New Cytotoxic Metabolites from the Sponge *Cacospongia scalaris*

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The sponge *Cacospongia scalaris* from Tarifa Island, Spain, contains in addition to five known compounds (**1**−**5**), the new scalarane sestertepenes 18-*epi*-scalaradial (**6**), 19-dihydroscalaradial (**7**), 12-*epi*-acetylscalarolide (**8**), and 16-acetylfuroscalarol (**9**) together with three uncommon norscalaranes norscalaral A (**10**), norscalaral B (**11**), and norscalaral C (**12**). The structures were elucidated by interpretation of spectral data. 18-*epi*-Scalaradial (**6**) represents the missing stereoisomer on structure−activity studies carried out with compounds of this series and did not react with methylamine. The new compounds isolated from *C. scalaris* showed significant *in vitro* cytotoxicity against five tumor cell lines with 18-*epi*-scalaradial showing the greatest activity (ED_{50} = 0.2 μg/mL).

Among the great array of structures of terpenoids of marine origin, the tetracyclic sesterterpenes with scalarane skeleton have proved to be one of the most interesting groups. The ecologic role of this group of compounds has been extensively studied.[1−5] In particular, those bearing a 1,4-dialdehyde moiety, of which scalaradial (**1**) is the most prominent example, have shown a remarkable antifeedant activity.[2] Structure−activity studies[6−8] have suggested that the key step involves the reaction between one aldehyde group of the terpenoid and the amine group of the receptor to form an imine. In addition, scalaradial (**1**) has been reported[9] to be a potent antiinflamatory agent with a similar inactivation profile to that of manoalide. Faulkner and Jacobs research groups, on a joint effort, have demonstrated that both manoalide and **1** initially react with the bee venom phospholipase A_2 (PLA_2) through the lysine residue of this enzyme to form an imine.[10]

Sponges of the *Cacospongia* genus have been reported to contain sestertepenes, C-21 furanoterpenoids and xanthenes.[11−13] This genus is an important source of scalarane sesterterpenes though these compounds have been isolated from several other genera of sponges of the Spongiidae and Thorectidae families.[11,14] The biological importance of scalarane terpenoids and the fact that *Cacospongia* genus is a source of these compounds prompted us to study specimens of *Cacospongia scalaris* from Tarifa Island in the Southern Coast of Spain. *C. scalaris* collected in the gulf of Naples, Italy, had been reported[15] to contain scalarin (**2**), the first compound possessing an scalarane skeleton to be described. Our specimen contained, in addition to scalarin (**2**), the known compounds scalaradial (**1**), 12-deacetoxyscalaradial (**3**), 19-deoxyscalarin (**4**), furoscalarol (**5**), together with four new scalaranes (**6**-**9**) and three new norscalaranes (**10**−**12**).

The use of a different extraction procedure (see Experimental Section) with respect to the previous study of *C. scalaris*[15] and of high performance liquid chromatography enabled us to isolate the less stable metabolite scalaradial (**1**) together with ten minor compounds (**3**−**12**). Chromatography of the acetone soluble material on silica gel followed by final purification using HPLC allowed isolation of the following compounds (in order of increasing polarity): 16-acetylfuroscalarol (**9**, 0.007% dry wt), 12-deacetoxyscalaradial (**3**, 0.008% dry wt), 19-deoxyscalarin (**4**, 0.005% dry wt), furoscalarol (**5**, 0.005% dry wt), scalaradial (**1**, 0.385% dry wt), 18-*epi*-scalaradial (**6**, 0.008% dry wt), norscalaral A (**10**, 0.008% dry wt), 12-*epi*-acetylscalarolide (**8**, 0.024% dry wt), norscalaral C (**12**, 0.004% dry wt), norscalaral B (**11**, 0.020% dry wt), 19-dihydroscalaradial (**7**, 0.008% dry wt), and scalarin (**2**, 0.141% dry wt). Comparison of spectral data with those reported in the literature allowed the identification of compounds **1**,[16,17] **2**,[15,18] **3**,[19] and **5**.[20,21] 19-Deoxyscalarin (**4**) had not been reported as a natural compound and was identified by comparison of spectral data with those reported for the synthetic derivative.[18]

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(1) Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 4976.
(2) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *Comp. Biochem. Physiol.* **1982**, *73B*, 471.
(3) Terem, B.; Scheuer, P. J. *Tetrahedron* **1986**, *42*, 4409.
(4) Rogers, S. D.; Paul, V. J. *Mar. Ecol. Prog. Ser.* **1991**, *77*, 221.
(5) Cimino, G.; Fontana, A.; Giménez, F.; Marín, A.; Mollo, E.; Trivellone, E.; Zubía, E. *Experientia* **1993**, *49*, 582.
(6) D'Ischia, M.; Prota, G.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 3295.
(7) Cimino, G.; Spinella, A.; Sodano, G. *Tetrahedron Lett.* **1984**, *25*, 4151.
(8) Cimino, G.; Sodano, G.; Spinella, A. *Tetrahedron* **1987**, *43*, 5401.
(9) de Carvalho, M. S.; Jacobs, R. S. *Biochem. Pharmacol.* **1991**, *42*, 1621.
(10) Potts, B. C. M.; Faulkner, D. J.; de Carvalho, M. S.; Jacobs, R. S. *J. Am. Chem. Soc.* **1992**, *114*, 5093.
(11) Faulkner, D. J. *Nat. Prod. Rep.* **1996**, *13*, 75 and previous reviews of this series.
(12) Carotenuto, A.; Conte, M. R.; Fattorusso, E.; Lanzotti, V.; Magno, S. *Tetrahedron* **1995**, *51*, 10751.
(13) Carotenuto, A.; Fattorusso, E.; Lanzotti, V.; Magno, S.; Mayol, L. *Liebigs Ann. Chem.* **1996**, 77.
(14) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 515.
(15) Fattorusso, E.; Magno, S.; Santacroce, C; Sica, D. *Tetrahedron* **1972**, *28*, 5993.
(16) Cimino, G.; De Stefano, S.; Minale, L. *Experientia* **1974**, *30*, 846.
(17) Cimino, G.; De Stefano, S.; Di Luccia, A. *Experientia* **1979**, *35*, 1277.
(18) Cimino, G.; De Stefano, S.; Minale, L.; Trivellone, E. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1587.
(19) De Rosa, S.; Puliti, R.; Crispino, A.; De Giulio, A.; Mattia, C. A.; Mazzarella, L. *J. Nat. Prod.* **1994**, *57*, 256.
(20) Cafieri, F.; De Napoli, L.; Fattorusso, E.; Santacroce, C.; Sica D. *Gazz. Chim. Ital.* **1977**, *107*, 71.
(21) Cimino, G.; Cafieri, F.; De Napoli, L.; Fattorusso, E. *Tetrahedron Lett.* **1978**, 2041.

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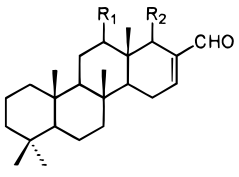

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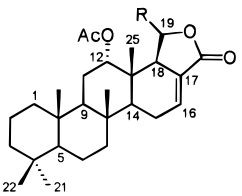
3 R_1 = H; R_2 = 18β-CHO
6 R_1 = 12α-OAc; R_2 = 18α-CHO
7 R_1 = 12α-OAc; R_2 = 18β-CH_2OH
13 R_1 = 12β-OAc; R_2 = 18β-CHO
14 R_1 = 12β-OAc; R_2 = 18α-CHO



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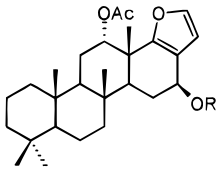
4 R= H



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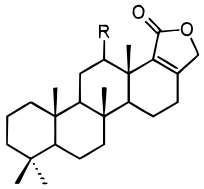
9 R= Ac



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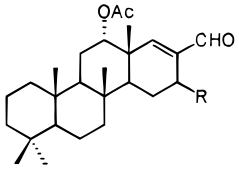

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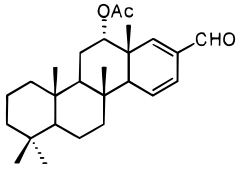
11 R= 16α-OH



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18-*epi*-Scalaradial (**6**) was obtained as an amorphous powder. The molecular formula, $C_{27}H_{40}O_4$, was obtained from the high resolution mass measurement. The infrared absorptions at 2852, 1685, and 1652 cm^{-1} were consistent with the presence of an α,*β*-unsaturated aldehyde. The ^1H NMR spectrum of **6** (Table 1) strongly resembled that of scalaradial (**1**). Thus, five singlets at *δ* 0.81, 0.82, 0.86, 0.90, and 0.94 were assigned to the five methyl groups of a scalarane skeleton. Two signals at *δ* 9.39 (s, 1H) and 9.54 (d, 1H, J = 4.3 Hz) were due to two aldehyde protons. The signal at *δ* 7.07 (dd, 1H, J = 4.3, 3.2 Hz) was assigned to an olefinic proton of the conjugated system. The signal at *δ* 4.88 (dd, 1H, J = 2.8, 2.8 Hz), typical of the proton geminal to an acetoxyl group, indicated an axial orientation for the acetoxyl at C-12 and therefore a configuration at this carbon identical to that of scalaradial (**1**). The main difference in the ^1H NMR spectrum of **6** with that of scalaradial (**1**) was observed on the H-18 signal that in the spectrum of **6** appeared at *δ* 3.21 (dd, 1H, J = 4.3, 0.9 Hz). Both the chemical shift and multiplicity of this signal clearly indicated that **6** was the C-18 epimer of scalaradial (**1**).

A comparison between the ^{13}C NMR data of 18-*epi*-scalaradial (**6**) (Table 1) with those reported[17] for scalaradial (**1**), 12-*epi*-scalaradial (**13**), and 12,18-di-*epi*-scalaradial (**14**) provided confirmation to the proposed structural assignments. The most significant differences were observed in C-14, C-19, and C-25 carbon signals. The upfield shift of C-14 resonance in **6** at *δ* 45.2 upon comparison with that of **1** (*δ* 49.4) and **14** (*δ* 48.1) is due to the *γ*-gauche shielding effects[22] of both axial acetoxyl and aldehyde groups in **6**. Because C-14 in 12-*epi*-scalaradial (**13**) lacks any of this *γ*-gauche interaction, it presents a higher chemical shift (*δ* 53.5). A similar rationale indicated that the C-25 angular methyl group in **6** at *δ* 21.8 is deshielded as compared with those of **1** (*δ* 15.2), **13** (*δ* 11.0), and **14** (*δ* 16.9) due to the absence of *γ*-gauche interactions between C-25 and the axial substituents at C-12 and C-18. Futhermore the resonance of the aldehyde appears upfield shifted at *δ* 195.9 upon comparison with those of **1** (*δ* 201.0), **13** (*δ* 200.0), and **14** (*δ* 199.4) as expected for an axial orientation of the aldehyde at C-18 and the acetoxyl group at C-12.

In vitro assays under biomimetic conditions by Cimino et al. have concluded that the stereochemistry at C-18 determines the ability of these 1,4-dialdehydes to react with methylamine.[8] 18-*epi*-Scaralaldial (**6**) represents the missing stereoisomer in these structure−activity experiments, and therefore we tested the ability of **6** to react with methylamine under the conditions described by Cimino. All our attempts were unfruitful, confirming that the larger distance between the axial aldehyde at C-18 and the aldehyde at C-17 blocks the reactions with primary amines. Furthermore, it has been reported that epimerization at C-18 causes a significant loss in potency as well as the ability to complete inactivate the bee venom PLA_2.[10] Consequently, 18-*epi*-scalaradial (**6**) might be expected to be less active than other members of this series upon antiinflamatory testing, though this point has yet to be demonstrated.

19-Dihydroscalaradial (**7**) was obtained as an amorphous powder. The molecular formula, $C_{27}H_{42}O_4$, was deduced from the high resolution mass measurement. The infrared spectrum contained a hydroxyl band centered at 3447 cm^{-1} together with the acetoxyl and the α,*β*-unsaturated carbonyl system bands at 1736, 1674, and 1632 cm^{-1}. It is worth noting that although TLC and HPLC analyses indicated that the compound was obtained with a high level of purity, the ^1H NMR spectrum showed a number of minor signals at *δ* 6.0−2.5 and duplicity on the methyl proton signals. However a careful analysis of the ^1H NMR, ^{13}C NMR, COSY, and HETCOR spectra allowed the structural elucidation of **7**.

Both ^1H and ^{13}C NMR spectra (Table 1) clearly indicated that **7** was an acetylated sesterterpene with scalarane skeleton. The signal at *δ* 5.13 (dd, 1H, J = 3.0, 2.6 Hz) that was correlated in the HETCOR with the ^{13}C NMR signal at *δ* 74.0 (d) was consistent with the presence of an α-acetoxy substituent at C-12. A comparison of the NMR data of **7** with those of **1**[16,17] and **3**[19] indicated that **7** bears the α,*β*-unsaturated aldehyde but lacks the second aldehyde signal, presenting instead two signals at *δ* 3.45 (ddd, 1H, J = 12.0, 7.1, 3.2 Hz) and 3.60 (dd, 1H, J = 12.0, 11.1 Hz), indicating the presence of a primary alcohol. Since the proton signals at *δ* 3.45 and 3.60 showed a cross peak in the COSY spectrum with the H-18 signal at *δ* 2.71 (m, 1H) which showed a long range coupling with the H-15 signals, the hydroxyl group must be located at C-19.

The ^{13}C NMR signals of C-9, C-14, and C-25 at *δ* 51.8 (d), 49.7 (d), and 14.5 (q) respectively, required axial orientation for the acetoxyl group at C-12 and equatorial for the hydroxymethylene group at C-18. A series of NOE difference spectroscopy experiments provided confirmation to the proposed stereochemistry, in particular, the enhancement of the H-14 proton signal upon irradiation of the H-18 signal and the enhancement of the C-25 methyl signal upon irradiation of one of the H-19 signals.

(22) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*, 3rd ed.; VCH: New York, 1989; p 183.

Table 1. NMR Data for Scalaranes 6–9[a,b]

	6		7		8		9	
no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.6	0.56 m; 1.58 m	39.6	0.62 m; 1.60 m	39.7	0.56 m; 1.57 m	39.6	0.62 m; 1.59 m
2	18.4[c]	1.38 m; 1.58 m	18.4[c]	1.39 m; 1.57 m	18.4[c]	1.37 m; 1.60 m	18.0[c]	1.42 m; 1.58 m
3	42.0[d]	1.14 m; 1.35 m	42.0[d]	1.13 m; 1.39 m	42.0[d]	1.10 m; 1.35 m	41.3[d]	1.13 m; 1.34 m
4	33.3		33.2		33.3		33.3	
5	56.4	0.85 m	56.4	0.82 m	56.7	0.82 m	56.6	0.87 m
6	18.0[c]	1.38 m; 1.58 m	18.0[c]	1.39 m; 1.57 m	18.1[c]	1.43 m; 1.55 m	18.5[c]	1.28 m; 1.43 m
7	41.3[d]	1.10 m; 1.76 dt (12.4, 3.2)	41.4[d]	1.00 m; 1.75 m	41.5[d]	1.02 ddd (12.6, 12.6, 4.1); 1.84 ddd (12.6, 3.3, 3.3)	41.9[d]	1.06 m; 1.80 m
8	36.8[e]		36.7[e]		38.6[e]		36.9[e]	
9	52.3	1.25 m	51.8	1.26 m	53.0	1.19 m	53.0	1.25 m
10	37.8[e]		36.8[e]		37.5[e]		37.2[e]	
11	22.4	1.67 ddd (14.9, 12.7, 2.4); 1.84 ddd (14.9, 3.2, 2.4)	22.5	1.67 m; 1.82 m	20.9	1.66 m; 2.00 m	21.7	1.71 m; 1.87 m
12	76.9	4.88 dd (2.8, 2.8)	74.0	5.13 dd (3.0, 2.6)	73.9	5.54 dd (2.9, 2.7)	73.2	5.41 dd (2.8, 2.8)
13	41.9		37.5[e]		36.9[e]		40.6	
14	45.2	2.18 dd (10.9, 6.9)	49.7	1.54 m	51.2	1.58 m	49.7	1.80 m
15	25.1	2.32 m; 2.61 m	24.8	2.32 m; 2.42 m	16.8	1.57 m; 1.90 m	24.9	2.23 dd (11.9, 6.7); 1.64 m
16	152.8	7.07 dd (4.3, 3.2)	156.1	6.97 m	24.9	2.30 m; 2.36 m	69.1	5.77 dd (9.4, 6.6)
17	136.0		142.9		133.0		116.4	
18	56.5	3.21 dd (4.3, 0.9)	47.0	2.71 m	159.7		158.2	
19	195.9	9.54 d (4.3)	59.7	3.45 ddd (12.0, 7.1, 3.2); 3.60 dd (12.0, 11.1)	169.8[f]		141.3	7.18 d (1.9)
20	191.5	9.39 s	197.2	9.38 s	70.8	4.54 d (16.9); 4.61 d (16.9)	108.5	6.18 d (1.9)
21	33.2	0.86 s	33.2	0.85 s	33.3	0.85 s	33.2	0.85 s
22	21.3	0.81 s	21.3	0.81 s	21.3	0.80 s	21.3	0.81 s
23	16.2[f]	0.82 s	16.2[f]	0.93 s	15.9[g]	0.81 s	15.9[f]	0.93[g] s
24	16.4[f]	0.94 s	16.2[f]	0.81 s	17.0[g]	0.90 s	17.2[f]	0.82[g] s
25	21.8	0.90 s	14.5	0.74 s	21.3	1.17 s	21.9	1.13 s
*C*H$_3$CO	21.3	2.02 s	21.2	2.12 s	21.2	1.95 s	21.2	1.84 s
CH$_3$*C*O	169.4		170.1		171.7[f]		170.2	
*C*H$_3$CO							21.4	2.10 s
CH$_3$*C*O							171.2	
O*H*				4.32 dd (11.1, 3.2)				

[a] Assignments were aided by COSY, APT, and HETCOR experiments. [b] Coupling constants are presented in hertz units. [c–g] Values with the same superscript in the same column may be interchanged.

It was therefore proposed that **7** was the alcohol that would arise by reduction of scalaradial (**1**) at C-19.

The minor signals observed in the NMR spectra of 19-dihydroscalaradial (**7**) were attributable to the pair of cyclic epimeric hemiacetals formed in solution by intramolecular nucleophilic attack of the C-19 hydroxyl group to the aldehyde.

12-*epi*-Acetylscalarolide (**8**) was isolated as an amorphous powder. The molecular formula, $C_{27}H_{40}O_4$, was obtained from the high resolution mass measurement. The ^{1}H NMR methyl singlets at δ 0.80, 0.81, 0.85, 0.90, and 1.17 that were correlated in the HETCOR experiment with the ^{13}C NMR signals at δ 21.3, 15.9, 33.3, 17.0, and 21.3, respectively, indicated that **8** was a scalarane sesterterpene. The infrared absorption at 1746 cm^{-1}, together with the ^{1}H NMR signals at δ 4.61 (d, 1H, J = 16.9 Hz) and 4.54 (d, 1H, J = 16.9 Hz) and the ^{13}C NMR signals δ 169.8 (s), 159.7 (s), 133.0 (s), and 70.8 (t), indicated the presence of an α,β-unsaturated γ-lactone ring and that **8** was therefore related to the pentacyclic scalaranes. The ^{1}H NMR signals at δ 1.95 (s, 3H) and δ 5.54 (dd, 1H, J = 2.9, 2.7 Hz) and the ^{13}C NMR signals at 171.7 (s), 73.9 (d) and 21.2 (q) indicated that **8** possessed a 12α-acetoxy substituent. These evidences together with the comparison of the NMR data of **8** (Table 1) with those of 12-acetylscalarolide (**15**)[23] confirmed that **8** was the C-12 epimer of the known compound **15**.

16-Acetylfuroscalarol (**9**) was isolated as a colorless oil. The molecular formula, $C_{29}H_{42}O_5$, was established from the high resolution mass meassurement. The presence of five methyl groups that in the ^{1}H NMR spectrum gave rise to five singlets at δ 1.13, 0.93, 0.85, 0.82, and 0.81, together with the presence of a disubstituted furan ring whose proton resonances appeared at δ 7.18 (d, 1H, J = 1.9 Hz) and 6.18 (d, 1H, J = 1.9 Hz) indicated that **9** was a pentacyclic sesterterpene related to furoscalarol (**5**). A comparison of the ^{1}H and ^{13}C NMR spectra of **9** (Table 1) with those of furoscalarol (**5**)[20,21] showed that **9** contained an additional acetyl group as indicated by the signal at δ 2.10 (s, 3H). The downfield shift of the H-16 signal, which in the alcohol **5** appears at δ 4.67 (dd, 1H, J = 9.4, 6.4 Hz) and in the acetate **9** at δ 5.77 (dd, 1H, J = 9.4, 6.6 Hz), indicated that **9** was the 16-acetyl derivative of furoscalarol (**5**). Acetylation of **5**, whose absolute configuration had been determined using the Horeau method,[21] yielded a compound identical in all respects to **9**, indicating that its absolute configuration is that represented in formula **9**. In the absence of an independent determination, the other new metabolites from *C. scalaris* are attributed to the same enantiomeric series on biogenetic grounds.

Norscalaral A (**10**) was isolated as an amorphous powder; the molecular formula, $C_{26}H_{40}O_4$, was obtained from the high resolution mass measurement. The infrared spectrum contained a hydroxyl band at 3518 cm^{-1}, a carbonyl band at 1738 cm^{-1}, and the α,β-unsaturated carbonyl bands at 1675 and 1660 cm^{-1}. The ^{1}H NMR spectrum of **10** (Table 2) showed the five singlets at δ 1.18, 0.91, 0.86, 0.82, and 0.81 that were correlated on the HETCOR experiment with the carbon signals at δ

(23) Bergquist, P. R.; Cambie, R. C.; Kernan, M. R. *Biochem. Syst. Ecol.* **1990**, *18*, 349.

Table 2. NMR Data for Norscalaranes A−C (10−12)[a,b]

	10		11		12	
no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.7	0.62 m; 1.56 m	39.7	0.63 m; 1.55 m	39.7	0.67 m; 1.56 m
2	18.5[c]	1.38 m; 1.60 m	18.5[c]	1.37 m; 1.57 m	18.4[c]	1.43 m; 1.64 m
3	42.0[d]	1.12 m; 1.38 m	42.0[d]	1.12 m; 1.34 m	42.0[d]	1.15 m; 1.40 m
4	33.3		33.3		33.3	
5	56.7	0.83 m	56.5	0.86 m	56.8	0.88 m
6	18.1[c]	1.41 m; 1.64 m	18.0[c]	1.39 m; 1.59 m	18.7[c]	1.47 m; 1.60 m
7	41.0[d]	1.00 m; 1.84 m	40.8[d]	1.10 m; 1.83 m	40.5[d]	1.01 m; 1.94 ddd (12.7, 3.2, 3.2)
8	37.0[e]		36.9[e]		36.9[e]	
9	52.9	1.22 m	52.9	1.35 m	52.3	1.30 m
10	37.2[e]		37.0[e]		37.1[e]	
11	22.0	1.72 m; 1.80 m	22.1	1.74 dd (12.6, 2.4); 1.79 m	22.2	1.70 m; 1.84 ddd (14.8, 3.0, 3.0)
12	76.2	5.05 dd (3.0, 2.8)	76.3	5.06 dd (2.8, 2.6)	75.3	5.01 dd (3.1, 2.4)
13	41.9		41.6		31.9	
14	47.3	1.51 m	44.3	1.93 m	51.4	2.57 m
15	25.5	1.47 m; 2.15 m	25.2	1.66 dd (13.7, 4.7); 1.88 m	118.8	6.32 ddd (9.8, 3.1, 1.4)
16	67.3	4.62 dd (9.0, 6.8)	61.9	4.58 d (4.5)	129.7	5.99 ddd (9.8, 2.3, 0.6)
17	140.5		139.9		136.4	
18	160.7	6.45 s	160.9	6.52 s	155.3	6.39 d (0.9)
19						
20	195.8	9.35 s	195.1	9.41s	191.2	9.36 d (0.6)
21	33.3	0.86 s	33.2	0.84 s	33.3	0.87 s
22	21.3	0.81 s	21.3	0.80 s	21.4	0.83 s
23	15.9[f]	0.82 s	15.9[f]	0.82 s	15.8[f]	0.83 s
24	17.1[f]	0.91 s	17.0[f]	0.89 s	16.5[f]	1.03 s
25	21.1	1.18 s	19.6	1.06 s	17.8	1.02 s
*C*H$_3$CO	21.2	2.03 s	21.4	2.05 s	21.4	2.02 s
CH$_3$*C*O	170.4		170.8		170.4	
O*H*		3.68 br s		2.56 br s		

[a] Assignments were aided by COSY, APT, and HETCOR experiments. [b] Coupling constants are presented in hertz units. [c−f] Values with the same superscript in the same column may be interchanged.

21.1, 17.1, 33.3, 15.9, and 21.3, respectively, suggesting that **10** possessed a tetracarbocyclic skeleton related to those above described. Considering that in the ^{1}H NMR spectrum appeared a singlet at δ 2.03 (s, 3H) assigned to an acetate group which accounts for two carbons of the molecular formula, **10** must be a norscalarane. The acetate was located at 12α upon observation of the geminal proton signal H-12eq at δ 5.05 (dd, 1H, J = 3.0, 2.8 Hz). The structure of ring D of the tetracyclic norsesterterpene skeleton was established as follows. A singlet at δ 9.35 (s, 1H) was assigned to an aldehyde attached to a quaternary carbon. A singlet at δ 6.45 (s, 1H) which was correlated on the HETCOR experiment with the carbon signal at δ 160.7 (d) together with the signal at δ 140.5 (s) were assigned to a trisubstituted double bond conjugated with the aldehyde. Finally, a signal at δ 4.62 (dd, 1H, J = 9.0, 6.8 Hz) was assigned to a proton geminal to a hydroxyl group. This signal showed allylic coupling in the COSY spectrum with the olefinic proton signal at δ 6.45 and vicinal coupling with the methylene protons signals at δ 2.15 (m, 1H) and 1.47 (m, 1H) which were, in addition, coupled with a methine proton signal at δ 1.51 (m, 1H). These data indicated the presence of a secondary alcohol connected both with the α,β-unsaturated aldehyde and with the methylene group as depicted in formula **10**. The stereochemistry at C-16 was defined upon observation of the value of the H-16 coupling constants (9.0 and 6.8 Hz) attributable to an axial proton and therefore indicating a β-orientation for the hydroxyl group at C-16.[21]

Norscalaral B (**11**) was isolated as an amorphous powder. The molecular formula, $C_{26}H_{40}O_4$, indicated that **11** was an isomer of norscalaral A (**10**). Furthermore, the ^{1}H NMR spectrum of **11** (Table 2) was very similar to that of **10** excepting the H-16 signal at δ 4.58 (d, 1H, J = 4.5 Hz), indicating an axial orientation of the hydroxyl group at C-16 which shifted the methine proton signal H-14 from δ 1.51 in **10** to δ 1.93 (m, 1H). It was therefore proposed that norscalaral B (**11**) was the C-16 epimer of norscalaral A (**10**).

Norscalaral C (**12**) was isolated as an amorphous powder. The molecular formula, $C_{26}H_{38}O_3$, was obtained from the high resolution mass measurement. Comparison of the ^{13}C NMR data of **12** (Table 2) with those of the norscalaranes **10** and **11** above described showed the absence of the signal corresponding to the carbon bearing the hydroxyl group and, in turn, the presence of two additional olefinic carbon signals at δ 129.7 (d) and 118.8 (d) that were correlated in the HETCOR experiment with the proton signals at 5.99 (ddd, 1H, J = 9.8, 2.3, 0.6 Hz) and 6.32 (ddd, 1H, J = 9.8, 3.1, 1.4 Hz). These data indicated that norscalaral C (**12**) was the corresponding dehydration product of the alcohols **10** and **11**.

In general 19-norscalaranes are uncommon natural products and up to now only the accounts from two marine sponges, *Hyrtios erecta* and *Collospongia auris*, have been reported.[11]

As a part of our investigations directed toward the search of new antitumor compounds from marine origin, the new compounds isolated from *C. scalaris* were tested against P-388 and SCHABEL mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma. The new compounds showed significant cytotoxicity toward the five tumor cell lines above mentioned with ED_{50} values that are reported in the Experimental Section between 1 and 5 μg/mL. 18-*epi*-Scalaradial (**6**) showed the strongest, though nonselective cytotoxicity toward P-388, Schabel, A-549, and HT-29 tumor cell lines (ED_{50} = 0.2 μg/mL).

Experimental Section

General. ^{1}H NMR and ^{13}C NMR spectra were made at 399.952 MHz and 100.577 MHz, respectively, using $CDCl_3$ as solvent. The resonance of residual chloroform at δ_H 7.26 and

δ_C 77.00 was used as internal reference for ^1H and ^{13}C spectra, respectively. In high performance liquid chromatography separations LiChrosorb silica 60 was used in normal phase mode using a differential refractometer and a UV detectors, and LiChrosorb RP-18 was used in reversed phase mode using a differential refractometer. All solvents were spectral grade or distilled from glass prior to use.

Collection, Extraction, and Isolation Procedures. The sponge *Cacospongia scalaris* (59 g dry wt) was collected by hand using SCUBA near Tarifa Island in July 1994 and was immediately frozen. The frozen tissue was extracted exhaustively with acetone at room temperature for 1 h. The filtered Me_2CO solution was evaporated under reduced pressure, and the aqueous residue was extracted with Et_2O. The solvent was evaporated to give an oil residue (6.4 g) which was chromatographed on a SiO_2 column using solvents of increasing polarity from hexane to diethyl ether and, subsequently, chloroform−methanol (8:2). Fractions eluted with 10% ether in hexane were further separated on normal phase HPLC eluting with hexane−EtOAc (95:5) to afford 16-acetylfuroscalarol (**9**, 4 mg, 0.007% dry wt). Fractions eluted with 20% ether in hexane were crystallized from EtOH to obtain 12-deacetoxyscalaradial (**3**, 5 mg, 0.008% dry wt). Fractions of the general chromatography eluted with 50% ether in hexane contained 19-deoxyscalarin (**4**, 3 mg, 0.005% dry wt) and furoscalarol (**5**, 3 mg, 0.005% dry wt). Crystallization in hexane−EtOAc of the fractions eluted with 70% ether in hexane yielded scalaradial (**1**, 227 mg, 0.385% dry wt). The mother liquors were subjected to normal phase HPLC separation, eluting with hexane−EtOAc (8:2) to yield 18-*epi*-scalaradial (**6**, 5 mg, 0.008% dry wt) and norscalaral A (**10**, 4.5 mg, 0.008% dry wt). Fractions eluted with 80% ether in hexane were subjected to normal phase HPLC separation eluting with hexane−EtOAc (7:3) to obtain 12-*epi*-acetylscalarolide (**8**, 14 mg, 0.024% dry wt), a mixture that was further separated by reversed phase HPLC eluting with CH_3CN-H_2O (9:1) to yield norscalaral C (**12**, 2.2 mg, 0.004% dry wt), and norscalaral B (**11**, 12 mg, 0.020% dry wt), and 19-dihydroscalaradial (**7**, 5 mg, 0.008% dry wt). More polar fractions of the general chromatography were crystallized in hexane−EtOAc to afford scalarin (**2**, 83 mg, 0.141% dry wt).

18-*epi*-scalaradial (6): amorphous powder; $[\alpha]_D = -37.9°$ ($c = 0.8$, $CHCl_3$); IR (film) 2852, 1736, 1685, 1652, 1236 cm^{-1}; UV (MeOH) λ_{max} 226 ($\epsilon = 6236$) nm; EIMS (70 eV) m/z (rel int) 428 (1), 369 (42), 340 (14), 258 (5), 205 (52), 191 (100), 176 (50), 137 (19), 123 (30); HREIMS obsd $m/z = 428.2910$ $(M)^+$, $C_{27}H_{40}O_4$ requires $m/z = 428.2926$; see Table 1 for NMR spectral data.

19-Dihydroscalaradial (7): amorphous powder; $[\alpha]_D = +50.5°$ ($c = 0.4$, $CHCl_3$); IR (film) 3447, 2853, 1736, 1674, 1632, 1243 cm^{-1}; UV (MeOH) λ_{max} 230 ($\epsilon = 2184$) nm; EIMS (70 eV) m/z (rel int) 431 (7), 413 (17), 370 (27), 352 (50), 258 (5), 205 (26), 191 (100), 137 (23), 123 (36); HREIMS obsd $m/z = 412.2977$ $(M\text{-}H_2O)^+$, $C_{27}H_{40}O_3$ requires $m/z = 412.2999$; see Table 1 for NMR spectral data.

12-*epi*-Acetylscalarolide (8): amorphous powder; $[\alpha]_D = +47.8°$ ($c = 0.9$, $CHCl_3$); IR (film) 1746, 1675, 1245 cm^{-1}; UV (MeOH) λ_{max} 216 ($\epsilon = 4871$) nm; EIMS (70 eV) m/z (rel int) 429 (27), 428 (3), 385 (38), 369 (100), 368 (18), 258 (3), 205 (43), 191 (24), 137 (5), 123 (9); HREIMS obsd $m/z = 428.2931$ $(M)^+$, $C_{27}H_{40}O_4$ requires $m/z = 428.2926$; see Table 1 for NMR spectral data.

16-Acetylfuroscalarol (9): oil; $[\alpha]_D = +15.0°$ ($c = 0.2$, $CHCl_3$); IR (film) 1744, 1243 cm^{-1}; UV (MeOH) λ_{max} 224 ($\epsilon = 3901$), 270 ($\epsilon = 672$) nm; EIMS (70 eV) m/z (rel int) 470 (3), 428 (1), 410 (1), 368 (8), 353(6), 197 (100), 158 (20), 145 (20), 137 (14), 123 (7); HREIMS obsd $m/z = 470.3075$ $(M)^+$, $C_{29}H_{42}O_5$ requires $m/z = 470.3032$; see Table 1 for NMR spectral data.

Norscalaral A (10): amorphous powder; $[\alpha]_D = +48.5°$ ($c = 0.2$, $CHCl_3$); IR (film) 3518, 2852, 1738, 1675, 1660, 1246 cm^{-1}; UV (MeOH) λ_{max} 225 ($\epsilon = 4913$) nm; EIMS (70 eV) m/z (rel int) 416 (16), 398 (2), 370 (7), 356 (11), 327 (34), 258 (6), 205 (8), 191 (100), 137 (12), 123 (23); HREIMS obsd $m/z = 416.2911$ $(M)^+$, $C_{26}H_{40}O_4$ requires $m/z = 416.2926$; see Table 2 for NMR spectral data.

Norscalaral B (11): amorphous powder; $[\alpha]_D = +5.2°$ ($c = 0.4$, $CHCl_3$); IR (film) 3438, 2869, 1737, 1686, 1655, 1247 cm^{-1}; UV (MeOH) λ_{max} 222 ($\epsilon = 6252$) nm; EIMS (70 eV) m/z 416 (28), 399 (38), 338 (52), 323 (41), 258 (3), 205 (23), 191 (100), 137 (34), 123 (52); HREIMS obsd $m/z = 416.2924$ $(M)^+$, $C_{26}H_{40}O_4$ requires $m/z = 416.2926$; see Table 2 for NMR spectral data.

Norscalaral C (12): amorphous powder; $[\alpha]_D = +22.3°$ ($c = 0.2$, $CHCl_3$); IR (film) 2853, 1744, 1704, 1692, 1666, 1242 cm^{-1}; UV (MeOH) λ_{max} 290 ($\epsilon = 5317$) nm; HREIMS obsd $m/z = 398.2813$ $(M)^+$, $C_{26}H_{38}O_3$ requires $m/z = 398.2821$; see Table 2 for NMR spectral data.

Acetylation of Furoscalarol (5). An excess of Ac_2O was added to a solution of **5** (3 mg) in dry pyridine. The mixture was kept overnight at room temperature, and the residual pyridine and Ac_2O were removed by distillation under reduced presure. The residue was purified on a small Si gel column using hexane−EtOAc (9:1) to obtain 16-acetylfuroscalarol (**9**) (1 mg).

Cytotoxicity Assays. The new compounds were tested against five tumor cell lines. The individual cell lines identifiers are given along with the corresponding ED_{50} (μg/mL) values for each compound tested.

18-*epi*-Scalaradial (6): P-388 (0.2), Schabel (0.2), A-549 (0.2), HT-29 (0.2), MEL-28 (0.5); **19-dihydroscalaradial (7):** P-388 (2), Schabel (2), A-549 (2), HT-29 (2), MEL-28 (2.5); **12-*epi*-acetylscalarolide (8):** P-388 (1), Schabel (1), A-549 (2), HT-29 (2), MEL-28 (2); **16-acetylfuroscalarol (9):** P-388 (2.5), Schabel (2.5), A-549 (5), HT-29 (2.5), MEL-28 (10); **norscalaral A (10):** P-388 (1), Schabel (1), A-549 (1), HT-29 (1), MEL-28 (2); **norscalaral B (11):** P-388 (2), Schabel (2), A-549 (2), HT-29 (2), MEL-28 (2); **norscalaral C (12):** P-388 (1.2), A-549 (2.5), HT-29 (5), MEL-28 (2.5).

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Supporting Information Available: Copies of the ^1H and ^{13}C NMR spectra of **6**−**12** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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