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Biotransformation of Sclareolide by Filamentous Fungi: Cytotoxic Evaluations of the Derivatives

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O esclareolido (**1**) foi incubado com oito diferentes espécies de fungos filamentosos usados convencionalmente para bio-oxidações. O composto **1** metabolizado pelo fungo *Aspergilus niger* em um meio de cultura A forneceu o 3-cetoesclareolido (**2**) e 3β-hidroxiesclareolido (**4**). Quando em um meio de cultura B (mais rico em nutrientes em relação ao meio de cultura A), foram obtidos os compostos **2**, **4**, e ainda 3α,6β-diidroxiesclareolido (**16**), 1-cetoesclareolido (**17**), 3-ceto-15-hidroxiesclareolido (**18**) e 3β,15-diidroxiesclareolido (**19**). Os produtos **16**-**19** resultantes da biotransformação de **1** são relatados como substâncias inéditas. A fermentação de **1** com *Cunninghamella blackesleeana* usando o meio de cultura A forneceu os compostos **2** e **4**, enquanto que empregando o meio de cultura B, forneceu os compostos **2**, **4**, **16** e **17**. Os compostos **2**, **4** e **17** foram obtidos também com *Curvularia lunata*. A biotransformação de **1** com *Beauveria bassiana* forneceu o composto **4** com rendimento satisfatório; com *Rhizopus oligosporus* e com *Mucor miehei* forneceu os compostos **2** e **4**, enquanto que com *R. nigricans* and *Fusarium moliniforme* os compostos **2**, **4** e **16** foram obtidos. A avaliação dos efeitos citotóxicos do composto **1** e dos produtos obtidos frente as linhagens de células cancerosas humanas selecionadas (U251, PC-3, K562, HCT-15, MCF-7 e SKUL-1) indicaram que o composto **16** (3α,6β- diidroxiesclareolido) apresenta um efeito citotóxico moderado (IC_{50} < 100 μM) contra a U251, a PC-3, a HCT-15 e a MCF-7.

Sclareolide (**1**) was incubated with eight different species of filamentous fungi conventionally used for bio-oxidations. Compound **1** was metabolized with *Aspergillus niger* in medium A to yield 3-ketosclareolide (**2**) and 3β-hydroxysclareolide (**4**), while in medium B (containing major number of nutrients with respect to medium A), compounds **2**, **4**, 3α,6β-dihydroxysclareolide (**16**), 1-ketosclareolide (**17**), 3-keto-15-hydroxysclareolide (**18**) and 3β,15-dihydroxysclareolide (**19**) were obtained. The biotransformation products **16**-**19** were found to be new substances. Fermentation of **1** with *Cunninghamella blackesleeana* using medium A afforded **2** and **4**, while using medium B yielded **2**, **4**, **16** and **17**. Compounds **2**, **4** and **17** were also obtained with *Curvularia lunata*. Biotransformation of **1** with *Beauveria bassiana* yielded **4** in satisfactory yield, with *Rhizopus oligosporus* and *Mucor miehei* afforded **2** and **4**, while with *R. nigricans* and *Fusarium moliniforme* yielded **2**, **4** and **16.** Cytotoxic evaluation of **1** and the obtained products against selected human cancer cell lines (U251, PC-3, K562, HCT-15, MCF-7 and SKUL-1) indicated that **16** (3α,6β-dihydroxysclareolide) displayed moderate cytotoxic (IC_{50} < 100 μM) against U251, PC-3, HCT-15 and MCF-7.

Keywords: biotransformation, sclareolide, filamentous fungi, microbiological oxidation

Introduction

The use of natural catalysts is now considered one of the most valuable routes for the synthesis of fine chemicals employing ecologically competitive procedures, particularly at industrial scale.[1-3] The selectivity and mildness of the biotransformations can be considered as advantages to similar, chemical-based methods[4-6] and this is evident for the selective oxidation of non-activated carbon atoms which is difficult to achieve by classical organic

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chemistry. Biotransformations are typically carried out using either whole cells or isolated enzymes. Although in recent years some enzymes responsible for fungal hydroxylation have been isolated, whole-cell fermentation is the technique most often employed.[7-9] A major challenge for the transformations using biocatalytic methods is to determine the appropriate microorganism and conditions, so, we proceeded with the screening using different fungal strains and media, with sclareolide (**1**, Figure 1) as substrate and then the processes were scaled up. In addition and in the search of new bioactive agents from natural products,[10,11] the cytotoxic evaluation against several human tumor cell lines of **1** and the obtained products (**2**, **4**, **16**-**19**) were carried up. Here we report our results.

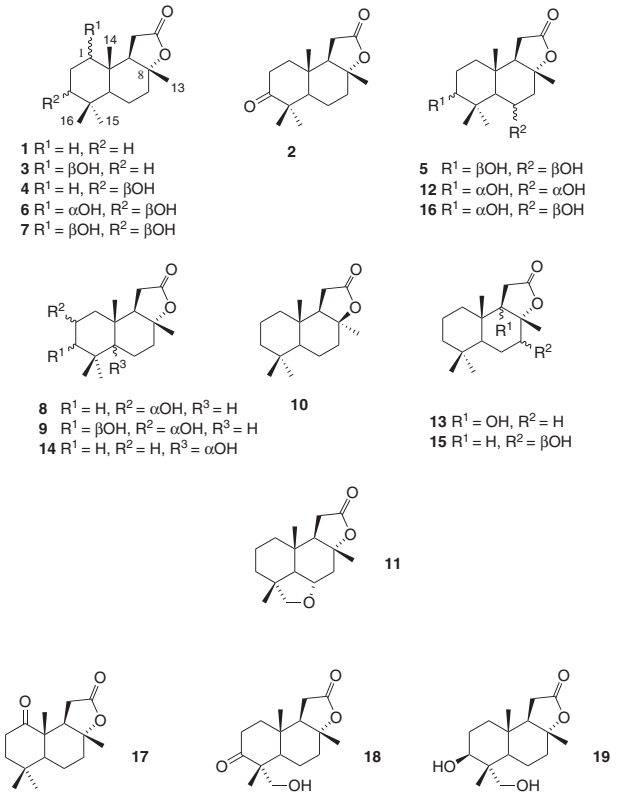

Figure 1. Chemicals structures of sclareolide (**1**) and their derivatives compounds **2**, **4**, **16**-**19**.

Sclareolide (**1**) is a natural product isolated from several plant species which displays antifungal,[12] phytotoxic[13] and cytotoxic[14] activities against several human tumor cell lines. This compound has also been used as starting material for the synthesis of various bioactive natural products.[15,16] Regarding the biotransformation of sclareolide (**1**) it has been previously reported that the incubation of **1** with *Mucor plumbeus* afforded 3-ketosclareolide (**2**), 1β-hydroxysclareolide (**3**) and 3β-hydroxysclareolide (**4**).[17] The bioconversion of **1** with *Cephalosporium aphidicola* gave **2, 4** and 3β,6β-dihydroxysclareolide (**5**).[18] The microbial transformation of **1** by *Curvularia lunata* yielded **2**-**4**, 1α,3β-hydroxysclareolide (**6**) and 1β,3β-dihydroxysclareolide (**7**).[19] The incubation of sclareolide with *Cunninghamella elegans* afforded **2**, **4**, **6** and **8**-**10**.[20] *Cunninghamella blackesleeana* metabolized compound **1** to afford **2**, **5**, **7** and **11**-**13**. Biotransformation of **1** with *C. echinulata* yielded 5-hydroxysclareolide (**14**) and 7β-hydroxysclareolide (**15**).[21]

Results and Discussion

A preliminary screening and preparative bioconversion of **1** with eight fungal species showed a widespread capacity to transform the starting material into more polar products in different yields (Table 1), which were separated by vacuum column chromatography.[22,23]

Table 1. Yields (%) of the isolated products of transformation of **1** with eight filamentous fungi[a]

	Products (%)						
Fungi	**1**[b]	**2**	**4**	**16**	**17**	**18**	**19**
A. niger [c]	6.5	22.7	37.4	-	-	-	-
A. niger [d]	2.8	11.7	27.7	5.9	10.1	12.4	9.4
C. blackesleeana [c]	6.5	18.8	57.3	-	-	-	-
C. blackesleeana [d]	5.3	7.2	20.6	28.3	8.7	-	-
C. lunata [d]	5.5	20.9	38	-	18.8	-	-
B. bassiana [c]	14.9	-	51.3	-	-	-	-
R. oligosporus [c]	17.3	12.1	32.1	-	-	-	-
R. nigricans [c]	18.9	12.9	28.5	4.5	-	-	--
M. miehei [c]	17.9	12.1	25.5	-	-	-	-
F. moliniforme [c]	10.3	16.1	28.9	32.3	-	-	-

[a]Yields calculated after purification. [b]Recovered starting material. [c]Using culture medium A. [d]Using culture medium B (see Experimental).

The crude organic extract obtained from incubation of sclareolide (**1**) with *A. niger* in culture medium A was separated by silica gel chromatographed to yield the known metabolites **2** and **4**. However, when the same microorganism was grown in culture medium B (containing major number of nutrients with respect medium A), compounds **2**, **4** and four new metabolites: **16**-**19** were obtained. Compound **2** was identified as the 3-ketosclareolide by comparison of its spectral data with those reported in the literature.[17,19] The ^{1}H NMR spectrum of **4** (Table 2) revealed a signal at δ_H 3.27 (dd, ^3J 11, 5 Hz, 1H,) indicative of an axial proton at C-3 (δ_C 78.6). The location of the hydroxyl group at C-3 was confirmed on the basis of HMBC experiments and this compound was identified as 3β-hydroxysclareolide (**4**).[17,19,20] Unambiguous and detailed ^{1}H NMR assignments (in comparison with previous reports[17]) are shown in Table 2.

Table 2. ^{1}H and ^{13}C NMR data (δ in ppm, multiplicite) of compounds **4**, **16-19** (500 and 125 MHz, $CDCl_3$). Coupling constants (J in Hz) in parenthesis

	4		**16**		**17**		**18**		**19**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	β_{ec}: 1.19, ddd (13.5, 13.5, 4)	37.8	β_{ec}: 1.18, ddt (14, 4, 3.5)	37.1		214.1	β_{ec}: 1.74, m	37.6	β_{ec}: 1.47, m	37.4
	α_{ax}: 1.45 m		α_{ax}: 1.68, ddd (14, 14, 4)				α_{ax}: 1.62, m		H-1α_{ax}: 1.24, m	
2	1.69, m	26.8	β_{ax}: 1.48, ddd (14, 4, 3)	26.0	β_{ax}: 2.68, ddd (16, 9, 5)	34.5	β_{ax}: 2.67, ddd (17, 13, 7)	34.8	1.74-1.68, m	26.4
			α_{ec}: 2.16, ddd (14.5, 4, 3)		α_{ec}: 2.29, ddd (16, 8, 4)		α_{ec}: 2.42, ddd (13, 7, 3)		1.74-1.68, m	
3	α_{ax}:3.27, dd (11, 5)	78.6	α_{ax}: 3.37, dd (3, 3)	72.6	α_{ax}: 1.69, m	39.2		216.1	α_{ax}: 3.70, dd (10, 5)	75.4
					β_{ec}: 1.84, m					
4		38.8		34.0		32.4		52.6		42.0
5	α_{ax}: 1.89, dd (11, 3)	55.3	α_{ax}: 1.54, d (3)	50.3	α_{ax}: 1.51, dd (12, 3)	53.8	α_{ax}: 2.04, dd (10, 2)	47.9	α_{ax}: 1.11, m	49.6
6	β_{ax}: 1.47, m	20.3	α_{ec}: 4.70, ddd (3, 3, 3)	69.0	β_{ax}: 1.59, dddd (12,12,12,3)	21.1	β_{ax}: 2.13, m	37.7	1.49-1.41, m	20.2
	α_{ec}:1.89, m				α_{ec}: 1.90 m		α_{ec} 2.13, m		1.74-1.68, m	
7	β_{ec}: 2.09, ddd (12, 3.5, 3.5)	38.4	β_{ec}: 2.04, dd (13, 4)	46.7	β_{ec}: 2.08, ddd (12, 3, 3)	37.2	β_{ec}: 1.65, m	21.0	β_{ec}:2.10-2.05, m	38.2
	α_{ax}:1.64, m		α_{ax}: 1.95, dd (13, 3)		α_{ax}: 1.68, m		H-7α_{ax}: 1.80, m		α_{ax}: 1.74-1.68, m	
8		86.1		86.3		85.6		85.6		85.9
9	α_{ax}: 1.92, dd (15, 6.5)	58.9	α_{ax}: 2.58, dd (14, 7 Hz)	51.8	α_{ax}: 2.16, dd (14, 7)	52.0	α_{ax}: 2.07, dd (15, 7)	58.1	α_{ax}: 1.95, dd (16, 7)	58.9
10		35.8		39.7		49.7		35.5		35.8
11	β_{ax}: 2.42, dd (16, 15)	28.7	β_{ax},: 2.46, dd (16, 14)	28.5	β_{ax}:2.96, dd (17, 14)	30.8	β_{ax}: 2.49, dd (16, 15)	28.6	β_{ax}: 2.42, dd (16, 15)	28.7
	α_{ec}: 2.24, dd (16, 6.5)		α_{ec}: 2.34, dd (16, 7)		α_{ec}: 2.53, dd (17, 7)		α_{ec}: 2.31, dd (16,7)		α_{ec}: 2.25, dd (16, 7)	
12		175.5		177.1		176.7		175.8		176.3
13	1.34, s	21.5	1.59, s	23.0	1.35, s	21.7	1.41, s	21.3	1.34, s	21.5
14	0.93, s	15.1	1.29, s	16.9	1.19, s	14.4	1.13, s	14.7	0.97, s	15.4
15	1.01, s	27.9	1.04, s	32.4	1.03, s	31.3	H-15_a: 3.76, d (11)	66.6	H-15_a: 3.72, (10.5)	70.9
							H-15_b: 3.39, d (11)		H-15_b:3.43, d (10.5)	
16	0.81, s	15.0	1.21, s	23.5	1.06, s	23.2	1.00, s	16.3	0.88, s	11.2

For compound **16**, the HRFABMS established a molecular formula of $C_{16}H_{26}O_4$ $[M+H]^+$, at *m/z* 283.1915, calculated for ($C_{16}H_{26}O_4$ + H) 283.1909. The ^{1}H NMR spectrum revealed two signals at δ_H 3.37 (dd, ^{3}J 3, 3 Hz, 1H) and δ_H 4.70 (ddd, ^{3}J 3, 3, 3 Hz, 1H), indicative of two equatorial hydrogens at C-3 (δ_C 72.6) and C-6 (δ_C 69.0), respectively. The locations of the hydroxyl groups at C-3 and C-6 in **16** were determined on the basis of HMBC experiments, in particular by the $16CH_3$– (δ_H 1.21) and the $15CH_3$– (δ_H 1.04) crosspeaks with C(3) (δ_C 72.6) and the crosspeaks between H-6 (δ_H 4.70) and C-4(δ_C 34.0), C-5 (δ_C 50.3), C-10 (δ_C 39.7), C-7 (δ_C 46.7), and C-8 (δ_C 86.3). COSY, HMQC and NOESY experiments allowed complete assignments for all protons and carbons (Table 2) and the structure was confirmed as 3α,6β-dihydroxysclareolide (**16**). The epimer at C(3) has been reported previously.[18]

The new metabolite **17** was obtained as a white crystalline solid. Its HRFABMS exhibited $[M+H]^+$ at *m/z* 265.1805, corresponding to the molecular formula $C_{16}H_{25}O_3$ (calculated for $C_{16}H_{24}O_3$ + H: 265.1804). The IR spectral data displayed absorptions for γ-lactone (1774 cm^{-1}) and ketone (1708 cm^{-1}). These data were similar to those for compound **2**.[17,18] The ^{13}C NMR data (Table 2) showed resonances for sixteen carbons and the DEPT experiments established the multiplicity for each carbon signal and they revelead the presence of four methyls, five methylenes, two methines and five quaternary carbons. The ^{1}H NMR (500 MHz) data of **17** (Table 2) showed singlets at δ_H 1.35, 1.19, 1.03 and 1.06 which were assigned to $13CH_3$–, $14CH_3$–, $15CH_3$– and $16CH_3$–, respectively, by direct comparison with similar compounds. In particular, $13CH_3$– is located downfield (δ_H 1.35) due to it is linked to the carbon closing the γ-lactone (C-8) and the signal at δ_H 1.19, which

showed NOESY crosspeak with H-11β (δ_H 2.96) and HMBC crosspeak with the signal at δ_C 52.00 (C-9) was assigned to $14CH_3$–. The location of the ketone at C-1 was determined by the HMBC crosspeak of the signal at δ_H 1.19 ($14CH_3$–) with the resonance at δ_C 214.1. Assignments for all hydrogens and carbons were done by COSY, HMQC, HMBC and NOESY experiments.

Compound **18** was obtained as white crystalline solid and its IR spectral data displayed absorptions at 3459 (O-H), 1773 (C=O) and 1174 (C-O) cm^{-1}. The HREIMS exhibited $[M+H]^+$ at *m/z* 281.1751 establishing the molecular formula $C_{16}H_{24}O_4$ (calculated for $C_{16}H_{24}O_4$ + H: 281.1753). The ^{13}C NMR spectrum of **18** (DEPT experiment) displayed resonances for sixteen carbon atoms, including three methyls, six methylenes, two methines and five quaternary carbons. Its ^1H NMR spectrum ($CDCl_3$, 500 MHz) showed three singlets at δ_H 1.41, 1.13 and 1.00 due the H-13, H-14 and H-16 methyls, respectively, due to the observed NOESY crosspeaks between C-13 and C-14 and between C-14 with C-16. A notable difference with the 4,4'-dimethyl products was the absence of one methyl group and the presence of an AB system (δ_H 3.76 and 3.39, ^2J 11 Hz), which was assigned to the methylene protons at C-15 (δ_C 66.6). The signal at δ_C 216.1 corresponds to a ketone carbonyl located at C-3 due to HMBC correlations of this carbon with the AB system (H-15_a and H-15_b), H-$2_{\beta ax}$ (δ_H 2.67), H-$2_{\alpha ec}$ (δ_H 2.42), H-$1_{\beta ec}$ (δ_H 1.74) and with $16CH_3$– (δ_H 1.00). The ^1H and ^{13}C NMR chemical shifts assigments for compound **18** (Table 2), confirmed this compound as 3-keto-15-hydroxysclareolide.

The other novel biotransformation product, **19**, was a crystalline solid. The IR spectral data displayed absorptions at 3489 (O-H) and 1767 (C=O) cm^{-1}. The HREIMS of **19** exhibited a molecular ion *m/z* 283.1908 corresponding to the molecular formula $C_{16}H_{26}O_4$ (calculated for $C_{16}H_{26}O_4$ + H: 283.1909). The ^{13}C NMR spectrum of **19** showed resonances for sixteen carbons including three methyls, six methylenes, three methines and four quaternary carbons. The ^1H NMR data of **19** (Table 2) showed singlets at δ_H 1.34, 0.97 and 0.88, assigned to $13CH_3$–, $14CH_3$–, $16CH_3$–, respectively, by comparison with the above described substances. Additionally, the signals at δ_H 3.72 and 3.43 (^2J 10, 5 Hz) established the presence of an AB system for the hydroxymethylene protons located at C-15 (δ_C 70.9) which overlapped with a doublet of doublet signal corresponding to H-$3_{\alpha ax}$ (δ_H 3.70, ^3J 10, 5 Hz). The signal at δ_C 75.4, assigned to C-3, showed HMBC correlations with the AB system for H-15_a and H-15_b, and with $16CH_3$– (δ_H 0.88). Assignments for all hydrogens and carbons for compound **19** were done by COSY, HMQC, HMBC and NOESY experiments and verified its structure as 3β,15-dihydroxysclareolide (Table 2).

An interesting variety of products and yields were obtained by the biotransformation of sclereolide (**1**) with different fungi. The transformation of **1** with *A. niger* in medium A (containing minor number of nutrients) produced **2** and **4**, while using medium B yielded six compounds (**2**, **4**, **16**-**19**) in variable yields (from 5.9% to 27.1%), affording a keto group at C-1 (**17**) for the first time. Using medium B with *A. niger*, *C. blackesleeana* and *C. lunata* yielded various products in relatively variable yields, while medium A produced fewer products, with better yields though (see Table 1). The transformation of **1** with *B. bassiana* afforded exclusively product **4** in acceptable yield (51.3%), in comparison with other biotransformations.[24] These results gave additional evidences of the importance of the species, strains and media employed in the structural diversity of the products and the yields of the biotransformations.

Sclareolide (**1**) and derivatives **2**, **4**, **16**-**19** were tested for in vitro cytotoxic activity against human cancer cell lines U251 (central nervous system), PC-3 (prostate cancer), K562 (leukemia), HCT-15 (colon), MCF-7 (breast) and SKUL-1 (lung) following standard procedures.[25] Compound **1** displayed activity against PC-3 tumor cell line (IC_{50} 71.12 ± 4.7 μM) and **16** displayed activity against U251 (IC_{50} 87.40 ± 5.4 μM), PC-3 (IC_{50} 34.47 ± 7.4 μM), HCT-15 (IC_{50} 92.23 ± 4.2 μM), MCF-7 (IC_{50} 89.11 ± 2.4 μM). All the other IC_{50} values were above a 100 μM and were considered not active.[25] It is interesting that the presence of the hydroxyl groups at C-3α and C-6β increased the cytotoxicity with respect to the starting material (**1**).

Experimental

General experimental procedures

Melting points were determined on a Fischer-Jones apparatus. Optical rotation were measured on a Perkin-Elmer 341 polarimeter. Infrared spectra are registred Nicolet Magna FT-IR 750 spectrometer. ^1H and ^{13}C NMR spectra were taken on a Varian Unity-plus 500 (at 500 and 125 MHz) instrument. EI-MS: Jeol JMS-AX505HA mass spectrometer and Jeol JMS-SX 102 A) for HREIMS. TLC spots were revealed by spraying with ceric ammonium sulfate, followed by heating. Vacuum column chromatographies were done following the reported procedures.[22,23] Column chromatography (CC) were performed using silica gel 70-230, TLC using silica gel $60F_{254}$ (Merck) plates and preparative TLC using silica gel $60F_{254}$ (Merck) Plates. Sclareolide was purchased from Sigma-Aldrich.

Microorganisms

A. niger (ATCC 16404), *B. bassiana* (ATCC 13144), *C. blackesleeana* (ATCC 8688a), *C. lunata* (ATCC 13432),

F. moliniforme (ATCC 10209), *M. miehei* (ATCC 16457), *R. oligosporus* (ATCC 22959), *R. nigricans* (ATCC 6227b), were obtained from the Instituto de Biotecnología, Universidad Nacional Autónoma de México, maintained on potato dextrose agar (PDA) and stored at 4 °C.

Media and culture conditions

The media were prepared by mixing the following ingredients in 1 L of distilled water. Medium A (YEPGA): (10 g) peptone, (10 g) yeast extract, (10 g) beef extract and (50 g) glucose. The pH was adjusted to 7 (NaOH 1 mol L^{-1}) before autoclaving. Medium B was prepared by mixing the following ingredients: glucose (10 g), glycerol (10 g), peptone (5 g), yeast extract (5 g), KH_2PO_4 (5 g) and NaCl (5 g) at pH 7 (adjusted with NaOH 1 mol L^{-1}) for *A. niger, C. blackesleeana, C. lunata.*

Incubation experiments

Firstly, the eight fungi were tested for their ability to metabolize **1** on the analytical scale (10 to 15 mg of substrate, 25 mL medium culture), and then the same biotransformation experiments were carried out on preparative scale (150 to 310 mg). Both analytical and preparative biotransformations were conducted following similar procedures. Erlenmeyer flasks (250 mL) containing 125 mL of medium A or the medium B were inoculated with a dense suspension (2 mL) of the corresponding fungi. Incubations were maintained at 25 °C with gyratory shaking (125 rpm) for 24 h (*A. niger, R. nigricans, R. oligosporus, M. miehei*), 48 h (*F. moniliforme*) or 72 h (*C. blackesleeana, C. lunata, B. bassiana*). Then, the substrates in acetone (5-10 mL) were added and the process continued for 14 days. These biotransformation experiments were monitored by TLC, including two controls, a "culture control" and a "substrate control" to eliminate the possibility that the isolated products were microbial secondary metabolites and/or that the culture media did not perform any chemical transformation on the substrate.

Recovery and purification of metabolites

Cultures were filtered and fungal cells were washed thoroughly with water and the filtrate and washings were combined, saturated with NaCl and extracted with CH_2Cl_2 (three times). The organic extracts were combined, dried with Na_2SO_4 and evaporated under reduced pressure. The organic residues were subjected to chromatography by VCC using gradient elution system with hexane/EtOAc. CC and preparative TLC allowed the final purification of the compounds.

*Biotransformation of **1** by A. niger in medium A*

After 24 h of inoculation with a dense suspension of the spores of *A. niger*, 20 erlenmeyer flask cultures (YEPGA) received 204.3 mg of sclareolide (**1**) in 10 mL of acetone. After incubation for 14 days the cultures were processed as indicated above to yield a crude dark oily residue (194.3 mg). Column chromatography of the organic extract yielded 3-ketosclareolide (**2**, 44.2 mg, 22.7%), 3β-hydroxysclareolide (**4**, 72.7 mg, 37.4%) and sclareolide (**1**, 12.6 mg, 6.5%).

*Biotransformation of **1** by A. niger in medium B*

The substrate **1** (210 mg) was dissolved in acetone (10 mL), distributed among 20 erlenmeyer flask cultures (medium B), previously (24 h) inoculated with a dense suspension of spores of *A. niger*. The biotransformation was allowed to proceed for 14 days and the cultures were processed as indicated above to obtain starting material **1** (6 mg, 2.8%), **2** (24.5 mg, 11.7%), **4** (58.2 mg, 27.7%), **16** (22.4 mg, 5.9%), **17** (21.2 mg, 10.1%) **18** (26 mg, 12.4%) and **19** (19.7 mg, 9.4%).

*3α,6β-Dihydroxysclareolide (**16**):* mp.188-190°C; $[\alpha]^{25}_D$ + 30.3 (*c* 0.30, $CHCl_3$); IR ν_{max}/ cm^{-1} ($CHCl_3$): 3618, 2933, 1758; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; HRFABMS $[M+H]^+$ Found: 283.1915. Calc. for $C_{16}H_{26}O_4$+H: 283.1909; EIMS *m/z* 267 (24%), 249 (56), 169 (83), 43 (100).

*1-Ketosclareolide (**17**)*: mp. 152-153 °C; $[\alpha]^{25}_D$ + 83.8 (*c* 0.11, $CHCl_3$); IR ν_{max}/cm^{-1} ($CHCl_3$): 2958, 1774, 1708, 1232, 1199, 925. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; HRFABMS $[M+H]^+$ Found: 265.1805. Calc. for $C_{16}H_{24}O_3$+H: 265.1804; EIMS *m/z* 264 (17%), 205 (86), 55(69), 43 (100).

*3-Keto-15-hydroxysclareolide (**18**)*: mp. 169-171 °C; $[\alpha]^{25}_D$ + 29.3 (*c* 0.20, MeOH); IR ν_{max}/cm^{-1} ($CHCl_3$): 3459, 2985, 2952, 1773, 1698, 1431, 1234, 1174, 920; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2; HRFABMS $[M+H]^+$ Found: 281.1751. Calc. for $C_{16}H_{24}O_4$+H: 281.1753); EIMS *m/z* 280 (12%), 278 (36), 277 (11), 250 (67), 235 (48), 81 (41), 43 (100).

*3β-15-Dihydroxysclareolide (**19**)*: mp. 157-158 °C; $[\alpha]^{25}_D$ + 49.2 (*c* 0.13, MeOH); IR ν_{max}/cm^{-1} ($CHCl_3$): 3489, 2944, 1767, 1603,1457, 1349, 1243, 920. ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 2. HRFABMS $[M+H]^+$ Found: 283.1908. Calc. for $C_{16}H_{26}O_4$+H: 283.1909; EIMS *m/z* 282 [M^+, 20%], 264 [M^+–H_2O, 41], 251

(57), 233 (56), 181 (12), 173 (30) 147 (69), 121 (64), 93 (52), 81 (41), 43 (100), 18 (36).

*Biotransformation of **1** by C. blackesleeana in medium B*

The substrate **1** (307.5 mg) was dissolved in acetone (15 mL), distributed among 20 erlenmeyer flask cultures (medium B), previously inoculated (72 h) with a dense suspension of spores of *C. blackesleeana*. The fermentation was allowed to proceed for 14 days, the cultures were processed as indicated above to obtain starting material **1** (16.3 mg, 5.3%), **2** (22.3, 7.2%), **4** (63.4 mg, 20.6%), 3α,6β-dihydroxysclareolide (**16**, 86.9 mg, 28.3%) and 1-ketosclareolide (**17**, 26.7 mg, 8.7%).

*Biotransformation of **1** by C. lunata, B. bassiana, R. oligosporus, R. nigricans, M. miehei and F. moliniforme*

Compound **1** (150 mg) was reacted with the microorganisms following the procedure described above, obtaining the results shown in Table 1.

Cytotoxic assays

Human tumor cell lines of central nervous system (U251), prostate cancer (PC-3), leukemia (K562), colon (HCT-15), breast (MCF-7), lung (SKLU-1) were supplied by the National Cancer Institute (NCI). The cytotoxic activities of **1**, **2**, **4**, **16**-**19** were determined using the protein-binding dye sulforhodamine B in a microculture assay to measure cell growth, following the protocols described in the literature.[18] Results were expressed as concentration giving 50% inhibition (IC_{50}). The IC_{50} values (mean ± standard error) were 100 μM, and those with minor values are reported in the text. The positive control was adriamycin ($IC_{50} = 0.32 \pm 0.02$ μM against U251).

Supplementary Information

Supplementary information data are available free of charge at http://jbcs.org.br as a PDF file.

Acknowledgments

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Supplementary Information

Biotransformation of Sclareolide by Filamentous Fungi: Cytotoxic Evaluations of the Derivatives

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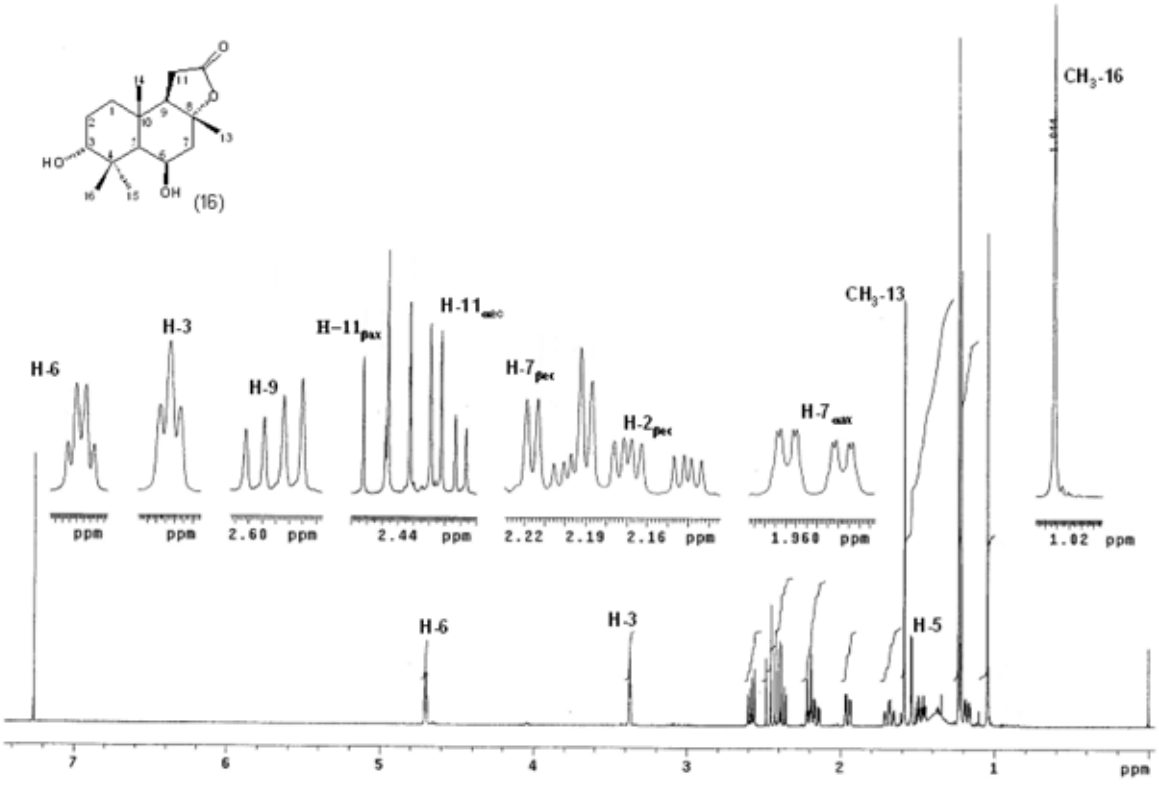

Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) of 3α,6β-dihydroxysclareolide (**16**).

*e-mail: delgado@unam.mx

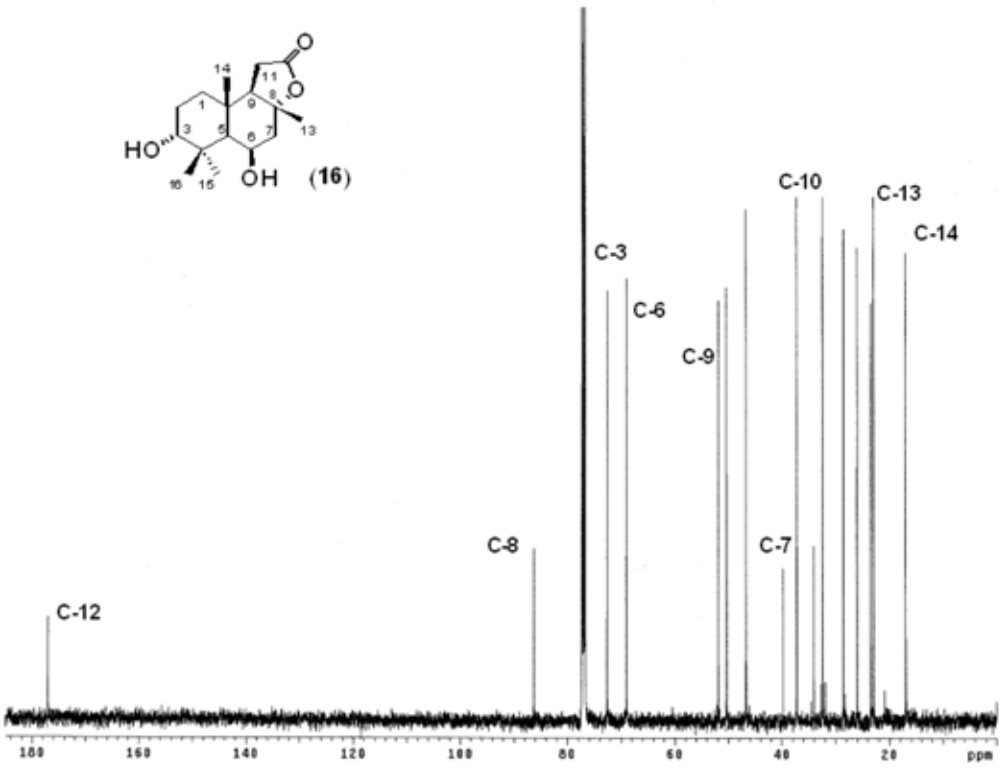

Figure S2. ^{13}C NMR (125 MHz, $CDCl_3$) of 3α,6β-dihydroxysclareolide (**16**).

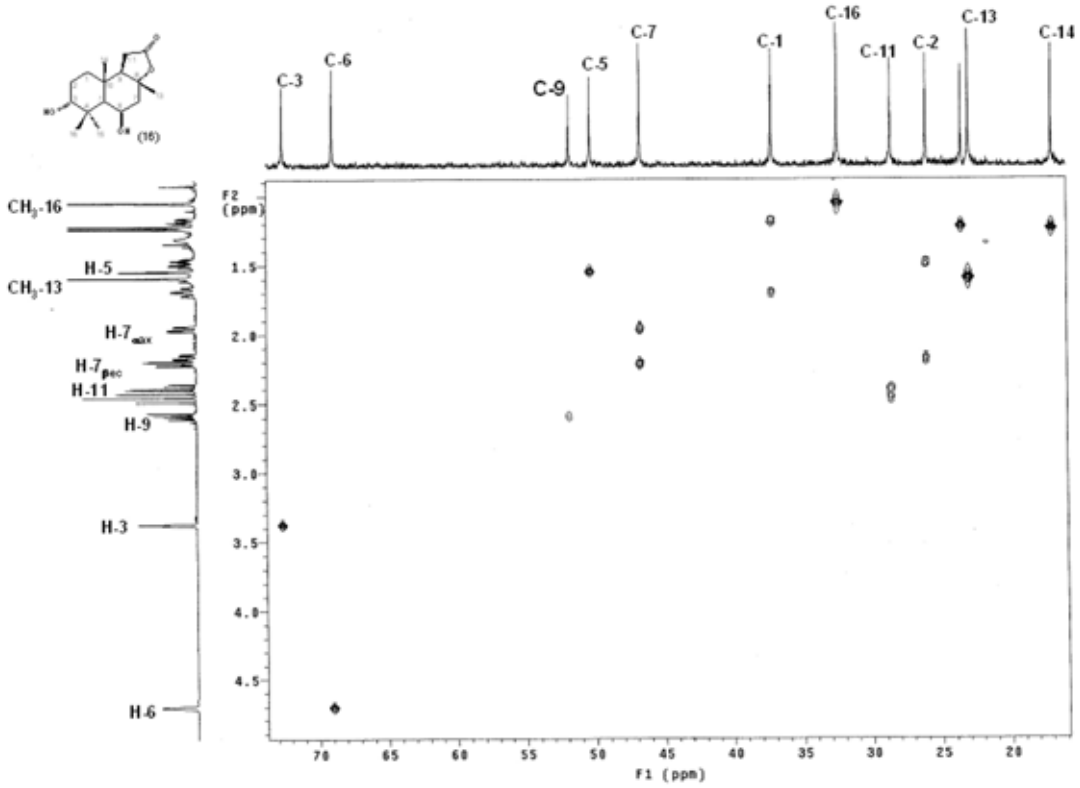

Figure S3. HSQC (500 MHz, $CDCl_3$) of 3α,6β-dihydroxysclareolide (**16**).

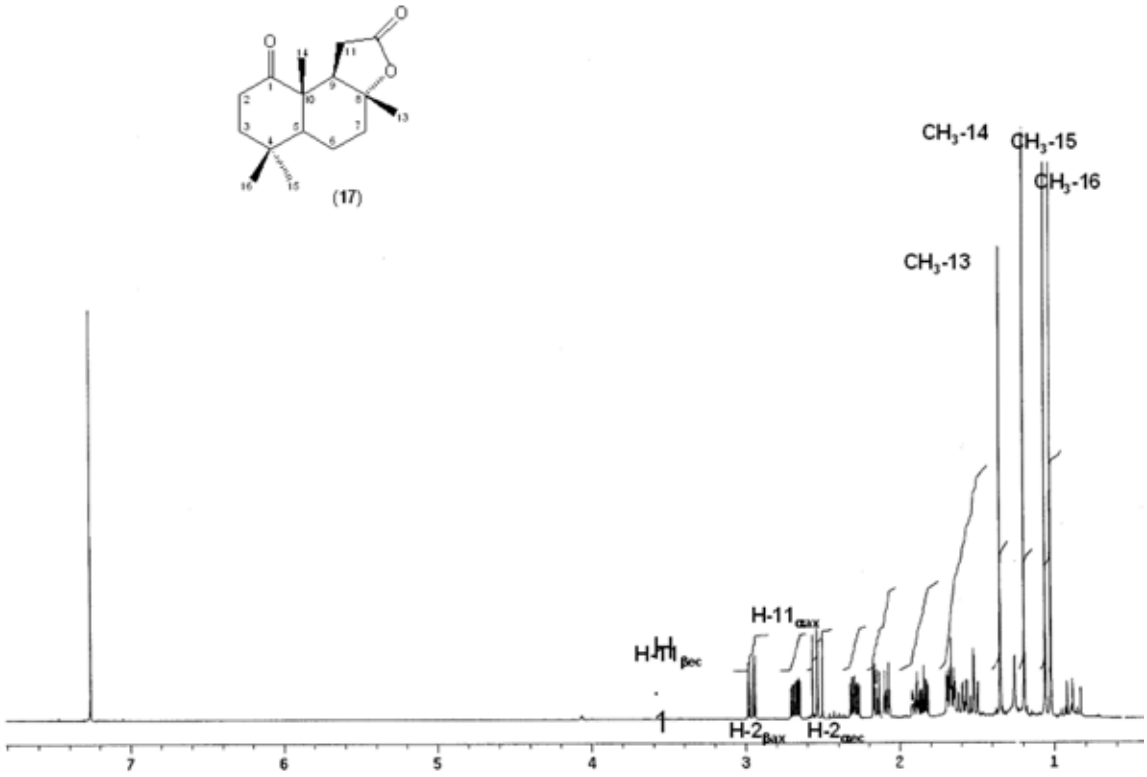

Figure S4. ^{1}H NMR (500 MHz, $CDCl_3$) of 1-ketosclareolide (**17**).

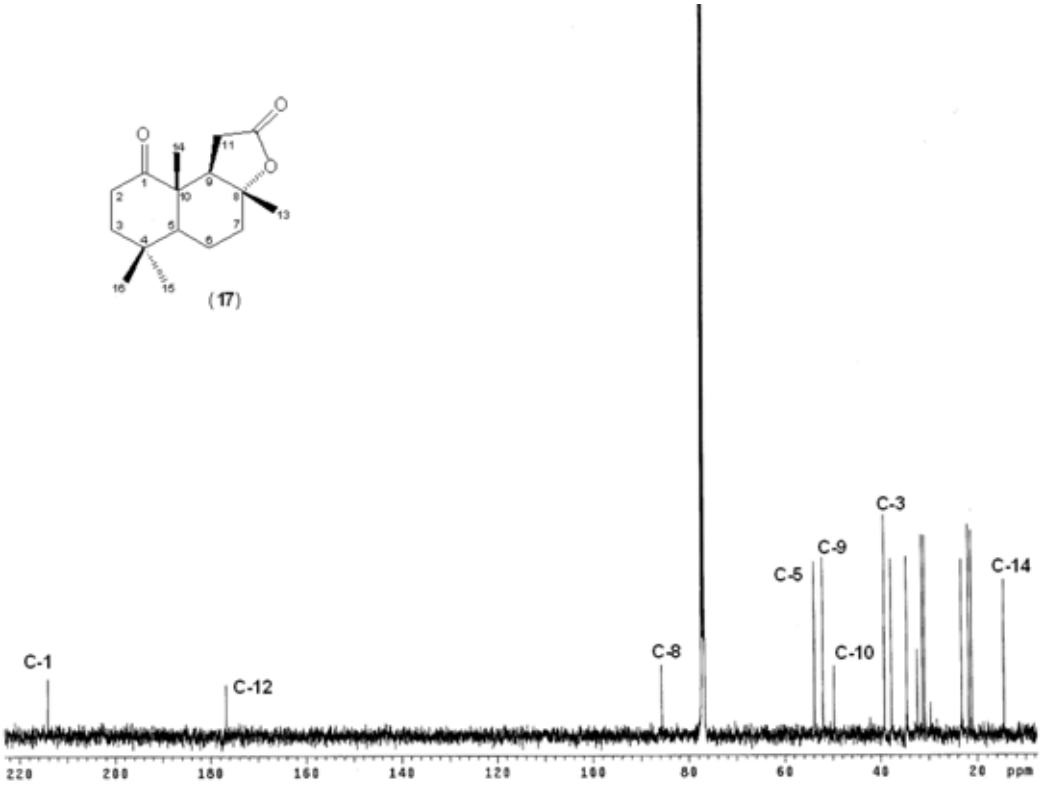

Figure S5. ^{13}C NMR (125MHz, $CDCl_3$) of 1-ketosclareolide (**17**).

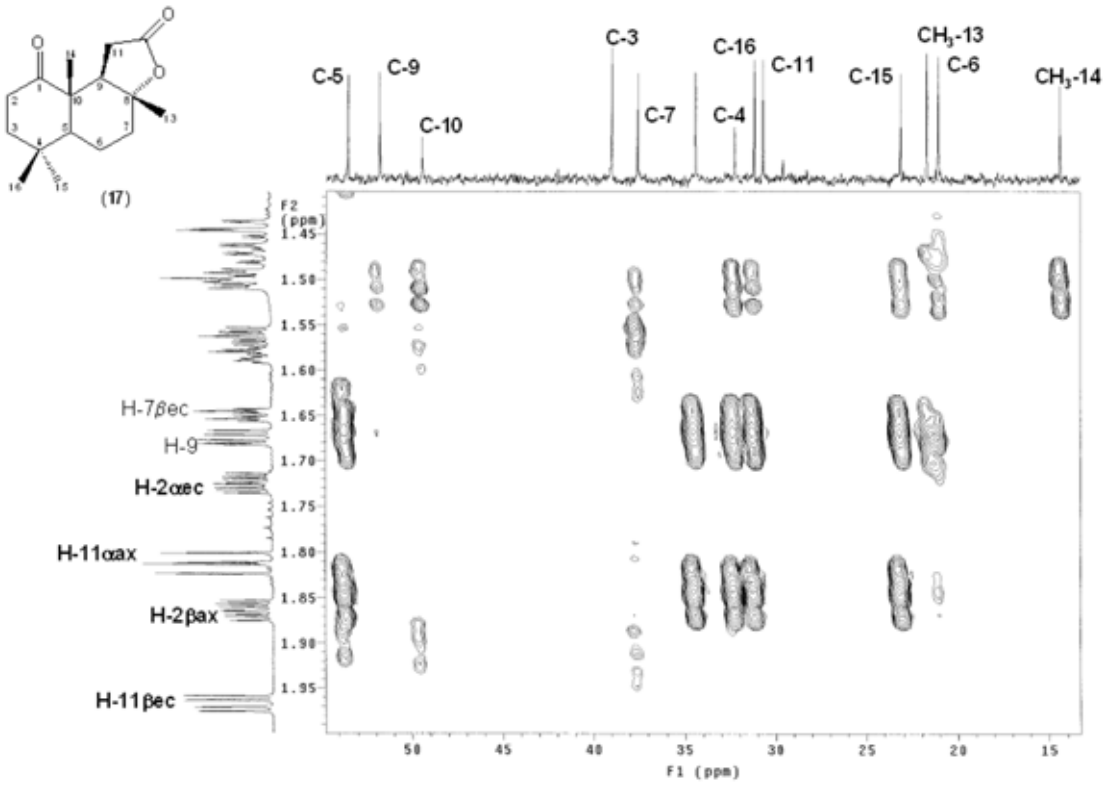

Figure S6. HMBC (500 MHz, $CDCl_3$) of 1-ketosclareolide (**17**).

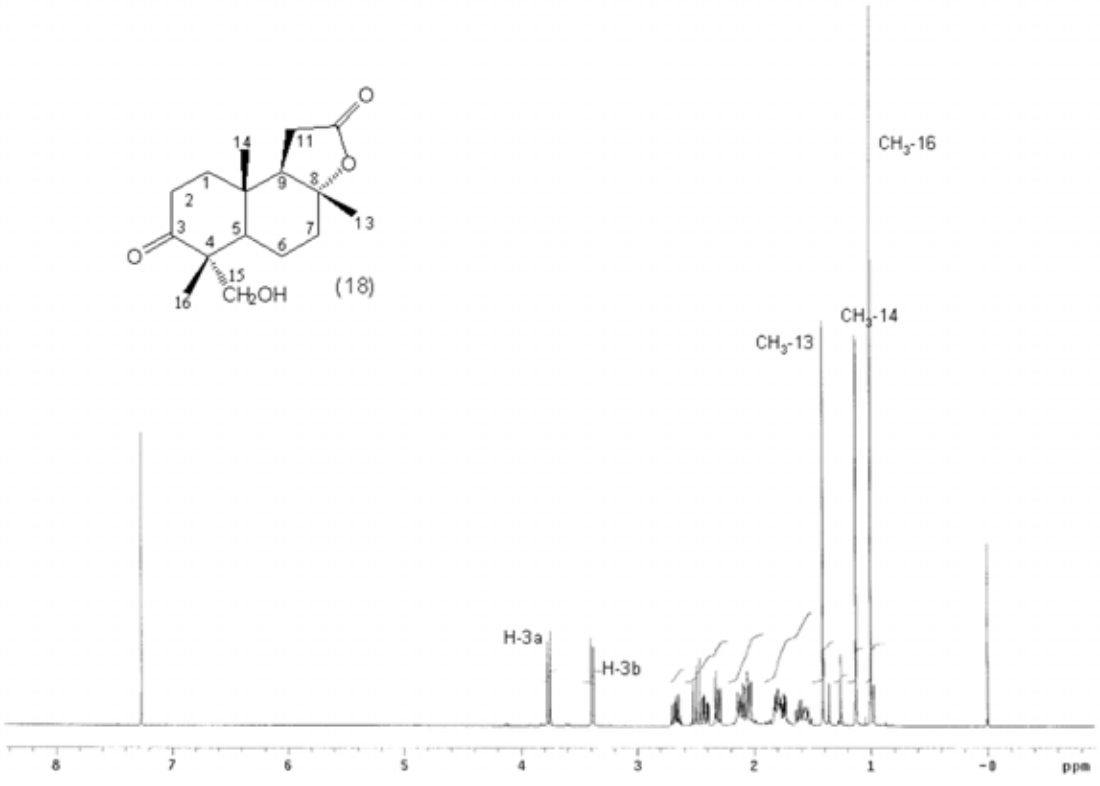

Figure S7. ^1H NMR (500MHz, $CDCl_3$) of 3-keto-15-hydroxysclareolide (**18**).

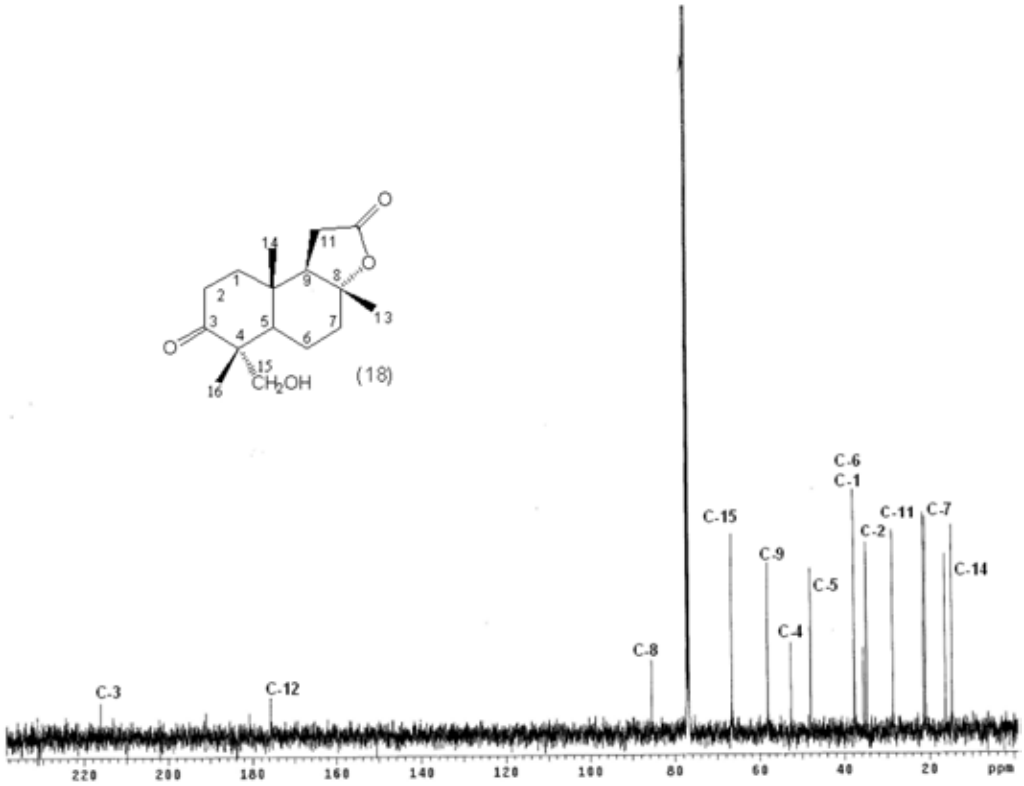

Figure S8. ^{13}C NMR (125 MHz, $CDCl_3$) of 3-keto-15-hydroxysclareolide (**18**).

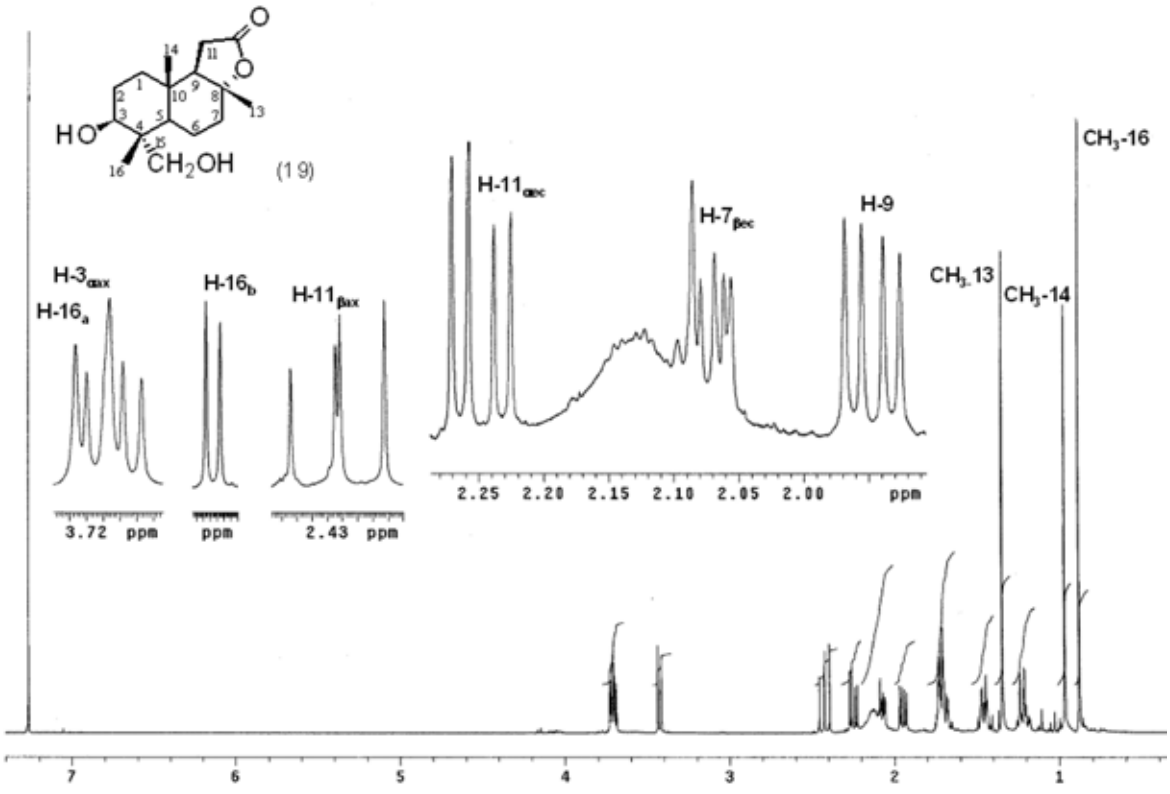

Figure S9. ^{1}H NMR (500 MHz, $CDCl_3$) of 3β,15-dihydroxysclareolide (**19**).

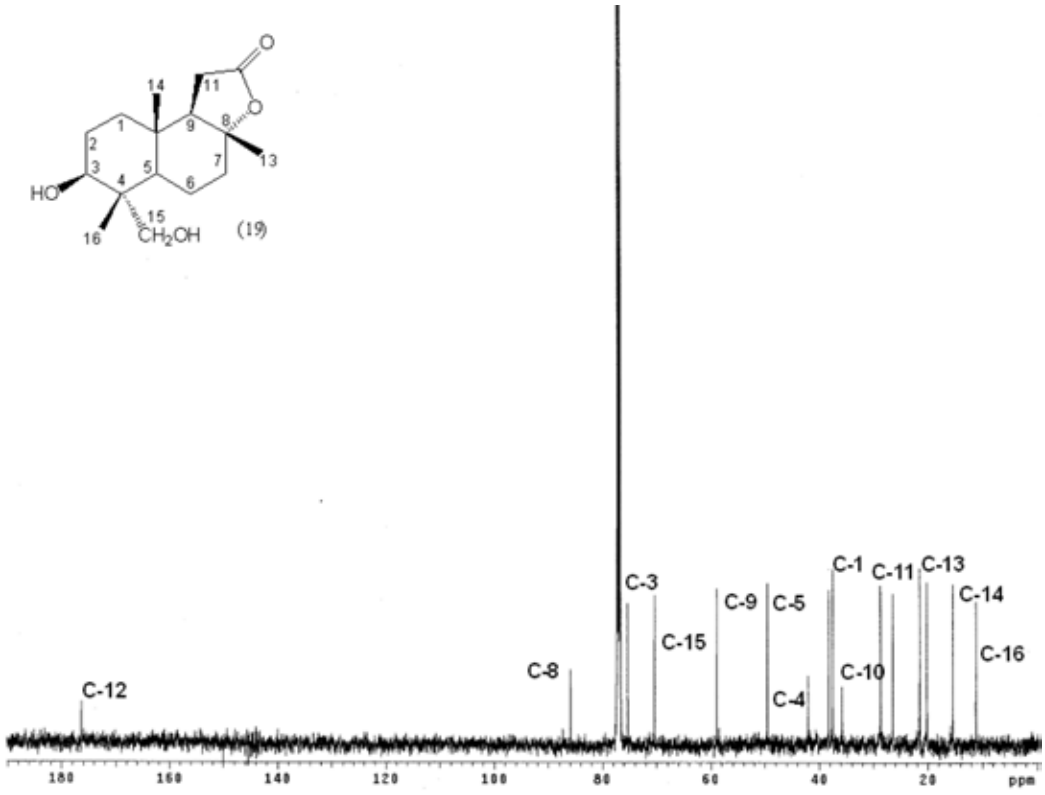

Figure S10. ^{13}C NMR (125 MHz, $CDCl_3$) of 3β,15-dihydroxysclareolide (**19**).

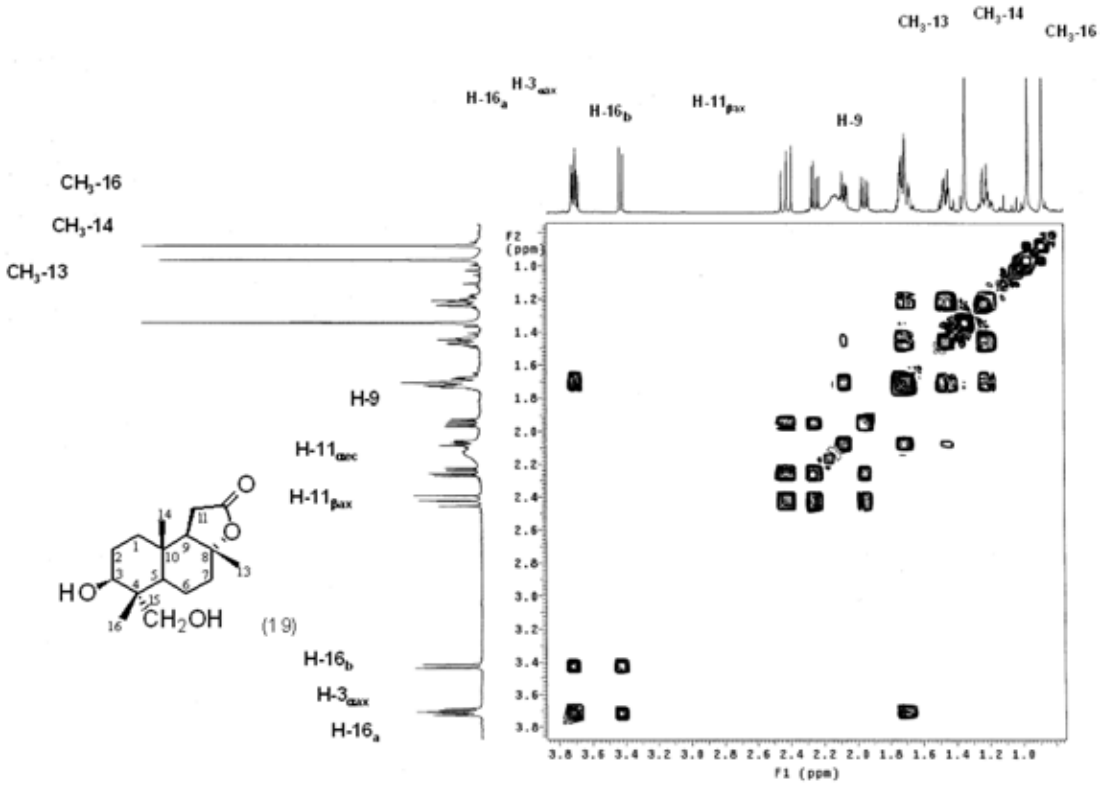

Figure S11. COSY Spectrum (500 MHz, $CDCl_3$) of 3β,15-dihydroxysclareolide (**19**).

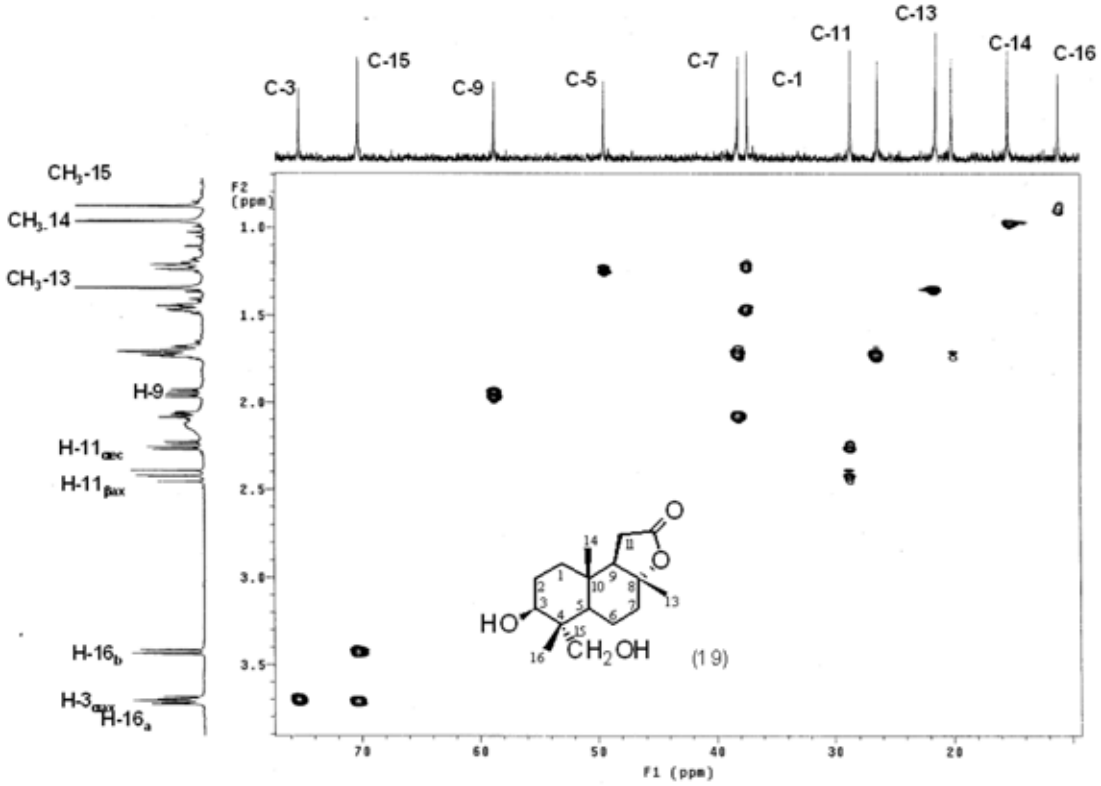

Figure S12. HSQC (500 MHz, $CDCl_3$) of 3β,15-dihydroxysclareolide (**19**).