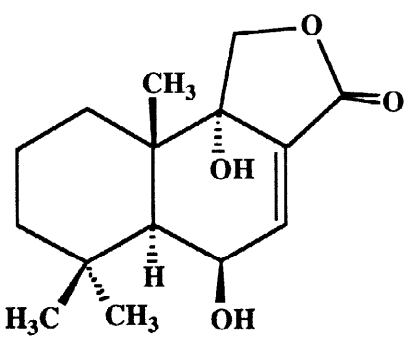
Compounds **1-4** were obtained from the organic extract of a 20 L *A. versicolor* seawater-based fermentation. The extract was subjected to cytotoxicity bioassay-guided fractionation employing, in sequence, chromatography on silica gel, Sephadex LH-20, and reversed-phase (C-18) HPLC leading to the isolation of four new drimane sesquiterpenoids related to lactones of the cinnamolide class.[18-20] Compound **1** was analyzed for the molecular formula $C_{22}H_{25}NO_8$ by mass spectral data [FABMS $(M + H)^+$ *m/z* = 432; HRFABMS $(M + H)^+$ *m/z* = 432.1551] and was responsible for essentially all of the HCT-116 colon carcinoma cell cytotoxicity



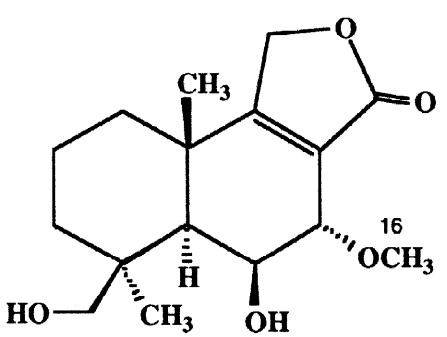
1, R = OH
3, R = H



2, R = H, R' = *p*-nitrobenzoyl
4, R = *p*-nitrobenzoyl, R' = H



5



6

present in the crude extract. The ^1H NMR spectrum of **1** contained two aliphatic methyl singlets, five signals between δ 3.57 and δ 6.10 assigned to protons on oxygenated carbons, one olefinic proton doublet at δ 6.72, and two distinctive 2H aromatic proton doublets characteristic of a *p*-disubstituted aromatic ring. Benzenoid UV absorption at 255 nm (ε 16900) confirmed the latter assignment. DEPT, HMBC, and HMQC NMR experiments permitted all proton and carbon assignments to be made (Tables 1 and 2). NMR data, overall, illustrated that **1** was related to the cinnamolide class of drimane lactones. Detailed comparison of the data for compound **1** with

those of the known compound pereniporin B (**5**)[21] supported this conclusion, but also revealed several significant differences from the known members of this class. First, the C-14 methyl group in **5** was found to be hydroxylated in **1**, as determined by the carbon signal at 65.0 ppm and the OH signal at δ 3.50 in the ^{1}H NMR spectrum. The hydroxyl group at C-6 in **5** is replaced by an unusual *p*-nitrobenzoyl ester group (see signals for C-1' - C-5', Table 2) which was confirmed by a long range HMBC correlation of H-6 with C-1'. The presence of the nitro moiety in **1** was further confirmed by IR absorptions characteristic for the NO_2 group (N=O stretching) at 1523 cm^{-1} and 1347 cm^{-1}. Key LREIMS fragments, corresponding to cleavage of the *p*-nitrobenzoyl ester, were observed at *m/z* 150 ($C_7H_4NO_3$ fragment) and at *m/z* 264 (M^+ - $C_7H_4NO_3$). These features accounted for the differences in elemental composition between **1** and **5**. The relative stereochemistry of compound **1** was assigned by analysis of NMR chemical shift and coupling constant values, and by NOESY NMR correlations. NOESY correlations between the 9α-OH proton and H-5, and between H-5 and H_3-13 helped to establish the orientation of these groups on the bottom face of the molecule. The ^{13}C NMR chemical shift value for C-9, at 77.5 ppm in **1**, was comparable to that for compound **5** (78.2 ppm in CD_3OD).[21] The remaining ^{1}H and ^{13}C NMR assignments were similar to those of **5** and other members of this class.[18, 19, 21, 22] Complete HMBC data for compounds **1-4** are included in the Experimental Section. On the basis of these data, the structure of compound **1** as shown, was confidently assigned as 9α,14-dihydroxy-6β-*p*-nitrobenzoylcinnamolide.[18-22]

Table 1. ^{1}H NMR Data for Compounds 1-3 in acetone-d_6.

Position	1	2	3
1α	2.25 (ddd; 13.5, 13.5, 2.5)	1.62 (m)	1.50 (ddd; 13.2, 13.2, 3.8)
1β	1.36 (m)	1.85 (m)	1.81 (m)
2α	1.50 (dt; 13.6, 3.4, 3.4)	1.64 (m)	1.46 (m)
2β	1.70 (m)	1.89 (br d; 13.8))	1.69 (m)
3α	0.96 (ddd; 13.2, 13.2, 2.5)	1.18 (m)	1.02 (ddd; 13.3, 13.3, 2.2)
3β	2.28 (ddd; 13.5,13.5, 4.1)	2.10 (br d; 13.3)	2.26 (br d; 13.5)
4	---	---	---
5	2.54 (d; 4.3)	2.16 (br s)	1.92 (d; 4.2)
6	6.10 (t; 4.3)	5.81 (br s)	6.15 (dd; 6.5, 4.1)
7	6.72 (d; 4.3)	4.38 (br s)	6.67 (t; 3.8)
8	---	---	---
9	---	---	3.04 (m)
10	---	---	---
11α	4.27 (d; 9.9)	4.91 (d; 17.5)	4.19 (t; 9.1)
11β	4.55 (d; 9.9)	5.02 (d; 17.5)	4.55 (t; 9.2)
12	---	---	---
13	1.10 (s)	1.17 (s)	1.12 (s)
14	3.57 (dd; 10.6, 4.4) 4.11 (dd; 10.5, 4.0)	3.56 (d; 10.5) 3.91 (d; 10.5)	3.55 (d; 10.8) 4.09 (d; 10.7)
15	1.38 (s)	1.72 (s)	1.27 (s)
1'	---	---	---
2'	---	---	---
3'/7'	8.28 (d; 8.8)	8.25 (d; 8.8)	8.29 (d; 8.9)
4'/6'	8.39 (d; 8.8)	8.36 (d; 8.8)	8.39 (d; 8.9)
5'	---	---	---
7-OH	---	4.96 (br s)	---
9-OH	4.95 (s)	---	---
14-OH	3.50 (br s)	3.51 (br s)	3.47 (br s)

Compound **2** was also analyzed for $C_{22}H_{25}NO_8$ and showed similar spectral features to those of **1** except for the replacement of the H-7 olefinic signal at δ 6.72 in **1**, with a broad singlet at δ 4.38 in **2.** These changes suggested that compound **2** differed significantly from **1** at C-7. The C-7 and C-8 signals in the ^{13}C NMR spectrum were shifted upfield to 63.7 ppm and 123.8 ppm, respectively, in **2** (as opposed to 132.2 ppm and 134.5 ppm, respectively, in **1**). Furthermore, the position of C-9 was shifted downfield to 174.0 ppm (from 77.5 ppm in **1**). These data, along with key HMBC correlations, such as H-1α and H_3-15 with C-9, and H-6 with C-8, are consistent with compound **2** being the isomeric allylic alcohol of **1** and having the structure shown. The ^1H and ^{13}C NMR results were also in agreement with literature values for known metabolites containing similar structural subunits.[21, 23] The relative stereochemistry of 7α,14-dihydroxy-6β-*p*-nitrobenzoylconfertifolin (**2**) is proposed on the basis of ^1H NMR *J*-values, NOESY data (see Figure 1), and by structural analogy to **1** and other known compounds.[21, 23]

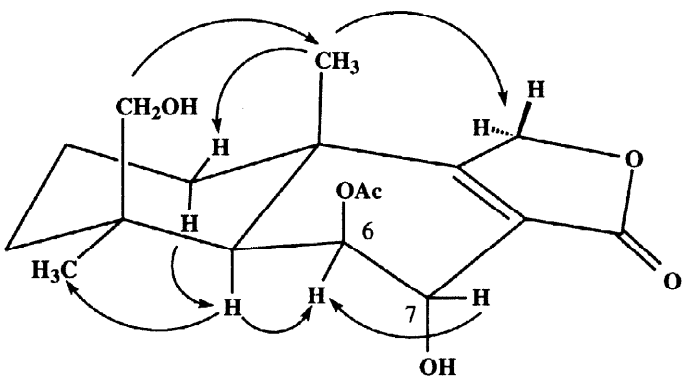


Figure 1. Key NOESY Correlations for 2.

Molecular models predict an approximately 80° dihedral angle between H-5 and H-6, and between H-6 and H-7 when the substituents at C-6 and C-7 are both pseudo-axial as shown in Figure 1. This observation is consistent with the lack of detectable coupling in the ^1H NMR spectrum between these two pairs of protons in compounds **2**,**4** and **5**. Only very weak coupling between H-5, H-6, and H-7 was detected in the COSY NMR spectrum.

Compound **3** has the formula $C_{22}H_{25}NO_7$ as determined by ^{13}C NMR and HRFABMS methods. The ^1H and ^{13}C NMR spectra of **3** were nearly identical to that of **1** except for the presence of an additional one-proton multiplet at δ 3.04, and the absence of the 9-OH signal (δ 4.95 in **1**) in **3**. These changes indicated that the 9-OH group in **1** is replaced by a proton in **3**. This was further confirmed by features of the ^{13}C NMR and DEPT spectra which revealed that C-9 (at 77.5 ppm in **1**) is characterized as a methine at 52.5 ppm in **3**. Analysis of the extensive NMR data for both **1** and **3** provided mutual support for the complete ^1H and ^{13}C NMR assignments for these compounds, since certain signals were better resolved in **3**, and others in **1**. Comparison of chemical shift values for the two compounds indicates that the 9-OH group in **1** causes a significant deshielding effect at H-1α and H-5, lending further support to the proposed relative stereochemistry as shown, with substituents at these three positions being on the α (less hindered) face of the molecule. The connectivity of 14-hydroxy-6β-*p*-nitrobenzoylcinnamolide (**3**) and all ^1H and ^{13}C NMR assignments were verified by

analysis of HMBC, HMQC, and COSY data.

Compound **4** analyzed for $C_{22}H_{25}NO_8$ by HRMS and combined spectral methods, the same molecular formula as **1** and **2**, yet the molecule was significantly more polar than either of these, showing only sparing solubility in MeOH. Extensive 2D NMR data in DMSO-d_6 (see Experimental Section) which showed long range HMBC correlations from H_2-14 to C-1', indicated that the *p*-nitrobenzoyl ester group in **4** is positioned at C-14. The increased polarity of 6β,7α–dihydroxy-14-*p*-nitrobenzoylconfertifolin (**4**) relative to **1-3** is explained by the presence of the diol functionality at C-6 - C-7.

Table 2. ^{13}C NMR Data for Compounds 1-3 in acetone-d_6.

C#	1(DEPT)	2(DEPT)	3(DEPT)
1	33.0 (CH_2)	39.1(CH_2)	41.8(CH_2)
2	18.3(CH_2)	19.1(CH_2)	18.7(CH_2)
3	36.8(CH_2)	37.0(CH_2)	37.0(CH_2)
4	40.0[a](C)	37.9(C)	40.3(C)
5	47.3(CH)	49.7(CH)	55.3(CH)
6	68.3(CH)	75.0(CH)	68.7(CH)
7	132.2(CH)	63.7(CH)	129.9(CH)
8	134.5(C)	123.3(C)	132.6(C)
9	77.5(C)	174.0[b](C)	52.5(CH)
10	40.1[a](C)	40.0(C)	35.6(C)
11	75.3(CH_2)	68.9(CH_2)	68.1(CH_2)
12	169.1(C)	174.0[b](C)	169.6(C)
13	27.4(CH_3)	27.8(CH_3)	27.1(CH_3)
14	65.0(CH_2)	65.3(CH_2)	65.0(CH_2)
15	21.7(CH_3)	24.0(CH_3)	17.1(CH_3)
1'	164.4(C)	165.3(C)	164.4(C)
2'	136.2(C)	137.5[c](C)	136.4(C)
3'/7'	131.9(CH)	131.7(CH)	131.9(CH)
4'/6'	124.6(CH)	124.5(CH)	124.7(CH)
5'	151.7(C)	152.5[c](C)	151.8(C)

[a,b] indicates that assignments for these carbon atoms may be interchanged. [c] indicates carbon shift assignments based on HMBC data.

Qualitative analysis of *A. versicolor* crude extracts by HPLC and TLC using pure compounds as standards indicated that compounds **2-4** were present in minor amounts. Determination of the yield of **1** in the crude extract was limited by the complexity of the mixture, but based upon the actual isolated amounts, compound **1** is produced at a concentration of 12 mg/L. The compounds **2-4** are minor metabolites produced in yields of approximately 2.0, 0.5 and 0.5 mg/L, respectively.

In the course of further studies of the chemistry of 9α,14-dihydroxy-6β-*p*-nitrobenzoylcinnamolide (**1**), it was observed that under mild basic conditions for ester hydrolysis (MeOH, K_2CO_3), **1** was converted to the product 6β,14-dihydroxy-7α-methoxyconfertifolin (**6**; yield ca. 65%) a new compound, plus methyl-*p*-nitrobenzoate. The structure and relative stereochemistry of **6** was deduced from ^1H and ^{13}C NMR data, LR and HRFABMS, and by analogy with **1-4** and other known compounds.[21, 23, 24] This result clearly indicates that **1** is susceptible to nucleophilic addition at C-7, leading to the elimination of the hydroxyl at C-9 to afford compound **6**. The resulting relative stereochemistry in **6** implies a preference for attack at C-7 on the less hindered (α) face of **1**. Based on this result it is likely that the addition proceeds through an S_N2' mechanism, however further studies would be necessary to rule out the possibility of addition-elimination via the resulting enol. These data lend further support for the proposed relative stereochemistry for **2** and **4**, and it follows that these two compounds may have originated via an analogous biosynthetic reaction mechanism.

The new lactone, 9α,14-dihydroxy-6β-*p*-nitrobenzoylcinnamolide (**1**) was found to be responsible for the majority of the antitumor activity present in the crude extract. Compound **1** was evaluated for antitumor activity in the National Cancer Institute's 60 cell-line panel[25] and showed a mean LC_{50} of 1.1 μg/mL. Compound **1** showed good cytotoxicity toward colon cancer cell lines HCC-2998, HCT-116, and CNS cancer cell line SNB-75, with LC_{50} values of 0.53, 0.44, and 0.44 μg/mL, respectively. This degree of cytotoxicity approximately mimics that of the antitumor agent etoposide (LC_{50} = 0.98 μg/mL vs. HCT-116). Of all cell lines tested, **1** showed the most potent activity toward the breast cancer cell line BT-549, with an LC_{50} = 0.27 μg/mL. Most notably, **1** showed selective toxicity against five renal cancer cell lines (786-0, ACHN, CAK-1, TK-10, and UO-31), with a mean LC_{50} of 0.51 mg/mL (range = 0.47 - 0.57 μg/mL). The cytotoxicity of sesquiterpene lactones, which have primarily been isolated from plants in the family Compositae, is believed to be explained on the basis of Michael addition of biological nucleophiles to the α,β-unsaturated lactone functionality.[26] This alkylation, presumably involving metabolically-essential biomolecules, ultimately results in cell death.[27] Although facile nucleophilic addition, perhaps involving a Michael type addition, to **1** has been clearly demonstrated (in the production of **6**), the selective toxicity of this compound to renal cancer cells is inconsistent with this simple model and may indicate a greater pharmacological potential than previously realized for members of this class of compounds.

EXPERIMENTAL

General. Preparative HPLC separations were accomplished using a 60A C_{18} column (8 μm particles, 10 mm × 25 cm) at 1.5 mL/min with RI detection. Gradient silica gel chromatography employed a two-chamber linear gradient apparatus coupled to a column packed with silica gel (60-200 mesh). TLC employed silica gel (0.25 mm) eluting with 9:1 CH_2Cl_2-MeOH. TLC spots were visualized by exposure to UV light at 254 nm or to a vanillin/H_2SO_4 (1% w/v) spray reagent. ^1H NMR data were obtained at 600 MHz while ^{13}C NMR data were obtained at 100 MHz. HMBC and HMQC data were obtained at 600 MHz (^1H dimension) and experiments were optimized for $^nJ_{CH}$ = 8 Hz and $^1J_{CH}$ = 150 Hz, respectively. Spectra were recorded in acetone-d_6 or DMSO-d_6 and ^1H and ^{13}C NMR chemical shifts were referenced using the corresponding solvent signals (e.g.,

δ 2.05 and 29.8 ppm for acetone-d_6). Optical rotations were recorded at 21° C in the solvents indicated. IR spectra were recorded as thin films on NaCl. Fast Atom Bombardment Mass Spectral data (FABMS) were obtained using an NBA matrix. The HCT-116 *in vitro* cytotoxicity bioassay is a standard 96-well microtiter plate assay using etoposide as a standard cytotoxin and crystal violet as the growth indicator.

Fungal Isolation, Fermentation, and Extraction. The producing strain of *Aspergillus versicolor* (CNC 327) was isolated from the surface of the green calcareous alga *Penicillus capitatus* collected using SCUBA from a patch reef at a depth of -25 m at Eleuthera Point, Bahamas Islands, in 1995. The fungus was grown in static liquid culture in 20 replicate 2.8 L Fernbach flasks each containing 1 L of a seawater-based medium comprised of 0.5% yeast extract, 0.5% peptone, 1.0% glucose, and 0.2% crab meal. Following a 21 day fermentation period, the mycelium and broth were separated by filtration and the broth extracted twice with equal volumes of EtOAc. The combined mycelial mats were freeze-dried and extracted twice with 4 L of 1:1 CH_2Cl_2:MeOH.

Isolation of Compounds 1-4. Both broth and mycelium extracts exhibited cytotoxicity against the human colon tumor cell line HCT-116 and therefore were combined (7.4 g). The combined extract was subjected to silica gel vacuum liquid chromatography (VLC)[28] over a prepacked column bed (10 × 2.5 cm). The column was eluted with a stepwise gradient of EtOAc (0-100%) in hexane, and a total of seven 500 mL or 1 L fractions were collected. Two fractions (40% and 60% EtOAc) were found to be cytotoxic and were combined (888 mg). To this combined material, ca. 5 mL of 3:1:1 hexane-toluene-MeOH was added in preparation for further separation by Sephadex LH-20 chromatography. At this point, there remained 310 mg of insoluble material which was subsequently triturated with ca. 5 mL of 3:1:0.5 hexane-toluene-MeOH to afford 284 mg of pure compound **1**. The soluble material (236 mg) was further fractionated by silica gel chromatography (2.5 × 12 cm) using a linear gradient of EtOAc (25-75%) in hexane. Fractions of similar composition as determined by TLC were pooled. The second fraction from this column (115 mg) was further purified by Sephadex LH-20 chromatography (2.5 × 33 cm) in 3:1:1 hexane-toluene-MeOH to yield 6.7 mg of compound **3**. The third fraction (63 mg) was also purified on Sephadex LH-20 under the same conditions to afford 14 mg of compound **2**. Fractions from the VLC experiment that eluted with 80% to 100% EtOAc were combined (1.1 g) and chromatographed on Sephadex LH-20 and silica gel using procedures similar to those described above. One resulting fraction (21 mg) was subjected to preparative reversed-phase HPLC (MeOH-H_2O) to yield compound **4** (2.6 mg), and an additional amount of compound **2** (25 mg).

9α,14-Dihydroxy-6β-*p*-nitrobenzoylcinnamolide (1): pale yellow solid; mp >193° (dec.); $[\alpha]_D$ -204° (c = 0.41, MeOH); UV (MeOH) 255 nm (ε 16900); IR (film on NaCl) 3420, 2944, 2866, 1754, 1723, 1527, 1345, 1268, 1243, 1097 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (H# => C#) H-1α => C-2, C-3, C-9, C-10, C-15; H-2α => C-1; H-3α => C-1, C-2, C-4, C-13, C-14; H-5 => C-3, C-4, C-6, C-9, C-10, C-13, C-14, C-15; H-6 => C-4, C-5, C-7, C-8, C-10, C-1'; H-7 => C-5, C-6, C-8, C-9, C-12; OH-9 => C-8, C-9, C-11; H-11α =>C-8, C-9, C-12; H-11β => C-9, C-10; H_3-13 => C-3, C-4, C-5, C-14; H_2-14 => C-3, C-4, C-13; OH-14 => C-4, C-14; H_3-15 => C-1, C-5, C-9, C-10; H-3' => C-1', C-5', C-7'; H-4' => C-2', C-5', C-6'; H-6' => C-2', C-4', C-5'; H-7' => C-1', C-3', C-5'. LRFABMS *m/z* = 432

[(M + H)$^+$; rel. int. 3.7], 359 (11), 265 (4.3), 247 (6.3), 234 (4.3), 199 (3.7), 165 (5.9); LREIMS *m/z* = 431 (M$^+$, rel. int. 0.1), 400 (0.9), 382 (3.6), 292 (6.6), 264 (3.3), 246 (7.5), 234 (9.1), 201 (9.2), 150 (100), 123 (35); HRFABMS (NBA), obsd. 432.1551 [(M + H)$^+$], calcd. for $C_{22}H_{25}NO_8$ + H$^+$, 432.1658.

7α,14-Dihydroxy-6β-*p*-nitrobenzoylconfertifolin (2): pale yellow solid; mp >200° (dec.); $[\alpha]_D$ -11° (c = 0.45, MeOH); UV (MeOH) 257 nm (ε 21100); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (H# => C#) H-1α => C-9; H-1β => C-2, C-3; H-2α => C-1; H-3α => C-2, C-13; H-3β => C-1, C-2; H-5 => C-3, C-4, C-6, C-9, C-13, C-14, C-15; H-6 => C-4, C-5, C-7, C-8, C-10, C-1'; H-7 => C-5, C-6, C8, C-9, C-12; H_2-11 => C-8, C-9, C-12; H_3-13 => C-3, C-4, C-5, C-14; H_2-14 => C-3, C-5, C-13; H-15 => C-1, C-5, C-9, C-10; H-3' => C-1', C-5', C-7'; H-4' => C-2', C-5', C-6'; H-6' => C-2', C-4', C5'; H-7' => C-1', C-3', C-5'. LRFABMS *m/z* = 432 [(M + H)$^+$; rel. int. 13], 414 (8.8), 391 (4.5), 369 (9.8), 341 (26), 313 (17), 267 (11), 257 (14), 239 (14), 176 (22); HRFABMS (NBA), obsd. 432.1661 [(M + H)$^+$], calcd. for $C_{22}H_{25}NO_8$ + H$^+$, 432.1658.

14-Hydroxy-6β-*p*-nitrobenzoylcinnamolide (3): pale yellow solid; mp 99-104°; $[\alpha]_D$ -76° (c = 0.54, MeOH); UV (MeOH) 256 nm (ε 8200); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations (H# => C#) H-1α => C-2, C-9, C-10, C-15; H-1β => C-5, C-10, C-15; H-2α => C-4, C-10; H-2β => C-1, C-3; H-3α => C-2, C-4, C-13, C-14; H-3β => C-1, C-2, C-4, C-5, C-14; H-5 => C-4, C-6, C9, C-10, C-13, C-14, C-15; H-6 => C-7, C-8, C-10, C-1'; H-7 => C-5, C-9, C-12; H-9 => C-1, C-7, C-8, C-10, C-11, C-15; H_2-11 => C-8, C-9, C-10, C-12; H-13 => C-3, C-4, C-5, C-14; H_2-14 => C-3, C-4, C-13; H_3-15 => C-1, C-5, C-9, C10; H-3' => C-1', C-5', C-7'; H-4' => C-2', C-5', C-6'; H-6' => C-2', C-4', C5'; H-7' => C-1', C-3', C-5'. LRFABMS *m/z* 415 (M$^-$; rel. int. 6.0), 385 (2.4), 342 (3.6), 325 (3.7), 255 (5.8), 244 (2.1), 222 (3.7), 188 (7.6), 166 (100); HRFABMS (NBA), obsd. 415.1646 (M$^-$), calcd. for $C_{22}H_{25}NO_7$, 415.1631.

6β,7α–Dihydroxy-14-*p*-nitrobenzoylconfertifolin (4): pale yellow solid; mp >230° (dec.); $[\alpha]_D$ +15° (c = 0.17, MeOH); UV (MeOH) 258 nm (ε 7600); ^{1}H NMR (DMSO-d_6): δ H-1α, 1.36 (ddd; 12.9, 12.9, 3.3); H-1β, 1.57 (br d; 12.3); H-2α, 1.52 (m); H-2β, 1.79 (m); H-3α, 1.10 (m); H-3β, 1.92 (br d; 13.7); H-5, 1.66 (br s); H-6, 4.16 (br s); OH-6, 5.02 (d; 3.8); H-7, 3.98 (d; 5.5); OH-7, 5.27 (d; 5.9); H_2-11, 4.97 (dd; 17.6, 1.6), 4.80 (d; 17.6); H_3-13, 1.12 (s); H_2-14, 4.83 (d; 11.4), 4.64 (d; 11.3); H_3-15, 1.46 (s); H-3'/H-7', 8.20 (d; 8.7); H-4'/H-6', 8.34 (d; 8.7); ^{13}C NMR (DMSO-d_6) C-1, 37.2; C-2, 17.8; C-3, 35.7; C-4, 37.5; C-5, 49.1; C-6, 70.3; C-7, 65.1; C-8, 122.4; C-9 [a], 172.9; C-10, 36.4; C-11 [b], 68.1; C-12 [a], 173.1; C-13, 27.3; C-14 [b], 68.2; C-15, 22.6; C-1', 164.4; C-2' [c], 135.5; C-3'/C-7', 130.6; C-4'/C-6', 124.0; C-5' [c], 150.5; ([a, b] indicates that assignments for these carbon atoms may be interchanged, [c] assignments for these carbon atoms based upon HMBC data); HMBC correlations (DMSO-d_6) (H# => C#) H-1α => C-15; H-1β => C-3, C-5; H-3α => C-2, C-5, C-14; H-3β => C-1, C-2, C-5; H-5 => C-1, C-6, C-9, C-13, C-14, C-15; H-6 => C-7, C-8, C-10; OH-6 => C-5; H-7 => C-5, C-6, C-8, C-9; OH-7 => C-7, C-8; H_2-11 => C-8, C-12; H_3-13 => C-3, C-4, C-5, C-14; H_2-14 => C-3, C-4, C-13, C-1'; H_3-15 => C-1, C-5, C-9, C-10; H-3' => C-1', C-5', C-7'; H-4' => C-2', C-5', C-6'; H-6' => C-2', C-4', C-5'; H-7' => C-1', C-3', C-5'; Electrospray MS *m/z* = 466 (M$^+$ + Cl$^-$; rel. int. 100), calcd. for $C_{22}H_{25}NO_8$ + Cl 466.1268.

Conversion of 9α,14-Dihydroxy-6β-*p*-nitrobenzoylcinnamolide (1) to 6β,14-Dihydroxy-7α-methoxyconfertifolin (6). Compound **1** (61 mg, 0.14 mmol) was dissolved in 1.5 mL of MeOH. To this solution was added 5 mg of K_2CO_3 (0.04 mmol) and the reaction was stirred for 1 h at RT. The mixture was concentrated to dryness and taken up in 2 mL MeOH, filtered, and the residue after evaporation separated by preparative reversed-phase (C-18) HPLC (isocratic elution, 55:45 MeOH-H_2O) to afford 27.8 mg of **6**.

6β,14-Dihydroxy-7α-methoxyconfertifolin (6): clear oil; ^{1}H NMR (CD_3OD): δ H-1α, 1.44 (ddd; 13.1, 13.1, 3.9); H-1β[a], 1.59 (m); H-2α[a], 1.56 (m); H-2β, 1.82 (m); H-3α, 1.28 (ddd; 13.7, 13.7, 4.5); H-3β, 1.69 (br d; 12.4); H-5, 1.62 (br s); H-6, 3.84 (t; 1.9); H-7, 4.36 (br s); H_2-11, 4.99 (dd; 17.7, 1.8), 4.89 (d; 17.9); H_3-13, 1.10 (s); H_2-14, 4.17 (d; 11.5), 3.33 (d; 11.9); H_3-15, 3.51 (s); H_3-16, 1.52 (s); ^{13}C NMR (CD_3OD) C-1[b], 39.7, C-2, 19.6, C-3, 38.5, C-4, 40.0, C-5, 51.5; C-6, 70.5; C-7, 75.4; C-8, 122.3; C-9[c], 176.2; C-10[b], 39.8; C-11, 68.1; C-12[c], 174.4; C-13, 28.7; C-14, 67.7; C-15, 58.7; C-16, 22.5; ([a-c] indicates that assignments for these atoms may be interchanged); LRCIMS (NH_3) *m/z* = 279 [$(M + H)^+$; rel. int. 100], 282 (10), 265 (25), 249 (11), 123 (11), 109 (12), 95 (11); HRCIMS (NH_3), obsd. 297.1708 [$(M + H)^+$], calcd. for $C_{16}H_{24}O_5 + H^+$ 297.1702.

ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health, National Cancer Institute, under grant CA44848. We appreciate the financial support provided by the NSF, Chemical Instrumentation Program, which allowed us to upgrade our NMR spectrometer. The support received from the University of California, Riverside, Mass Spectrometry Facility is gratefully acknowledged, as is Dr. David Porter (Department of Botany, University of Georgia) for fungal identification. We thank Dr. Kenneth M. Snader, NCI, Division of Cancer Treatment, for providing cell line testing data for compound **1**. The authors would like to especially thank Mr. Chris Kauffman and Ms. Orell Petillo for their technical assistance.

REFERENCES

1. Fenical, W. *Chem. Rev.* **1993**, *93*, 1673.
2. Bernan, V.S.; Greenstein, M.; Maiese, W.M. *Adv. Appl. Microbiol.*. **1997**, *43*, 57.
3. Okami, Y. *J. Mar. Biotechnol.* **1993**, *1*, 59.
4. Davidson, B.S. *Curr. Opin. Biotechnol.* **1995**, *6*, 284.
5. Cuomo, V., Palomba, I., Perritti, A., D'Ambrosio, M., Pietra, F. *J. Mar. Biotchnol.*. **1995**, *2*, 199.
6. Guerriero, A., D'Ambrosio, M., Cuomo, V., Vanzanella, F., Pietra, F. *Helv. Chim. Acta* **1988**, *71*, 57.
7. Kobayashi, M., Uehara, H., Matsunami, K., Aoki, S., Kitagawa, I. *Tetrahedron Lett.* **1993**, *34*, 7925.
8. Poch, G.K., Gloer, J.B. *J. Nat. Prod.* **1989**, *52*, 257.
9. Numata, A., Takahashi, C., Matsushita, T., Miyamoto, T., Kawai, K., Usami, Y., Matsunura, E., Inoue, M., Ohishi, H., Shingu, T. *Tetrahedron Lett.*. **1992**. *33*, 1622.
10. Cheng, X.-C., Varoglu, M., Abrell, L., Crews, P., Lobkovsky, E., Clardy, *J. Org. Chem.* **1994**, *59*,

6344.

11. Kohlmeyer, J.; Kohlmeyer, E. In *Marine Mycology, The Higher Fungi.* Academic Press: New York, 1979, pp. 54-69.
12. Numata, A., Takahashi, C., Ito, Y., Takada, T., Kawai, K., Usami, Y., Matsumura, E., Imachi, M., Ito, T., Hasegawa, T. *Tetrahedron Lett.*. **1993**, *34*, 2355.
13. Chen, C., Imamura N., Nishijima, M., Adachi, K., Sakai, M., Sano, H. *J. Antibiot.* **1996**, *49*, 998.
14. Takahashi, C., Numata, A., Yamada, T., Minoura, K., Enomoto, S., Konishi, K., Nakai, M., Matsuda, C., Nomoto, K. *Tetrahedron Lett.* **1996**, *37*, 655.
15. Numata, A., Takahashi, C., Ito, Y., Minoura, K., Yamada, T., Matsuda, C., Nomoto, K. *J. Chem. Soc. Perkin Trans. I* **1996**, *3*, 239.
16. Takahashi, C., Numata, A., Ito, T., Matsunura, E., Araki, H., Iwaki, H., Kushida, K. *J. Antibiot.*. **1994**, *47*, 1242.
17. Smith, G., Ives, L.D., Nagelkerken, I.A., Ritchie, K.B. *Nature* **1996**, *383*, 487.
18. Canonica, L.; Corbella, A.; Jommi, G.; Krepinsky, J.; Ferrari, G.; Casagrande, C. *Tetrahedron Lett.* **1967**, *23*, 2137.
19. Canonica, L.; Corbella, A.; Gariboldi, P.; Jommi, G.; Krepinsky, J.; Ferrati, G.; Casagrande, C. *Tetrahedron* **1969**, *25*, 3895.
20. During the course of this work, we became aware that compound **1** had also been isolated from a marine strain of the fungus *Aspergillus insulicola.* Unfortunately, this compound had already been called insulicolide A. see: Rahbaek, L., Christophersen, C., Frisvad, J., Abstract # P111, Annual Meeting of the American Society of Pharmacognosy, Iowa City, IA, July, 1997. In the final preparation of this manuscript this paper appeared, see: Rahbaek, L., Christophersen, C., Frisvad, J., Bengaard, H.S., Larsen, S., Rassing, B.R. *J. Nat. Prod.* **1997**, *60*, 811.
21. Kida, T.; Shibai, H.; Seto, H. *J. Antibiot.* **1986**, *39*, 613.
22. Ayer, W.A.; Trifonov, L.S. *J. Nat. Prod.* **1992**, *55*, 1454.
23. Gould, R.O.; Simpson, T.J.; Walkinshaw, M.D. *Tetrahedron Lett.* **1981**, *22*, 1047.
24. Hashimoto, T.; Tori, M.; Asakawa, Y. *Phytochemistry* **1989**, *28*, 3377.
25. Boyd, M.R.; Paull, K.D. *Drug Development Res.* **1995**, *34*, 91.
26. Beekman, A.C.; Woerdenbag, H.J.; van Uden, W.; Pras, N.; Konings, A.W.T.; Wikstrom, H.V.; Schmidt, T.J. *J. Nat. Prod.* **1997**, *60*, 252.
27. Cassady, J.M.; Suffness, M. In *Anticancer Agents Based on Natural Products Models.* Cassady, J.M. and Doures, J.D., Eds.; Academic Press: New York, 1980, pp. 201-269.
28. Coll, J.C., Bowden, B.F. *J. Nat. Prod.* **1986**, *49*, 934.