Terretonins E and F, Inhibitors of the Mitochondrial Respiratory Chain from the Marine-Derived Fungus *Aspergillus insuetus*[#]

M. Pilar López-Gresa,*[,†] Nuria Cabedo,[‡] M. Carmen González-Mas,[§] Maria Letizia Ciavatta,[⊥] Conxita Avila,[∥] and Jaime Primo[‡]

Instituto de Biología Molecular y Celular de Plantas (IBMCP), CSIC-UPV, Ciudad Politécnica de la Innovación, Edificio 8E, Laboratory 3.02, 46022 Valencia, Spain, Centro de Ecología Química Agrícola (CEQA), UPV, Campus de Vera, Edificio 6C, 46022 Valencia, Spain, Centro de Citricultura, Instituto Valenciano de Investigaciones Agrarias (IVIA)-Fundación Agroalimed, Carretera Náquera-Moncada, Km 4.5, 46113 Moncada, Valencia, Spain, Istituto di Chimica Biomolecolare (ICB), CNR, 80078 Pozzuoli, Italy, and Departament de Biologia Animal (Invertebrats), Facultat de Biologia, Universitat de Barcelona, Avenida Diagonal 645, 08028 Barcelona, Spain

Received February 16, 2009

Two new meroterpenoids, terretonins E and F (**1**, **2**), together with three known compounds, aurantiamine (**3**), linoleic acid, and uridine, were isolated as fermentation products of the marine-derived fungus *Aspergillus insuetus*, which was associated with the sponge *Petrosia ficiformis.* Structures of all isolates were elucidated employing spectroscopic methods, mainly by two-dimensional NMR techniques. Compounds **1**−**3** showed activity as inhibitors of the mammalian mitochondrial respiratory chain.

In the search for novel and bioactive molecules, terrestrial fungi have yielded many biologically active compounds. More recently, marine fungi have become an important research area of natural products with significant value due to the diversity in chemical structures and biological activities.[1,2] However, compared with other marine organisms, relatively few investigations of the secondary metabolites from marine fungi have been reported.[3] Fungi isolated from various organisms in the marine environment, e.g., from mangroves,[4] algae,[5] mollusks,[6] and particularly sponges,[7] have been examined for their secondary metabolite content.

As part of our research into marine fungi, we report results regarding the secondary metabolite chemistry of *Aspergillus insuetus*, isolated from a specimen of the Mediterranean sponge *Petrosia ficiformis*. This study led to the isolation of two new compounds, **1** and **2**, together with three known compounds previously isolated from other fungal sources[8−10] (Figure 1). The two new compounds are structurally related to the known mycotoxin terretonin[11] and terretonins A−D[12] isolated from *A. terreus*. Here, the isolation, structure elucidation, and biological activity of these fungal metabolites, named terretonin E (**1**) and terretonin F (**2**), are described.

Chemical studies of the organic extract obtained from the culture broth of *A. insuetus* afforded two new meroterpenoids, terretonin E (**1**) and terretonin F (**2**), along with the known compound aurantiamine (**3**). Linoleic acid and uridine were also isolated from the dichloromethane and methanol mycelium extracts of *A. insuetus,* respectively. The previously known compounds were identified by comparing their spectroscopic and physical data (^{1}H and ^{13}C NMR, MS, and $[\alpha]_D$) with literature values.[8,13,14] The new molecules, **1** and **2**, were characterized as described below (Table 1).

The molecular formula of terretonin E (**1**) was $C_{26}H_{32}O_9$, as deduced from the sodiated molecular ion peak at *m/z* 511.1954 in its HR-ESIMS spectrum, indicating 11 degrees of unsaturation. The ^{13}C NMR spectrum of **1** contained resonances for nine sp^2 carbons, four of them at δ 113.1 (C-22), 132.5 (C-5), 142.1 (C-12), and 143.4 (C-6) belonging to two double bonds. The more deshielded signals at δ 212.6 (C-3) and 204.0 (C-17) were ascribed to two ketone groups, and the signal at δ 195.2 (C-7) was assigned to an α,β-unsatured ketone. The other two sp^2 carbon resonances at δ 170.0 (C-15) and 167.1 (C-25) indicated the presence of two ester carbonyls. The remaining unsaturations required by the molecular formula of **1** were thus attributed to four rings. Signals at δ 78.9 and 69.7 ppm were assigned to sp^3 carbons linked to oxygen. Thus, initial analysis of NMR spectroscopic data of **1** illustrated structural features that were reminiscent of the terretonins; however, only two of the three typical methylene resonances present in all of the known structures of the terretonin family were observed in the DEPT spectrum of **1**. The ^{1}H NMR spectrum of **1** showed the presence of an exomethylene group (δ 5.04, H-22a and δ 4.89, H-22b), three methines (δ 4.78, H-16, δ 4.04, H-1, and δ 2.75, H-9), a methoxy group (δ 3.82, H$_3$-1′), two methylenes (δ 2.84,

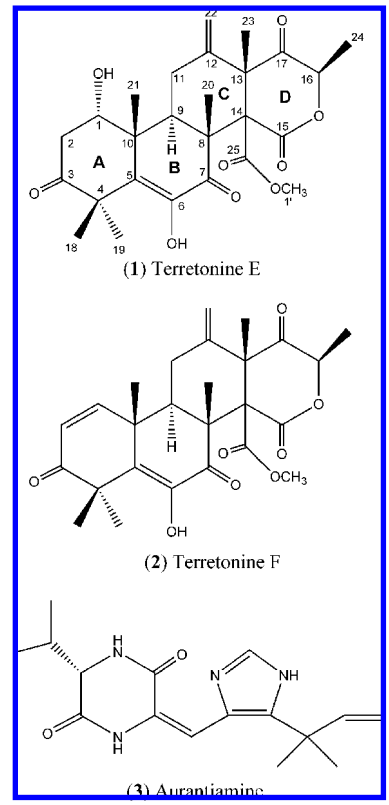

Figure 1. Metabolites (**1**−**3**) isolated from *Aspergillus insuetus*.

[#] This paper is dedicated to Prof. E. Primo Yúfera *in memoriam.*

* To whom correspondence should be addressed. Tel: +34963879058. Fax: +34963879059. E-mail: mplopez@ceqa.upv.es.
[†] Instituto Biología Molecular y Celular de Plantas, CSIC-UPV.
[‡] Centro de Ecología Química Agrícola, UPV.
[§] Centro de Citricultura, IVIA.
[⊥] Istituto per la Chimica Biomolecolare, CNR.
[∥] Facultat Biologia, UB.

10.1021/np900085n CCC: $40.75
Published on Web 04/24/2009

Table 1. NMR Spectroscopic Data of Terretonins E (**1**) and F (**2**)

	terretonin E (**1**)			terretonin F (**2**)		
position	δ_C[a], mult.[b]	δ_H[c] (*J* in Hz)	HMBC[d]	δ_C[a], mult.[b]	δ_H[c] (*J* in Hz)	HMBC[d]
1	69.7, CH	4.04*eq*, d (5.7)	3, 5	150.6, CH	6.80, d (10.2)	3, 5, 6, 9, 10
2	43.3, CH_2	2.84*ax*, dd (19.8, 5.7)	3, 9	127.4, CH	6.11, d (10.2)	4, 10
		2.70*eq*, m	1, 3, 10			
3	212.6, qC			204.1, qC		
4	47.9, qC			48.0, qC		
5	132.5, qC			133.2, qC		
6	143.4, qC			140.0, qC		
7	195.2, qC			194.5, qC		
8	48.8, qC			49.3, qC		
9	38.8, CH	2.75, m	10, 20, 21	44.1, CH	2.18, dd (13.8, 3.0)	1, 8, 10, 20, 21
10	43.1, qC			39.1, qC		
11	27.8, CH_2	2.55*ax*, t (13.8)	8, 9, 12, 22	28.0, CH_2	2.66*ax*, t (13.8)	9, 12, 22
		2.30*eq*, dd (13.8, 3.0)	8, 9, 12, 13, 22		2.45*eq*, dd (13.8, 3.0)	8, 9, 12, 13, 22
12	142.1, qC			143.0, qC		
13	54.8, qC			54.8, qC		
14	65.9, qC			65.6, qC		
15	170.0, qC			169.9, qC		
16	78.9, CH	4.78, q (6.6)		78.8, CH	4.78, q (6.7)	24
17	204.0, qC			202.9, qC		
18	21.3, CH_3	1.48, s	3, 4, 5, 19	20.9, CH_3	1.54, s	3, 4, 5, 19
19	24.3, CH_3	1.62, s	3, 4, 5, 18	26.4, CH_3	1.58, s	3, 4, 5, 18
20	19.4, CH_3	1.80, s	7, 8, 9, 14	18.9, CH_3	1.80, s	7, 8, 9, 14
21	16.7, CH_3	1.02, s	1, 2, 5, 9, 10	25.7, CH_3	1.27, s	1, 5, 9, 10
22	113.1, CH_2	5.04, 4.89, brs	11, 13	113.3, CH_2	5.10, 4.93, brs	11, 13, 23
23	21.7, CH_3	1.86, s	12, 13, 14, 15	21.8, CH_3	1.86, s	12, 13, 14, 15
24	17.8, CH_3	1.58, d (6.6)	16, 17	17.8, CH_3	1.55, d (6.7)	16, 17
25	167.1, qC			166.9, qC		
1′	53.2, CH_3	3.82, s	25	53.3, CH_3	3.82, s	25
OH-6		6.72, s	5, 6, 7		6.68, s	5, 6, 7

[a] Bruker 75 MHz, $CDCl_3$, values are reported in ppm referenced to $\underline{C}DCl_3$ (δ ^{13}C 77.0). [b] Multiplicity deduced by DEPT. [c] Bruker 600 MHz, $CDCl_3$, values are reported in ppm referenced to $C\underline{H}Cl_3$ (δ ^{1}H 7.26). [d] HMBC correlations are from proton(s) stated to the indicated carbon.

H-2*ax*, δ 2.70, H-2*eq*, and δ 2.55, H-11*ax*, δ 2.30, H-11*eq*), five singlet methyls (δ 1.86, H_3-23, δ 1.02, H_3-21, δ 1.62, H_3-19, δ 1.48, H_3-18, and δ 1.80, H_3-20), and one doublet methyl (δ 1.58, H_3-24). Analysis of the ^{1}H−^{1}H COSY spectrum of **1** showed correlations between the doublet methyl (H_3-24, J = 6.6 Hz) and the more deshielded methine (δ 4.78, H-16, q, J = 6.6 Hz), indicating that this position was substituted the same as in terretonin C.[12] The signal of the oxygenated methine at δ 4.04 (H-1, d, J = 5.7 Hz) had cross-peaks in the ^{1}H−^{1}H-COSY spectrum with both protons at δ 2.84 (H-2*ax*, dd, J = 19.8, 5.7 Hz) and 2.70 (H-2*eq*, m) attributable to a methylene near a carbonyl group, showing another important spin system; in addition, a long-range correlation in the HBMC experiments of C-3 (δ 212.6) and C-5 (δ 132.5) with the proton at δ 4.04 positioned the hydroxy group at C-1. The presence of the carboxylic methyl ester at position C-14 (δ 65.9) in terretonin E (**1**) was determined according to spectroscopic data of other fungal meroterpenoids such as citreohybridones[15−18] and andrastins[19] (Figure 2).

The relative configuration of terretonin E (**1**) was determined by the analysis of ^{1}H−^{1}H coupling constants and NOE difference experiments. The axial orientation of the hydroxy group at C-1 was suggested by both the coupling constant of the proton H-1 (δ 4.04, d, J = 5.7 Hz), in agreement with an equatorial orientation of H-1, and the downfield chemical shift of H-9 at δ 2.75 relative to the same proton in terretonin A (δ 1.55).[12] In addition, the significant NOE effects between H-2*eq* (m, δ 2.70) and H-1 supported the equatorial orientation of H-1. Irradiation of methyl H_3-21 (δ 1.02) caused the enhancement of H_3-18 (δ 1.48), H_3-20 (δ 1.80), H-11*ax* (δ 2.55), H-2*ax* (δ 2.84), and H-1 (δ 4.04) signals, indicating that all of these protons were oriented on the same side of the molecule. Irradiation of methyl H_3-23 (δ 1.86) produced only a cross-peak with H-11*ax*, suggesting that this methyl was oriented to the same (β) side. The lack of NOE correlations for the carbomethoxy group prevented assignment of the configuration at C-14. Finally, the configuration of C-16 was proposed on the basis of the close similarity of the ^{1}H and ^{13}C NMR chemical shifts of C-16, H-16, C-24, and H_3-24 with terretonin C,[12] and no NOE effect was observed between H-16 (δ 4.78) and H_3-23 (δ 1.86). Our attempt to establish the absolute configuration of the secondary alcohol (OH-1) in terretonin E by derivatization with the modified Mosher's method[20,21] failed, probably due to the steric hindrance near the axial hydroxy group.

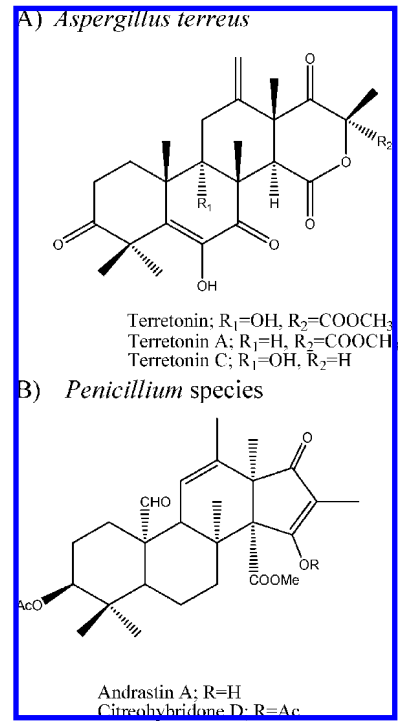

Figure 2. Meroterpenoids isolated from fungi.

The structure of terretonin F (**2**) was established by comparison of its NMR and MS spectra with those of **1**. HR-ESIMS (493.1851

$[M + Na]^+$) for compound **2** rendered $C_{26}H_{30}O_8$ as its molecular formula, which provides a molecular mass 18 units less than that for **1**. Comparison of all spectroscopic data of compound **2** with those derived from **1** showed that both structures were very similar, with differences only noticeable in the lower field region. Compound **2** showed the doublet methyl group on C-16 and the methyl ester on C-14, identical to compound **1**. Compound **2** showed, however, the presence of an additional double bond on the basis of the signals at δ 150.6 and 127.4 in its ^{13}C NMR spectrum, corresponding to sp^2 carbons C-1 and C-2, respectively. The ^1H NMR spectrum of **2** also showed two new signals at δ 6.80 (H-1) and 6.11 (H-2), instead of the signal at δ 4.04 (H-1), which were attributed to the olefinic protons of a disubstituted double bond. These protons were located at positions 1 and 2, according to HMBC spectral data: δ 6.80 (H-1) showed correlations with δ 204.1 (C-3), 133.2 (C-5), 140.0 (C-6), 44.1 (C-9), and 39.1 (C-10), and δ 6.11 (H-2) correlated with δ 48.0 (C-4). Dehydration of compound **1** could explain the formation of compound **2**. On the basis of the high degree of identity of ^{13}C NMR chemical shifts and proton coupling constants of **2** with those of **1**, and also considering the possibility that **2** is biogenetically derived from compound **1**, we suggested for compound **2** the same relative configuration as for **1**.

Compounds **1** and **2** are new members of the terretonin group, mycotoxins[11,22] that are structurally related to some known mitochondrial electron-transfer chain inhibitors such as the benzopyran meroterpenoid polyalthidin[23] or the tigliane diterpene 4,20-dideoxyphorbol-12,13-bis(isobutyrate).[24] In this study, we have assayed the natural isolates (**1**−**3**, uridine) as respiratory chain inhibitors by measuring the NADH oxidase activity in beef heart submitochondrial particles (SPM).[25]

Compounds **1** and **2** were found to be inhibitors of the integrated chain (NADH oxidase activity) with IC_{50} values of 3.90 ± 0.4 and 2.97 ± 1.2 μM, respectively. The ability of these compounds to inhibit the mitochondrial respiratory chain could explain, at least partly, the toxicity attributed to terretonins. Compound **2** was slightly more active than **1**, but not sufficiently to establish preliminary structure−activity relationships. Aurantiamine (**3**) and uridine, which are close structural analogues of the mitochondrial respiratory chain inhibitor flavacol,[26] were also tested at this level. Compound **3** was 5 times less potent than **2** with an IC_{50} value of 15.1 ± 1.8 μM, whereas the nucleoside uridine did not show inhibitor activity at all. Linoleic acid was previously evaluated by Tormo and co-workers[27] as an inhibitor of the mammalian mitochondrial complex I.

Taking into account these results, **1** and **2** showed midrange potency with respect to the most potent respiratory inhibitors, such as rotenone (IC_{50} of 5.1 nM),[24] and some *Annonaceous* acetogenins, as laherradurin (IC_{50} of 0.18 nM).[28,29] The IC_{50} values of **1** and **2** are in the same range as polyalthidin (IC_{50} of 4.4 μM), the most potent known meroterpenoid.[23] The knowledge of new inhibitors of the mammalian mitochondrial respiratory chain is very useful for basic biomedical research of this electron chain. That is interesting because many degenerative diseases such as Parkinson's and Huntington's diseases are associated with alterations in the functioning of this complex.[30,31] Finally, it is important to note that compounds **1** and **2**, like other mitochondrial inhibitors, may serve as leads for the development of new insecticides based on this mechanism of action.[32,33] In conclusion, two new inhibitors of the mammalian mitochondrial respiratory chain have been isolated from the marine-derived fungus *Aspergillus insuetus*. Previously this type of meroterpenoid had been found exclusively in *A. terreus*.[11,12]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. UV spectra were obtained using a Shimadzu UV-2101PC spectrophotometer. IR spectra were recorded on a Biorad FTS 155 FT-IR spectrophotometer. ^1H, ^{13}C, and $^1H-^1H$ COSY NMR spectra were recorded on a Bruker AV 300 MHz instrument. Multiplicities of ^{13}C signals were assigned by DEPT experiments. For HSQC, HMBC NMR experiments and NOEDIFF irradiations a Bruker 600 MHz spectrometer was used. High-resolution ESIMS (electrospray) data were obtained on a Micromass Q-TOF Micro coupled with a HPLC Waters Alliance 2695. The instrument was calibrated using a PEG mixture from 200 to 1000 MW (resolution specification 5000 fwhm, deviation <5 ppm rms in the presence of a known lock mass). Silica gel chromatography was performed using precoated Merck F_{254} plates and Merck Kieselgel 60 powder. Isolation and purification were carried out by a Waters HPLC system with a 600 pump and both a 2996 photodiode array detector (PDA) and ELSD 2420 detector.

Fungal Material. The fungal isolate was recovered from the surface of the sponge *Petrosia ficiformis* collected in Punta de Santa Ana in the Mediterranean Sea (Blanes, Spain), during June 2006 and was classified by "Colección Española de Cultivo Tipo (CECT)", of the Universitat de València as *Aspergillus insuetus*. For the molecular analysis, two genomic regions, the internal transcribed spacer (ITS) region and β-tubulin genes (GenBank accession number EU076356.1 and EU076371.1, respectively), were amplified and sequenced as previously described.[34]

A sample of the strain is deposited in the international culture collection "Colección Española Cultivo Tipo" of the Universitat de València, coded as CECT 20745, and in the "Centro de Ecología Química Agrícola" of the Universidad Politécnica de Valencia, coded as HM9 and kept in agar slants with potato dextrose agar (PDA) as culture medium.

The strain was seeded in Petri dishes with PDA culture medium in seawater and incubated for 7 days at 28 °C. Then, a solution of Tween 80 (0.05%) in sterile distilled water was used to obtain a suspension containing ca. 10^6 conidia/mL. This suspension was poured into an Erlenmeyer flask containing antibiotic test broth (1:9 v/v). The mixture was incubated for 22 days, in the dark, at 25 °C in a total volume of 5 L.

Extraction and Isolation. After incubation the mycelium was removed from the culture broth by filtration. Both the broth and the mycelium were studied. First, the broth (5 L) was partially evaporated under vacuum to 1 L, and it was extracted with CH_2Cl_2/EtOAc, 1:1 (3 × 1 L). The resulting organic extract was dried under reduced pressure to obtain a brown solid (0.5 g), which was partitioned by column chromatography on silica gel using stepwise gradient elution from 70% hexane in ethyl acetate, to 100% ethyl acetate, to 100% methanol. The volume eluted in each step was 0.5 L, and 10 fractions were obtained and evaporated to dryness. Moreover, the dried mycelium (12 g) was first extracted with CH_2Cl_2 (3 × 1 L) to give 1 g of dichloromethane extract and then with CH_3OH (3 × 1 L) to obtain 11 g of methanol extract, where the compounds linoleic acid and uridine were isolated, respectively.

Purification of the resulting fractions of the organic culture broth allowed us to obtain the pure compounds **1**−**3** as follows. The fraction F-3 (47 mg), eluted with hexane/EtOAc (8:2), was subjected to flash chromatography on silica gel (1:100, w/w) using as mobile phase hexane/EtOAc (8:2). Aliquots of 3 mL were collected and pooled in eight fractions according to their similarity by TLC. Semipreparative HPLC of subfraction SF3-4 (6.9 mg) was performed using a Tracer Excel 120 ODS-B C18 column, 5 μm (25.0 × 1 cm), and CH_3CN/H_2O (1:1) as mobile phase with a flow of 2 mL/min. From this subfraction the less polar compound terretonin F (**2**, 1.2 mg) was isolated with a retention time (t_R) of 32.52 min. Fraction F-5 (14 mg), eluted also with hexane/EtOAc (8:2), was directly subjected to semipreparative HPLC, employing the above reversed-phase column and a gradient from 100% H_2O to 100% CH_3CN in 20 min, to give terretonin E (**1**, 5.2 mg) with a t_R of 24.74 min. Finally, fraction F-7 (21.5 mg), eluted with 100% EtOAc, was purified by a silica gel column (CH_2Cl_2/CH_3OH, 97:3) to afford aurantiamine (**3**, 5.4 mg) in the subfraction SF-7-2.

The ^1H NMR spectrum of the dichloromethane extract of the mycelium showed linoleic acid (1 g) as the major compound. To obtain the most polar compound, 1.2 g of the methanol extract of the mycelium was submitted to a Sephadex LH-20 column (CH_2Cl_2/CH_3OH, 1:2) to give 13 fractions. Fraction 10 (210 mg) was submitted to further purification on a silica gel column (CH_2Cl_2/CH_3OH, 9:1). The subfraction SF10-3 (20.4 mg) was subjected to HPLC purification with

H_2O/CH_3OH 30% gradient in 30 min, affording uridine (3.1 mg) with a t_R of 21.62 min.

Terretonin E (1): white powder; $[\alpha]_D^{25}$ −31 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 276 (2.60) nm; IR (film) ν_{max} 3517, 3402 (br), 2929, 1754, 1729, 1719, 1665, 1635, 1440, 1391, 1361, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 1; HRESIMS *m/z* 511.1954 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_9Na$, 511.1944).

Terretonin F (2): white powder; $[\alpha]_D^{25}$ −19 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (2.73), 276 (2.75) nm; IR (film) ν_{max} 3397 (br), 2924, 1754, 1734, 1719, 1669, 1630, 1445, 1386, 1356, 1226 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 1; HRESIMS *m/z* 493.1851 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_8Na$, 493.1838).

Biological Assays. NADH and other biochemical reagents were purchased from Sigma-Aldrich Chemical Co. Stock solutions (30 mM in absolute EtOH) of **1**−**3** and uridine were prepared and kept in the dark at −20 °C. Inverted submitochondrial particles (SMP) from beef heart were obtained following Fato's method[35] by extensive ultrasonic disruption of frozen−thawed mitochondria to produce open membrane fragments where permeability barriers to substrates were lost, and they were stored at −70 °C at 28 mg/mL (protein measured by the biuret method). The beef heart SMP were transferred in glass test tubes, diluted to 0.5 mg/mL in 250 mM sucrose and 10 mM Tris-HCl buffer, pH 7.4, and treated with 300 μM NADH to activate complex I before starting experiments. Aliquots of the stocks solutions (1 μL) were added successively to 500 μL of the SMP preparations with 5 min of incubation on ice after each addition (ethanol never exceeded 2% of the total volume). After each incubation, an aliquot of these incubated SMP (25 μL) was diluted to 6 μg/mL in 50 mM potassium phosphate buffer (pH 7.4) and 1 mM EDTA, in a cuvette at 22 °C, always in the presence of 75 μM NADH. Immediately, NADH oxidase activity was measured as the aerobic oxidation of NADH. Reaction rates were calculated for each inhibitor (at increasing concentrations) from the linear decrease of NADH concentration ($\lambda = 340$ nm, $\varepsilon = 6.22$ mM^{-1} cm^{-1}) measured in an end-window photomultiplier spectrophotometer ATI-Unicam UV4-500. The inhibitory concentration (IC_{50}) was taken as the final compound concentration in the assay cuvette that yielded 50% inhibition of the NADH oxidase activity. Data from individual titrations were used to assess the means and standard deviations of three assays for each compound.

Acknowledgment. We acknowledge ICB and ITQ NMR Service (D. Melck and E. Mateos) and E. Estornell and V. Romero for biological assay support. We would like to thank the R&D&I Linguistic Assistance Office, Universidad Politécnica de Valencia (Spain), for granting financial support for the linguistic revision of this paper. This work has been supported through European Social Fund (ESF).

Supporting Information Available: 1D and 2D NMR spectra for terretonins E and F. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Abdel-Wahab, M. A.; Asolkar, R. N.; Inderbitzin, P.; Fenical, W. *Phytochemistry* **2007**, *68*, 1212–1218.
(2) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143–163.
(3) Daferner, M.; Anke, T.; Sterner, O. *Tetrahedron* **2002**, *58*, 7781–7784.
(4) Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561–1566.
(5) Abdel-Lateff, A.; Klemke, C.; Konig, G. M.; Wright, A. D. *J. Nat. Prod.* **2003**, *66*, 706–708.
(6) Ciavatta, M. L.; Lopez-Gresa, M. P.; Gavagnin, M.; Nicoletti, R.; Manzo, E.; Mollo, E.; Guo, Y. W.; Cimino, G. *Tetrahedron* **2008**, *64*, 5365–5369.
(7) Bringmann, G.; Lang, G.; Steffens, S.; Schaumann, K. *J. Nat. Prod.* **2004**, *67*, 311–315.
(8) Larsen, T. O.; Frisvad, J. C.; Jensen, S. R. *Phytochemistry* **1992**, *31*, 1613–1615.
(9) Gaby, W. L.; Hadley, C.; Kaminski, Z. C. *J. Biol. Chem.* **1957**, *227*, 853–861.
(10) Wu, S. H.; Luo, X. D.; Ma, Y. B.; Liu, J. K.; Wu, D. G.; Zhao, B.; Lu, Y.; Zheng, Q. T. *J. Nat. Prod.* **2000**, *63*, 534–536.
(11) Springer, J. P.; Dorner, J. W.; Cole, R. J.; Cox, R. H. *J. Org. Chem.* **1979**, *44*, 4852–4854.
(12) Li, G. Y.; Li, B. G.; Yang, T.; Yin, J. H.; Qi, H. Y.; Liu, G. Y.; Zhang, G. L. *J. Nat. Prod.* **2005**, *68*, 1243–1246.
(13) Matsufuji, H.; Chino, M.; Takeda, M. *J. Agric. Food Chem.* **2004**, *52*, 3601–3605.
(14) Yalcin, F. N.; Ersoz, T.; Akbay, P.; Calis, I.; Donmez, A. A.; Sticher, O. *Turk. J. Chem.* **2003**, *27*, 703–711.
(15) Kosemura, S.; Matsunaga, K.; Yamamura, S.; Kubota, M.; Ohba, S. *Tetrahedron Lett.* **1991**, *32*, 3543–3546.
(16) Kosemura, S.; Miyata, H.; Yamamura, S.; Albone, K.; Simpson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 135–139.
(17) Kosemura, S.; Matsuo, S.; Yamamura, S. *Phytochemistry* **1996**, *43*, 1231–1234.
(18) Kosemura, S. *Tetrahedron Lett.* **2002**, *43*, 1253–1256.
(19) Shiomi, K.; Uchida, R.; Inokoshi, J.; Tanaka, H.; Iwai, Y.; Omura, S. *Tetrahedron Lett.* **1996**, *37*, 1265–1268.
(20) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Tetrahedron Lett.* **1989**, *30*, 3147–3150.
(21) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(22) Turner, W. B.; Aldrige, D. C. *Fungal Metabolites II*; Academic Press: London, GB, 1983; pp 482−484.
(23) Zafra-Polo, M. C.; González, M. C.; Tormo, J. R.; Cortes, D. *J. Nat. Prod.* **1996**, *59*, 913–916.
(24) Betancur-Galvis, L.; Palomares, E.; Marco, J. A.; Estornell, E. *J. Ethnopharmacol.* **2003**, *85*, 279–282.
(25) Degli Esposti, M.; Ghelli, A.; Ratta, M.; Cortes, D.; Estornell, E. *Biochem. J.* **1994**, *301*, 161–167.
(26) López-Gresa, M. P.; González, M. C.; Primo, J.; Moya, P.; Romero, V.; Estornell, E. *J. Antibiot.* **2005**, *58*, 416–419.
(27) Tormo, J. R.; Zafra-Polo, M. C.; Serrano, A.; Estornell, E.; Cortes, D. *Planta Med.* **2000**, *66*, 318–323.
(28) Bermejo, A.; Figadère, B.; Zafra-Polo, M. C.; Barrachina, I.; Estornell, E.; Cortes, D. *Nat. Prod. Rep.* **2005**, *22*, 269–303.
(29) Tormo, J. R.; Estornell, E.; Gallardo, T.; González, M. C.; Cave, A.; Granell, S.; Cortes, D.; Zafra-Polo, M. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 681–684.
(30) Weiss, H.; Friedrich, T.; Hofhaus, G.; Preis, D. *Eur. J. Biochem.* **1991**, *197*, 563–576.
(31) Hague, S. M.; Klaffke, S.; Bandmann, O. *J. Neurol. Neurosurg. Psychiatry* **2005**, *76*, 1058–1063.
(32) Lümmen, P. *Biochim. Biophys. Acta* **1998**, *1364*, 287–296.
(33) Isman, M. B. *Annu. Rev. Entomol.* **2006**, *51*, 45–66.
(34) Houbraken, J.; Due, M.; Varga, J.; Meijer, M.; Frisvad, J. C.; Samson, R. A. *Stud. Mycol.* **2007**, 107–128.
(35) Fato, R.; Estornell, E.; Bernando, S.; Palloti, F.; Parenti-Castelli, G.; Lenaz, G. *Biochemistry* **1996**, *35*, 2705–2716.

NP900085N