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Sesterterpene metabolites from the sponge *Hyatella intestinalis*

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Abstract—The sponge *Hyatella intestinalis* from the Gulf of California contains the new scalarane-related sesterterpenes hyatelones A–C and hyatolides A–B, together with the new scalaranes hyatolides C–E, hyatelactam, 12-*O*-deacetyl-19-*epi*-scalarin and the new norscalarane 12-*O*-deacetylnorscalaral B. The structures of the new metabolites have been established by spectroscopic analysis of the natural products and, in some instances, of their acetyl derivatives. The new compounds hyatelone A, 19,20-di-*O*-acetylhyatelone B, hyatolide A, 20-*O*-acetylhyatolide C, hyatelactam, and 12-*O*-deacetylnorscalaral B have shown activity as growth inhibitors of several tumor cell lines.

1. Introduction

Among the natural terpenoids, the C_{25} derivatives or sesterterpenes are the least common group, with most of the compounds described from marine organisms, in particular from sponges of the Order Dictyoceratida.[1] From a structural point of view, a significant group of marine-derived sesterterpenes displays a tetracarbocyclic framework formed by six-membered rings. Within this group, scalarin (**1**)[2] is the parent compound of the scalarane skeleton, which possess C-19 and C-20 linked to C-18 and C-17 of ring D, respectively. On the other hand, furoscalarol (**2**)[3] was the first member of a more unusual series of scalarane-related compounds whose framework is characterized by possessing a two-carbons unit (C-19 and C-20) linked to C-17 of ring D. From a biomedical point of view, a number of scalaranes and related sesterterpenes has shown interesting properties, recent examples of which include antibacterial[4] and cytotoxic activities.[5]

As a part of our project directed to the search for cytotoxic metabolites from sponges, we have studied specimens of the species *Hyatella intestinalis* whose extracts showed growth inhibitory activity of the tumor cell lines A-549 and HT-29. Previous studies of this sponge collected in Australia and the Indo-Pacific region have yielded spongiane diterpenes,[6] scalaranes,[7] and sesquiterpene-quinones.[8] In addition, macrolides,[9] likely of symbiotic origin, and ansa farnesyl quinols[10] have been obtained from unidentified species of the genus *Hyatella* collected from the Indonesian and Kenyan coasts, respectively. Herein we report the chemical study of *H. intestinalis* from the Gulf of California, that has yielded the new sesterterpenes hyatelones A–C (**3**–**5**), hyatolides A–E (**6**–**10**), hyatelactam (**11**), 12-*O*-deacetyl-19-*epi*-scalarin (**12**), and 12-*O*-deacetylnorscalaral B (**13**). The sponge also contained the known compounds furoscalarol (**2**),[3] norscalaral A,[11] norscalaral B (**14**),[11] deoxoscalarin,[12] 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide,[13] 12-*epi*-12-*O*-acetylscalarolide,[11] 12-*epi*-12-*O*-deacetyl-19-deoxyscalarin,[14] and scalarin (**1**).[2]

2. Results and discussion

Specimens of *H. intestinalis* were collected by hand using SCUBA diving, liophylized, and exhaustively extracted with acetone/MeOH (1:1). After evaporation of the solvent under reduced pressure, the residue was partitioned between H_2O and Et_2O and the resulting organic extract was subjected to column chromatography. Fractions eluted with hexane/Et_2O (60:40 to 30:70) and $CHCl_3$/MeOH (80:20) were subjected to repeated HPLC separations to yield the new compounds **3**–**13**. Compounds **4**, **5**, **8**, and **10** were characterized as their acetyl derivatives **4a**, **5a**, **8a**, and **10a**, respectively.

2.1. Hyatelones A–C (3–5)

Hyatelone A (**3**) was obtained as a solid whose molecular formula $C_{27}H_{40}O_6$ was determined by HRCIMS. The ^{13}C NMR spectrum displayed 27 signals (Table 1), two of them attributable to an acetoxyl group [δ 169.6 (CH_3COO–) and 21.3 (CH_3COO–)]. The remaining 25 carbon signals, together with the five singlets observed in the ^1H NMR spectrum at

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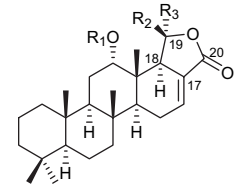

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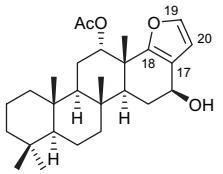
12 R_1 = R_3 = H, R_2 = OH



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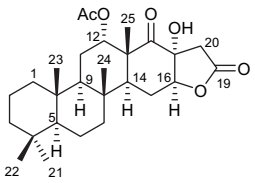

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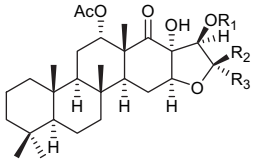
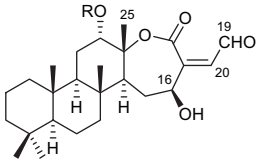


4 R_1 = R_3 = H, R_2 = OH
4a R_1 = Ac, R_2 = OAc, R_3 = H
5 R_1 = R_2 = H, R_3 = OH
5a R_1 = Ac, R_2 = H, R_3 = OAc



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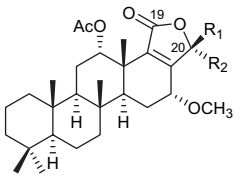
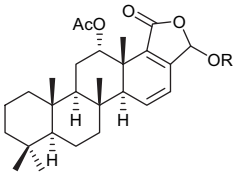


8 R_1 = OH, R_2 = H
8a R_1 = OAc, R_2 = H
9 R_1 = H, R_2 = OH



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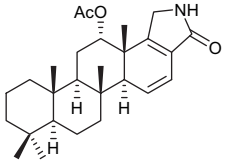
10a R = Ac



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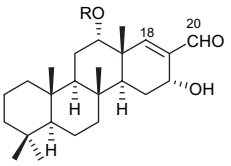


13 R = H
14 R = Ac

δ 1.27 (3H), 0.93 (3H), 0.86 (3H), 0.82 (3H), and 0.81 (3H), suggested that **3** was a sesterterpene. In particular, the presence of a scalarane or related tetracarbocyclic skeleton was deduced from the ^{13}C NMR signals due to three methines [δ 56.5 (C-5), 52.3 (C-9), and 44.0 (C-14)] and four quaternary sp^3 carbons [δ 51.1 (C-13), 38.2 (C-8), 36.9 (C-10), and 33.3 (C-4)]. The NMR signals at δ_C 72.8 (d)/δ_H 5.31 (1H, dd, J=3.5 and 2.2 Hz) were assigned to a methine bearing the acetoxyl group. This methine was located at C-12 based on the HMBC correlation between the carbon at δ 72.8 and Me-25 (δ 1.27). The presence of a ketone carbonyl at C-18 was deduced from the ^{13}C NMR signal at δ 210.1 that showed HMBC correlations with H-14 and Me-25 (Fig. 1). The NMR spectra also showed signals due to the carbonyl of an ester [δ_C 172.2 (s)] and two carbons bearing oxygenated functions [δ_C 85.1 (d)/δ_H 4.56 (dd, J=11.5 and 6.6 Hz) and δ_C 78.9 (s)]. These data together with an isolated methylene [δ_C 42.2 (t)/δ_H 2.71 (1H, d, J=17.8 Hz) and 2.51 (1H, d, J=17.8 Hz)] and the hydroxyl function observed in the IR spectrum (3463 cm^{-1}) were consistent with the presence of a β-hydroxy-γ-lactone unit involving carbons C-16, C-17, C-19, and C-20 of a scalarane-related framework. This proposal was confirmed by the HMBC correlations between the methylene proton at δ 2.71 (H-20) and the carbonyl at C-18 and between the oxymethine carbon at δ 85.1 (C-16) and H-14 (Fig. 1).

In the NOESY spectrum the Me-24 (δ 0.93) was correlated with Me-23 (δ 0.82) and Me-25 (δ 1.27), while the methine proton H-9 (δ 1.24) was correlated with the methines H-5 (δ 0.82) and H-14 (δ 1.69). These data were in agreement with an all *trans*-fusion of rings A–B–C–D of the tetracarbocyclic skeleton, being Me-23, Me-24, and Me-25 axially oriented to the β-face, whereas H-5, H-9, and H-14 are axially oriented to the α-face. On the other hand, the coupling constants of H-12 (dd, J=3.5 and 2.2 Hz) indicated an equatorial (β) orientation for H-12. This assignment was supported by the NOESY correlations of H-12 with H-11eq, H-11ax, and Me-25. The α-orientation of H-16 was deduced from the NOESY cross peaks of this proton with H-15α and H-14, while the α-orientation of the hydroxyl at C-17 was deduced from the correlations of the proton H-20 at δ 2.71 with H-15β and Me-25. All these data led to propose structure **3** for hyatelone A.

Hyatelones B (**4**) and C (**5**) were obtained as a mixture that could not be separated by HPLC under all the assayed conditions. Treatment of the mixture with Ac_2O/Py followed by HPLC yielded 19,20-di-*O*-acetylhyatelone B (**4a**) and 19,20-di-*O*-acetylhyatelone C (**5a**). The NMR spectra of **4a** were related to those of hyatelone A (**3**) except for the absence of the signals due to the lactone carbonyl at C-19 and the methylene at C-20, showing in turn the signals of two oxymethines [δ_C 94.7 (d)/δ_H 6.42 (1H, d, J=4.2 Hz) and δ_C 79.9 (d)/δ_H 5.20 (1H, d, J=4.2 Hz)] together with two additional acetyl groups. The HMBC cross peaks of C-16 (δ 84.1) with the protons at δ 6.42 and 5.20 confirmed the location of the oxymethines at C-19 and C-20, respectively, each one linked to one acetyl group. The α-orientation of H-19 and H-20 was proposed from the NOESY correlations of both protons with H-16, which in turn showed cross peaks with H-15α and H-14. All these data defined the structure **4a** for 19,20-di-*O*-acetylhyatelone B, and hence structure **4** for the natural compound hyatelone B. The NMR spectra of compound **5a** were almost identical to those of **4a** except for the signals around the five-membered ring. In particular, H-19 and H-20 appeared as two singlets at δ 6.07 (s) and 5.12 (s), suggesting that **5a** differed from **4a** in the stereochemistry at C-19 and/or C-20. In the NOESY spectrum no cross peak was observed between H-19 and H-16 while the methyl

Table 1. NMR data for compounds **3**, **4a**, and **6**[a,b]

	3		**4a**		**6**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.7	1.58 (m) β, 0.60 (ddd, 13.7, 13.7, 3.7) α	39.7	1.55 (m) β, 0.60 (ddd, 13.4, 13.4, 4.2) α	39.7	1.58 (m) β, 0.68 (ddd, 13.2, 13.2, 3.3) α
2	18.4	1.62 (m), 1.40 (m)	18.4	1.57 (m), 1.43 (m)	18.4	1.58 (m), 1.38 (m)
3	41.8	1.40 (m) β, 1.12 (ddd, 13.2, 13.2, 3.5) α	41.9	1.37 (m) β, 1.11 (ddd, 13.4, 13.4, 4.2) α	41.8	1.38 (m) β, 1.15 (m) α
4	33.3		33.3		33.2	
5	56.5	0.82 (m)	56.5	0.85 (m)	56.2	0.86 (m)
6	18.0	1.62 (m) α, 1.40 (m) β	18.0	1.62 (m) α, 1.43 (m) β	18.4	1.58 (m) α, 1.38 (m) β
7	41.0	1.87 (m) β, 1.10 (ddd, 12.6, 12.6, 4.0) α	40.7	1.85 (ddd, 12.5, 3.3, 3.0) β, 1.07 (m) α	41.3	1.90 (m) β, 1.15 (m) α
8	38.2		37.9		38.8	
9	52.3	1.24 (m)	52.2	1.24 (m)	51.2	1.43 (dd, 13.1, 2.0)
10	36.9		36.9		36.9	
11	21.1	1.87 (m) α, 1.67 (m) β	21.6	1.88 (ddd, 14.9, 3.3, 2.7) α, 1.67 (ddd, 14.9, 13.4, 2.4) β	23.1	1.86 (ddd, 14.8, 3.7, 2.0) α, 1.58 (m) β
12	72.8	5.31 (dd, 3.5, 2.2)	74.1	5.27 (dd, 3.3, 2.4)	74.7	5.11 (dd, 3.7, 2.2)
13	51.1		52.1		85.4	
14	44.0	1.69 (dd, 13.0, 1.2)	44.3	1.71 (d, 12.8)	50.9	2.06 (dd, 12.9, 3.2)
15	25.0	2.43 (dd, 12.8, 6.6) α, 1.55 (m) β	24.8	2.25 (dd, 12.8, 7.3) α, 1.77 (ddd, 12.8, 12.8, 10.7) β	33.8	2.37 (ddd, 13.4, 3.2, 3.2) α, 1.79 (ddd, 13.4, 12.9, 10.8) β
16	85.1	4.56 (dd, 11.5, 6.6)	84.1	4.20 (dd, 10.7, 7.3)	69.9	4.44 (br dd, 10.8, 2.7)
17	78.9		81.5		158.0	
18	210.1		212.1		165.2	
19	172.2		94.7	6.42 (d, 4.2)	191.1	9.96 (d, 7.7)
20	42.2	2.71 (d, 17.8) β, 2.51 (d, 17.8) α	79.9	5.20 (d, 4.2)	130.2	6.52 (dd, 7.7, 1.5)
21	33.2	0.86 (s)	33.2	0.86 (s)	33.2	0.87 (s)
22	21.3	0.81 (s)	21.3	0.81 (s)	21.4	0.81 (s)
23	16.1	0.82 (s)	16.0	0.82 (s)	16.3	0.80 (s)
24	17.5	0.93 (s)	17.7	0.92 (s)	15.9	0.85 (s)
25	20.2	1.27 (s)	19.2	1.25 (s)	21.9	1.47 (s)
***C*H$_3$COO-(12)**	21.3	2.00 (s)	21.1	1.97 (s)	21.2	2.14 (s)
CH$_3$COO-(12)	169.6		169.7		169.8	
***C*H$_3$COO-(19)**			20.5[c]	2.06 (s)		
CH$_3$*C*OO-(19)			168.9			
***C*H$_3$COO-(20)**			20.9[c]	2.06 (s)		
CH$_3$*C*OO-(20)			169.0			

[a] ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 MHz and 150 MHz, respectively.
[b] Assignments were aided by COSY, HSQC, HMBC, and NOESY experiments.
[c] Signals with the same superscript in the same column may be interchanged.

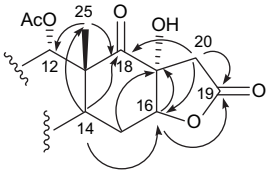


Figure 1. Selected HMBC correlations observed for hyatelone A (**3**).

group of the acetoxyl function at C-19 was correlated with H-20. All these data suggested a β-orientation of H-19 and an α-orientation of H-20, and therefore structure **5a** for 19,20-di-*O*-acetylhytelone C. To the best of our knowledge, hyatelones A–C (**3**–**5**) are the first scalarane-related sesterterpenes containing a carbonyl group at C-18 and a five-membered ring fused to C-16, C-17 of ring D. This novel positioning of the heterocyclic ring represents a significant structural departure from the sestertepenes so far reported, exemplified by furoscalarol (**2**)[3] and scalarolbutenolide.[15]

2.2. Hyatolides A (6) and B (7)

The molecular formula of hyatolide A (**6**), $C_{27}H_{40}O_6$, was determined by HRCIMS. The ^{13}C NMR spectrum displayed 27 signals, two of them corresponding to an acetoxyl group [δ 169.8 (CH_3COO–) and 21.2 (CH_3COO–)]. The remaining 25 signals together with the five methyl singlets in the ^{1}H NMR spectrum at δ 1.47, 0.87, 0.85, 0.81, and 0.80, indicated that **6** was also a sesterterpene. Furthermore, the analysis of the COSY, HSQC, HMBC, and NOESY spectra allowed us to assign the ^{1}H and ^{13}C NMR signals corresponding to rings A–B–C of a scalarane-related compound bearing oxygenated functions at C-12 and C-13. Thus, the presence of the secondary acetoxyl group at C-12 was inferred from the ^{13}C NMR signal at δ 74.7 (d) that was correlated in the HSQC spectrum with the signal at δ 5.11 (1H, dd, J=3.7 and 2.2 Hz) and in the HMBC spectrum with the signals of H-9, H-11eq, and Me-25. On the other hand, the ^{13}C NMR signal at δ 85.4 (s), corresponding to a fully substituted carbon linked to an oxygenated function, was assigned to C-13 upon observation of its HMBC correlations with the signals of H-12, H-14, and Me-25 (Fig. 2). The remaining signals of the ^{13}C NMR spectrum were assigned to an aldehyde [δ 191.1 (d)], the carbonyl of an ester [δ 165.2 (s)], a conjugated-trisubstituted double bond [δ 158.0 (s) and 130.2 (d)], an oxygenated methine [δ 69.9 (d)], and a methylene [δ 33.8 (t)]. These data together with the hydroxyl function observed in the IR spectrum (3375 cm^{-1}) were

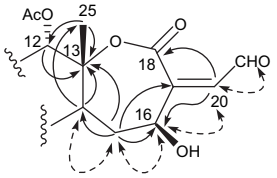


Figure 2. Selected HMBC (plain arrows) and COSY (dashed arrows) correlations observed for hyatolide A (**6**).

accommodated in a seven-membered lactone as follows: the aldehyde proton [δ 9.96 (1H, d, J=7.7 Hz)] was coupled with the olefinic proton [δ 6.52 (1H, dd, J=7.7 and 1.5 Hz)], which showed HMBC correlations with the carbonyl of the ester and with the oxymethine carbon (Fig. 2). These data indicated the presence of an α,β-unsaturated aldehyde whose carbon β was linked both to the carbonyl of the ester and to the oxygenated methine. This latter one was identified as C-16 since the oxymethine proton at δ 4.44 (1H, dd, J=10.8 and 2.7 Hz) showed in the COSY spectrum cross peaks with the methylene protons at δ 2.37 (H-15α) and 1.79 (H-15β), which in turn were coupled with H-14. Consequently, the ester carbonyl had to be located at C-18, the double bond at C-17, C-20, and the aldehyde at C-19. The correlations observed in the NOESY spectrum determined that the stereochemistry of the ring junctions and at C-12 was identical to that described for compound **3**. The NOESY cross peaks of H-16 with H-15α and H-14 indicated the α-orientation for H-16. Finally, the *Z* geometry of the double bond was supported by the correlation between the olefinic proton H-20 and H-15β. All these data led to propose structure **6** for hyatolide A.

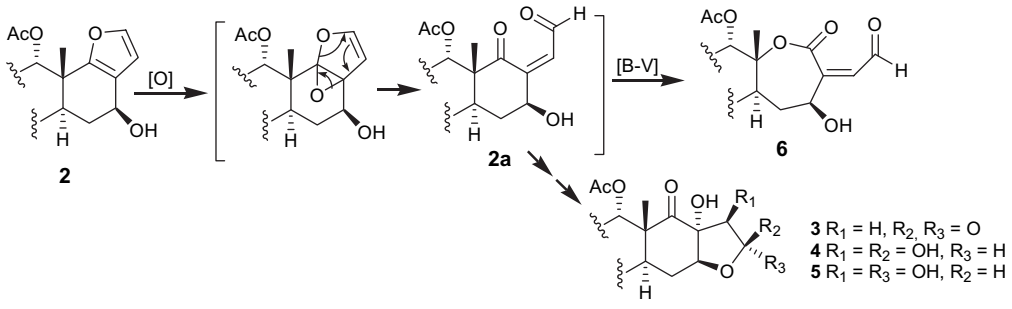
Hyatolide B (**7**) displayed NMR spectra similar to that of compound **6**, except for the absence of the signals due to the acetyl group and the ^{1}H and ^{13}C chemical shifts of the methine C-12 [δ_C 73.9/δ_H 3.88 (dd, J=3.4 and 2.4 Hz)]. These data implied that hyatolide B (**7**) was the 12-*O*-deacetyl derivative of **6**.

The structural features of the seven-membered lactone present in hyatolides A and B (**6** and **7**) are rather unusual. This ε-lactone moiety could be biosynthetically derived from a furan-containing precursor, as shown in Scheme 1. Thus, the transformation of furoscalarol (**2**) into the dicarbonyl intermediate **2a**, followed by a Baeyer–Villiger oxidation of the ketone carbonyl, could lead to hyatolide A (**6**). The involvement of a furan precursor is in agreement with the report on the obtention of a C_{15} compound containing a ε-lactone by reaction of the furanosesquiterpene furodysin with singlet oxygen.[16] Furthermore, hyatelones A–C (**3**–**5**) could also be derived from furoscalarol (**2**) via the dicarbonyl intermediate **2a**.

2.3. Hyatolides C–E (8–10)

Hyatolides C (**8**) and D (**9**) were obtained as a mixture (ca. 2.5:1). In the ^{1}H NMR spectrum, signals due to the major component **8** included six singlets, two of them at δ 1.95 (3H) and 3.44 (3H) were assigned to an acetoxyl and a methoxyl group, respectively, while the remaining singlets at δ 1.15 (3H), 0.91 (3H), 0.85 (3H), and 0.81 (6H) were appropriated for the methyl groups of a scalarane sesterterpene. In addition, the spectrum showed three deshielded methine protons at δ 6.11 (1H, s), 5.51 (1H, dd, J=3.2 and 2.7 Hz), and 4.05 (1H, dd, J=4.5 and 1.0 Hz). Treatment of the mixture with Ac_2O/Py followed by HPLC separation led to the derivative **8a**, whose ^{1}H NMR (Table 2) exhibited the singlet of an additional acetyl group and the methine singlet significantly downfield shifted [δ 6.89 (s) in **8a**, δ 6.11 (s) in **8**]. Similar to the compounds above described, the signal at δ 5.49 (dd, J=2.9 and 2.3 Hz) indicated the location of one axial acetoxyl group at C-12 of **8a**. The attachment of the methoxyl group at C-16 was deduced from the proton signal at δ 3.92 (1H, dd, J=4.6 and 1.2 Hz, H-16) that was correlated in the HMBC spectrum with C-14 (δ 45.8) and the *O*-methyl group (δ 57.4) (Fig. 3). Furthermore, the coupling constants of H-16 indicated an equatorial (β) orientation for this proton. The signals at δ_C 91.1 (d)/δ_H 6.89 (1H, s) were assigned to an acetylated hemiacetal methine. This methine together with a carbonyl [δ 167.9 (s)] and a tetrasubstituted double bond [δ 154.0 (s) and 140.1 (s)] defined the presence of a γ-acetoxy-α,β-unsaturated-γ-lactone involving carbons C-17 to C-20 of the scalarane framework. The location of the double bond at C-17 and C-18 was supported by the HMBC correlations of the olefinic carbons at 154.0 (s, C-17) and 140.1 (s, C-18) with H-15 and Me-25, respectively (Fig. 3). Furthermore, the ^{13}C chemical shifts of C-17 and C-18 indicated the location of the carbonyl at C-19 and defined the regiochemistry of the lactone.

The NOESY correlations were in agreement with the all *trans*-fused A–B–C–D rings system and the equatorial (β) orientation of H-12 (cross peaks with H-11eq, H-11ax, and Me-25) and H-16 (cross peaks with H-15ax and H-15eq). The α-orientation of H-20 was based on the NOESY correlations of H-20 with H-16 and the methoxyl group. All these data were consistent with the structure **8a** and therefore with



Scheme 1. Proposed biosynthetic pathway for the formation of hyatolide A (**6**) and hyatelones A–C (**3**–**5**) from furoscalarol (**2**).

Table 2. NMR data for compounds **8a**, **11**, and **13**[a,b]

	8a		**11**[c]		**13**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.6	1.59 (m) β, 0.57 (m) α	39.8	1.57 (m) β, 0.60 (ddd, 13.6, 13.6, 4.8) α	39.8	1.61 (m) β, 0.81 (m) α
2	18.4	1.59 (m), 1.42 (m)	18.4	1.59 (m), 1.43 (m)	18.5	1.58 (m), 1.43 (m)
3	41.9	1.36 (m) β, 1.10 (m) α	41.9	1.38 (m) β, 1.14 (ddd, 13.4, 13.2, 4.4) α	42.0	1.37 (m) β, 1.14 (m) α
4	33.3		33.3		33.2	
5	56.4	0.86 (dd, 12.4, 2.3)	56.7	0.86 (m)	56.6	0.91 (dd, 12.5, 2.4)
6	18.0	1.59 (m) α, 1.42 (m) β	17.8	1.61 (m) α, 1.45 (m) β	18.0	1.58 (m) α, 1.43 (m) β
7	41.1	1.82 (ddd, 12.5, 3.3, 3.3) β, 1.09 (m) α	40.7	1.96 (ddd, 13.0, 3.3, 3.3) β, 1.03 (m) α	40.9	1.82 (m) β, 1.12 (m) α
8	37.0		37.3		37.1	
9	52.6	1.24 (dd, 13.2, 2.3)	52.0	1.29 (dd, 13.2, 2.4)	52.3	1.39 (m)
10	36.8		36.8		37.0	
11	21.1	2.03 (ddd, 15.0, 2.9, 2.3) α, 1.66 (ddd, 15.0, 13.2, 2.3) β	21.8	1.91 (ddd, 14.7, 3.2, 2.4) α, 1.67 (ddd, 14.7, 13.2, 2.2) β	25.1	1.81 (m) β, 1.70 (ddd, 14.5, 3.1, 2.9) α
12	73.5	5.49 (dd, 2.9, 2.3)	73.5	5.06 (dd, 3.2, 2.2)	74.5	3.90 (dd, 2.9, 2.9)
13	39.2		42.2		42.8	
14	45.8	1.81 (dd, 13.1, 1.9)	52.5	2.68 (br s)	43.3	1.88 (dd, 13.4, 2.0)
15	21.7	2.13 (br d, 14.7) α, 1.59 (m) β	129.2	5.97 (dd, 9.7, 2.4)	25.2	1.89 (br d, 14.1) α, 1.63 (m) β
16	69.6	3.92 (dd, 4.6, 1.2)	117.9	6.33 (dd, 9.7, 3.1)	62.0	4.59 (dd, 4.9, 1.4)
17	154.0		129.2		139.6	
18	140.1		159.8		162.5	6.72 (s)
19	167.9		44.2	3.88 (d, 19.8) β, 3.83 (d, 19.8) α	—	
20	91.1	6.89 (s)	172.6		195.1	9.48 (s)
21	33.2	0.85 (s)	33.2	0.86 (s)	33.2	0.84 (s)
22	21.3	0.81 (s)	21.5	0.82 (s)	21.3	0.82 (s)
23	15.9	0.81 (s)	15.9	0.83 (s)	16.1	0.85 (s)
24	16.9	0.91 (s)	18.8	1.05 (s)	17.1	0.89 (s)
25	19.5	1.16 (s)	17.5	1.03 (s)	19.9	1.01 (s)
***C*H$_3$COO-(12)**	20.9	1.96 (s)	21.3	2.11 (s)		
CH$_3$*C*OO-(12)	169.9		170.2			
***C*H$_3$COO-(20)**	20.7	2.16 (s)				
CH$_3$*C*OO-(20)	169.1					
OMe	57.4	3.39 (s)				

[a] ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 MHz and 150 MHz, respectively.
[b] Assignments were aided by COSY, HSQC, HMBC, and NOESY experiments.
[c] The NH signal was clearly observed when the ^{1}H NMR spectrum was recorded in $(CD_3)_2CO$, see Section 3.

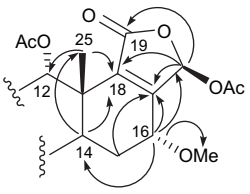


Figure 3. Selected HMBC correlations observed for 20-*O*-acetylhyatolide C (**8a**).

structure **8** for the natural metabolite hyatolide C. Although the acetyl derivative of **9** could not be recovered, the analysis of the NMR spectra of the natural mixture of **8** and **9**, allowed us to identify hyatolide D (**9**) as the C-20 epimer of **8**. Thus, in the NOESY spectrum the signal due to H-20 of **9** [δ 5.93 (s)] was only correlated with H-16 [δ 4.08 (br d, J=4.3 Hz)], in agreement with a β-orientation of H-20.

The ^{1}H NMR spectrum of hyatolide E (**10**) was related to those of compounds **8** and **9**, except for the absence of the signal due to the methoxyl group and the presence of the signals of a disubstituted double bond at δ 6.39 (1H, dd, J=9.7 and 2.7 Hz) and 6.31 (1H, dd, J=9.7 and 3.1 Hz). Complete characterization of this compound was achieved after acetylation and HPLC purification to yield compound **10a**. The comparison of the NMR spectra of **10a** with those of **8a** indicated that both compounds possessed an identical A–B–C rings system, and the same butenolide unit. Therefore, the disubstituted double bond of **10a** had to be located at C-15, C-16, conjugated with the butenolide ring. This assignment was fully confirmed by the HMBC correlations of the olefinic proton H-15 (δ 6.41) with C-13 and C-17 whereas the proton H-16 (δ 6.19) was correlated with C-14 and C-18. The stereochemistry at C-20 remains undetermined since no correlations were observed in the NOESY spectrum neither for H-20 nor for its geminal acetyl group. All these data allowed us to define structure **10a** and therefore structure **10** for the natural metabolite hyatolide E.

2.4. Hyatelactam (11)

Hyatelactam (**11**) possessed the molecular formula $C_{27}H_{39}NO_3$, determined from HRCIMS. A comparison of the NMR data of **11** with those of compound **10a** above described, indicated that **11** also displayed a scalarane framework containing an axial acetoxyl group at C-12 and a 15,17-diene moiety. The remaining signals of the spectra were assigned to a carbonyl [δ_C 172.6 (s)] and to a methylene

[δ_C 44.2 (t)/δ_H 3.88 (1H, d, J=19.8 Hz) and 3.83 (1H, d, J=19.8 Hz)], that together with the nitrogen atom of the molecular formula, were accommodated in a γ-lactam ring fused to C-17 and C-18 of ring D. Differing from **10a**, the most deshielded olefinic carbon signal [δ 159.8 (s)] was assigned to C-18 from the HMBC correlations of this signal with H-14 (δ 2.68) and Me-25 (δ 1.03). Therefore the carbonyl of the lactam ring had to be located at C-20. This assignment was further supported by the NOESY cross peaks of the methylene proton at δ 3.88 (H-19β) with H-12 and Me-25. All these data led to propose structure **11** for hyatelactam. After the isolation of molliorin A, possessing a *N*-(2-methylbutyl)pyrrole ring,[17] hyatelactam (**11**) represents the second example of a nitrogen-containing scalarane.

2.5. 12-*O*-Deacetyl-19-*epi*-scalarin (12)

The NMR spectra of 12-*O*-deacetyl-19-*epi*-scalarin (**12**) were quite similar to those of scalarin (**1**),[2] also isolated in this study. However, several diagnostic differences were observed. The most evident were the absence of the signals due to the acetyl group, the upfield shift of H-12 at δ 4.00 (br s) and the ^{13}C shift of C-19 at δ 106.6 (d) [δ 97.9 (d) in **1**]. Furthermore, the signal assigned to the hemiacetal proton H-19 [δ 5.57, d, J=6.7 Hz] was correlated in the NOESY spectrum with H-18, which in turn was correlated with H-14. These correlations indicated the α-orientation of H-18 and H-19, and therefore, compound **12** possessed at C-19 an opposite configuration to that of scalarin (**1**). All these data led to define the structure 12-*O*-deacetyl-19-*epi*-scalarin for compound **12**.

2.6. 12-*O*-Deacetylnorscalaral B (13)

The five singlets due to methyl groups in the ^{1}H NMR spectrum of compound **13** (δ 1.01, 0.89, 0.85, 0.84, and 0.82), together with the presence in the ^{13}C NMR spectrum of the signals corresponding to three methines [δ 56.6 (C-5), 52.3 (C-9), and 43.3 (C-14)] and four quaternary sp^3 carbons [δ 42.8 (C-13), 37.1 (C-8), 37.0 (C-10), and 33.2 (C-4)], indicated that **13** possessed a tetracarbocyclic skeleton related to the scalaranes above described. However, the molecular formula $C_{24}H_{38}O_3$, deduced from HRCIMS, together with the 24 carbons counted in the ^{13}C NMR spectrum were consistent with a norscalarane structure for **13**. In particular, the NMR spectra of **13** were closely similar to those of norscalaral B (**14**),[11] except for the absence of the signals due to the acetyl group at C-12 and the upfield shift of H-12 at δ 3.90. Therefore, compound **13** was 12-*O*-deacetylnorscalaral B.

2.7. Cytotoxic activity

The new compounds hyatelone A (**3**), 19,20-diacetylhyatelones B (**4a**) and C (**5a**), hyatolides A (**6**) and B (**7**), 20-*O*-acetylhyatolide C (**8a**), hyatelactam (**11**), 12-*O*-deacetyl-19-*epi*-scalarin (**12**), and 12-*O*-deacetylnorscalaral B (**13**) were tested in assays directed to detect cytotoxic activity against the tumor cell lines MDA-MB-231 (breast carcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Compounds **3**, **4a**, **6**, **8a**, **11**, and **13** showed mild activity with GI_{50} values of 4.0–9.3 μg/mL (Table 1), while compounds **5a**, **7**, and **12** were inactive against the three lines tested.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. IR spectra were recorded with a Perkin–Elmer FTIR System Spectrum BX. UV spectra were registered on a Philips PU 8710 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz, respectively, on a Varian INOVA 600 spectrometer using $CDCl_3$ or $(CD_3)_2CO$ as solvent. ^{1}H and ^{13}C NMR chemical shifts were referenced using the corresponding solvent signals [δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 2.04 and δ_C 29.8 for $(CD_3)_2CO$]. COSY, HSQC, and HMBC were performed using standard VARIAN pulse sequences. Low-resolution mass spectra were recorded on a Finningan Voyager GC8000^{top} spectrometer. High-resolution mass spectra were recorded on a Autospec-Q spectrometer. Column chromatography was carried out using Merck Silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrospher Si-60 (Merck) columns in normal phase mode and with LiChrosorb RP-18 (Merck) columns in reversed phase mode using a differential refractometer RI-71. All solvents were spectral grade or distilled prior to use.

3.2. Collection, extraction, and isolation procedure

The sponge *H. intestinalis* was collected by hand using SCUBA at the Gulf of California and liophylized (263.3 g). The material was extracted with acetone/MeOH (1:1, 6.5 L) and the solvent concentrated under reduced pressure to give a residue that was suspended in H_2O and extracted with Et_2O (4×775 mL). The Et_2O extract (5.9 g) was chromatographed on a silica gel column using solvents of increasing polarities from hexane to Et_2O and subsequently $CHCl_3$/MeOH (80:20) and MeOH. The fraction eluted with hexane/Et_2O (60:40) was subjected to normal phase HPLC (hexane/EtOAc, 70:30) to give furoscalarol (**2**, 5 mg, 1.9×10^{-3}%), hyatolide A (**6**, 1.8 mg, 6.8×10^{-4}%), hyatelone A (**3**, 2.0 mg, 7.6×10^{-4}%), and a mixture of hyatelones B and C (**4** and **5**, 4.5 mg, 1.7×10^{-3}%). The fraction of the general chromatography eluted with hexane/Et_2O (50:50) was subjected to HPLC (hexane/EtOAc, 75:25) to give norscalaral A (2 mg, 7.6×10^{-4}%), deoxoscalarin (11.6 mg, 4.4×10^{-3}%), hyatolide B (**7**, 4.5 mg, 1.7×10^{-3}%), 12α-acetoxy-19β-hydroxy-15,17-dien-20,19-olide (10.1 mg, 3.8×10^{-3}%), and 12-*epi*-12-*O*-deacetyl-19-deoxyscalarin (13.5 mg). The fraction of the general chromatography eluted with hexane/Et_2O (30:70) was subjected to repeated HPLC separations (hexane/EtOAc, 70:30; MeOH/H_2O, 85:15 to 95:5) to yield 12-*epi*-12-*O*-acetylscalarolide (8.5 mg, 3.2×10^{-3}%), hyatolide E (**10**, 2.2 mg, 8.4×10^{-4}%), further amounts of 12-*epi*-12-*O*-deacetyl-19-deoxyscalarin (27.6 mg, 0.016% overall), norscalaral B (**14**, 3.1 mg, 1.2×10^{-3}%) and a mixture of hyatolides C and D (**8** and **9**, 3.0 mg, 1.1×10^{-3}%). The fraction of the general chromatography eluted with Et_2O contained scalarin (**1**, 264 mg). The fraction of the general chromatography eluted with $CHCl_3$/MeOH (80:20) was chromatographed on a silica gel column eluted with mixtures of $CHCl_3$/MeOH of increasing polarities from 99.5:0.5 to 92:8. The fraction eluted with $CHCl_3$/MeOH (99.5:0.5) contained further amounts of **1** (311 mg, 0.22% overall). The fraction eluted

with $CHCl_3$/MeOH (99:1) was subjected to repeated HPLC separations (MeOH/H_2O, 90:10; MeCN/H_2O, 80:20) to give 12-*O*-deacetylnorscalaral B (**13**, 1.8 mg, 6.8×10^{-4}%), hyatelactam (**11**, 4.5 mg, 1.7×10^{-3}%) and 12-*O*-deacetyl-19-*epi*-scalarin (**12**, 1.8 mg, 6.8×10^{-4}%).

3.2.1. Hyatelone A (3). Amorphous solid; $[\alpha]_D$ +72.0 (*c* 0.1, $CHCl_3$); IR (film) 3463, 2927, 1784, 1738, 1716, 1249 cm^{-1}; ^1H and ^{13}C NMR (Table 2); EIMS (70 eV) *m*/*z* (rel int.) 461 ([M+H]$^+$, 4), 444 (10), 427 (3), 418 (19), 400 (61), 386 (53), 372 (63), 368 (55), 357 (37), 191 (99), 81 (100); HRCIMS (+) Obsd *m*/*z*=461.2916, (M+H)$^+$, $C_{27}H_{41}O_6$ requires *m*/*z*=461.2903.

3.2.2. Hyatelones B (4) and C (5). ^1H NMR (600 MHz, $CDCl_3$) δ 5.55 (br s, H-19)/5.22* (br s, H-19), 5.28 (br s H-12), 4.35* (dd, *J*=10.5 and 7.3 Hz, H-16)/3.98 (dd, *J*=11.1 and 6.9 Hz, H-16), 4.09* (br s, H-20)/3.84 (br s, H-20), 1.98* (s, C*H*$_3$COO–)/1.97 (s, C*H*$_3$COO–), 1.23 (s, Me-25)/1.19* (s, Me-25), 0.89 (s, Me-24), 0.85 (s, Me-21), 0.80 (s, Me-23 and Me-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 215.3/214.6* (s, C-18), 169.9/169.8* (s, CH_3*C*OO–), 102.6*/96.7 (d, C-19), 84.3*/82.5 (d, C-16), 83.6*/83.4 (s, C-17), 82.6*/78.8 (d, C-20), 74.6 (C-12), 56.7/56.6* (d, C-5), 52.4 (d, C-9), 52.1 (s, C-13), 44.8*/44.7 (d, C-14), 41.9 (t, C-3), 41.0 (t, C-7), 39.6 (t, C-1), 37.9 (s, C-8), 36.9 (s, C-10), 33.3 (s, C-4), 33.2 (q, Me-21), 25.6/25.4* (t, C-15), 21.6 (t, C-11), 21.3 (q, Me-22), 21.2 (q, *C*H$_3$COO–), 19.0/18.8* (q, Me-25), 18.4 (t, C-2), 18.0 (t, C-6), 17.8/17.7* (q, Me-24), 16.0 (q, Me-23). For each duplicated signal, the value with asterisk corresponds to compound **5**. Treatment of the mixture of **4** and **5** with Ac_2O/Py and subsequent HPLC separation (hexane/EtOAc, 65:35) yielded compounds **4a** (1.8 mg) and **5a** (2.8 mg).

3.2.2.1. 19,20-di-*O*-Acetylhyatelone B (4a). Amorphous solid; $[\alpha]_D$ +97.0 (*c* 0.1, $CHCl_3$); IR (film) 2926, 1760, 1743, 1717, 1239 cm^{-1}; ^1H and ^{13}C NMR (Table 2); EIMS (70 eV) *m*/*z* (rel int.) 562 ([M]$^+$, 1), 503 (12), 485 (4), 460 (7), 442 (25), 390 (100), 191 (82); HRCIMS(+) Obsd *m*/*z*=562.3149 (M)$^+$, $C_{31}H_{46}O_9$ requires *m*/*z*=562.3141.

3.2.2.2. 19,20-di-*O*-Acetylhyatelone C (5a). Amorphous solid; $[\alpha]_D$ +122.0 (*c* 0.05, $CHCl_3$); IR (film) 2925, 1760, 1750, 1706, 1232, 1214 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.07 (1H, s, H-19), 5.27 (1H, dd, *J*=3.3 and 2.2 Hz, H-12), 5.12 (1H, s, H-20), 4.40 (1H, dd, *J*=10.9 and 7.6 Hz, H-16), 2.29 (1H, dd, *J*=13.2 and 7.6 Hz, H-15α), 2.11 (3H, s, C*H*$_3$COO-19), 2.02 (3H, s, C*H*$_3$COO-20), 1.97 (3H, s, C*H*$_3$COO-12), 1.89 (1H, ddd, *J*=15.0, 3.3, and 3.0 Hz, H-11α), 1.85 (1H, ddd, *J*=12.8, 3.3, and 3.3 Hz, H-7β), 1.76 (1H, d, *J*=12.5 Hz, H-14), 1.66 (1H, m, H-11β), 1.60 (1H, m, H-6α), 0.86 (3H, s, H-21), 1.56 (3H, m, H-1β, H-2, and H-15β), 1.43 (2H, m, H-2 and H-6β), 1.37 (1H, m, H-3β), 1.22 (1H, m, H-9), 1.21 (3H, s, H-25), 1.12 (1H, ddd, *J*=13.5, 13.5, and 4.1 Hz, H-3α), 1.06 (1H, ddd, *J*=12.8, 12.8, and 3.8 Hz, H-7α), 0.91 (3H, s, H-24), 0.85 (1H, m, H-5), 0.82 (3H, s, H-23), 0.81 (3H, s, H-22), 0.61 (1H, ddd, *J*=13.2, 13.2, and 3.3 Hz, H-1α); ^{13}C NMR (150 MHz, $CDCl_3$) δ 212.9 (s, C-18), 169.7[a] (s, CH_3*C*OO-19), 169.5[a] (s, CH_3*C*OO-12), 168.3 (s, *C*H$_3$COO-20), 98.3 (d, C-19), 85.7 (d, C-16), 82.8 (d, C-20), 82.0 (s, C-17), 74.3 (d, C-12), 56.5 (d, C-5), 52.2 (d, C-9), 52.0 (s, C-13), 44.0 (d, C-14), 41.9 (t, C-3), 40.8 (t, C-7), 39.7 (t, C-1), 37.8 (s, C-8), 36.9 (s, C-10), 33.3 (s, C-4), 33.2 (q, C-21), 24.7 (t, C-15), 21.6 (t, C-11), 21.3 (q, C-22), 21.1[b] (2×q, *C*H$_3$COO-19 and *C*H$_3$COO-12), 20.7[b] (q, *C*H$_3$COO-20), 19.1 (q, C-25), 18.4 (t, C-2), 18.0 (t, C-6), 17.7 (q, C-24), 16.0 (q, C-23), Signals with the same superscript may be interchanged; EIMS (70 eV) *m*/*z* (rel int.) 563 ([M+H]$^+$, 2), 503 (42), 485 (8), 460 (9), 442 (21), 390 (100), 191 (62); HRCIMS (+) Obsd *m*/*z*=562.3129 (M)$^+$, $C_{31}H_{46}O_9$ requires *m*/*z*=562.3141.

3.2.3. Hyatolide A (6). Amorphous solid; $[\alpha]_D$ +27.0 (*c* 0.1, $CHCl_3$); IR (film) 3375, 2926, 1740, 1715, 1260 cm^{-1}; UV (MeOH) 220 (ε 6707) nm; ^1H and ^{13}C NMR (Table 2); EIMS (70 eV) *m*/*z* (rel int.) 400 ([M−AcOH]$^+$, 2), 382 (4), 367 (5), 288 (16), 271 (14), 191 (100); HRCIMS (+) Obsd *m*/*z*=461.2931 (M+H)$^+$, $C_{27}H_{41}O_6$ requires *m*/*z*=461.2903.

3.2.4. Hyatolide B (7). Amorphous solid; $[\alpha]_D$ +14.0 (*c* 0.18, $CHCl_3$); IR (film) 3435, 2928, 1735, 1717 cm^{-1}; UV (MeOH) 220 (ε 6115) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 9.97 (1H, d, *J*=7.7, H-19), 6.58 (1H, dd, *J*=7.7 and 1.5 Hz, H-20), 4.42 (1H, dd, *J*=11.2, 3.5, and 1.5 Hz, H-16), 3.88 (1H, dd, *J*=3.4 and 2.4 Hz, H-12), 2.38 (1H, ddd, *J*=13.4, 3.5, and 3.1 Hz, H-15α), 2.13 (1H, dd, *J*=13.0 and 3.1 Hz, H-14), 1.91 (1H, ddd, *J*=14.3, 3.4, and 2.1 Hz, H-11α), 1.86 (1H, ddd, *J*=12.5, 3.3, and 3.3 Hz, H-7β), 1.80 (1H, ddd, *J*=13.4, 13.0, and 11.2 Hz, H-15β), 1.67 (1H, dd, *J*=13.0 and 2.1 Hz, H-9), 1.63 (1H, m, H-1β), 1.59 (2H, m, H-2 and H-6α), 1.50 (1H, ddd, *J*=14.3, 13.0, and 2.4 Hz, H-11β), 1.44 (1H, m, H-2), 1.41 (3H, s, H-25), 1.38 (2H, m, H-3β and H-6β), 1.16 (2H, m, H-3α and H-7α), 0.92 (1H, m, H-5), 0.88 (1H, m, H-1α), 0.86 (3H, s, H-21), 0.82 (3H, s, H-24), 0.80 (6H, s, H-22 and H-23); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.9 (d, C-19), 130.5 (d, C-20), 165.7 (s, C-18), 157.8 (s, C-17), 89.3 (s, C-13), 73.9 (d, C-12), 70.0 (d, C-16), 56.0 (d, C-5), 49.7 (d, C-14), 49.5 (d, C-9), 41.8 (t, C-3), 41.3 (t, C-7), 39.6 (t, C-1), 38.8 (s, C-8), 36.8 (s, C-10), 34.2 (t, C-15), 33.2 (q, C-21), 33.2 (s, C-4), 23.9 (t, C-11), 21.6 (q, C-25), 21.2 (q, C-22), 18.5 (2×t, C-2 and C-6), 16.4 (q, C-23), 15.8 (q, C-24); EIMS (70 eV) *m*/*z* (rel int.) 418 ([M]$^+$, 3), 400 (4), 385 (5), 357 (9), 329 (5), 191 (77), 69 (100); HRCIMS (+) Obsd *m*/*z*=418.2734 (M)$^+$, $C_{25}H_{38}O_5$ requires *m*/*z*=418.2719.

3.2.5. Hyatolides C (8) and D (9). ^1H NMR (600 MHz, $CDCl_3$) δ 6.11 (s, H-20)/5.93* (s, H-20), 5.51 (dd, *J*=3.2 and 2.7 Hz, H-12)/5.50* (t, 2.6 Hz, H-12), 4.08* (br d, *J*=4.3 Hz, H-16)/4.05 (dd, *J*=4.5 and 1.0 Hz, H-16), 3.46* (s, –OC*H*$_3$)/3.44 (s, –OC*H*$_3$), 2.13* (m, H-15α)/2.12 (m, H-15α), 1.98* (s, C*H*$_3$COO–)/1.95 (s, C*H*$_3$COO–), 1.85* (dd, *J*=12.8 and 1.3 Hz, H-14)/1.78 (dd, *J*=12.9 and 1.7 Hz, H-14), 1.56 (m, H-15β), 1.15 (s, Me-25), 0.92* (s, Me-24)/0.91 (s, Me-24), 0.85 (s, Me-21), 0.81(s, Me-23 and Me-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9/169.3* (s, C-19), 169.9 (s, CH_3*C*OO–), 155.6/152.5* (s, C-17), 142.0*/139.4 (s, C-18), 97.2*/95.4 (d, C-20) 74.0*/73.6 (d, C-12), 72.0*/69.7 (d, C-16), 56.5 (d, C-5), 52.8*/52.7 (d, C-9), 45.9/45.6* (d, C-14), 42.0 (t, C-3), 41.3 (t, C-7), 39.7 (t, C-1), 39.0 (s, C-13), 37.0 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, Me-21), 21.8 (q, Me-22), 21.3 (t, C-15), 21.2 (q, *C*H$_3$COO–), 20.9 (t, C-11), 19.6/19.3* (q, Me-25), 18.4 (t, C-2), 18.1 (t, C-6), 17.0*/16.9 (q, Me-24), 15.9 (q,

Me-23). For each duplicated signal, the value with asterisk corresponds to compound **9**. Treatment of the mixtures of **8** and **9** with Ac_2O/Py and subsequent HPLC separation (hexane/EtOAc, 80/20) led to recover compound **8a** (1.8 mg).

3.2.5.1. 20-*O*-Acetylhyatolide C (8a). Amorphous solid; $[\alpha]_D$ +13.0 (*c* 0.1, $CHCl_3$); IR (film) 2927, 1780, 1740, 1238 cm^{-1}; UV (MeOH) 210 (ε 7396) nm; ^1H and ^{13}C NMR (Table 3); EIMS (70 eV) *m*/*z* (rel int.) 516 ($[M]^+$, 1), 474 (47), 456 (18), 442 (21), 414 (57), 396 (23), 384 (27), 382 (70), 365 (89), 337 (32), 191 (44), 69 (100); HRCIMS (+) Obsd *m*/*z*=485.2910 $(M+H-CH_3OH)^+$, $C_{29}H_{41}O_6$ requires *m*/*z*=485.2903; Obsd *m*/*z*=457.2952 $(M+H-AcOH)^+$, $C_{28}H_{41}O_5$ requires *m*/*z*=457.2954.

Table 3. Cytotoxicity assay results for sesterterpenes from *H. intestinalis* (GI_{50} values in μg/mL)

	3	4a	6	8a	11	13
MDA-MB-231	5.4	5.3	4.8	4.0	—	4.9
A-549	—	9.3	5.1	7.2	—	4.5
HT-29	9.2	5.1	5.0	6.2	8.1	4.2

3.2.6. Hyatolide E (10). ^1H NMR (600 MHz, $CDCl_3$) δ 6.39 (dd, *J*=9.7 and 2.7 Hz, H-15), 6.31 (dd, *J*=9.7 and 3.1 Hz, H-16), 6.00 (s, H-20), 5.48 (dd, *J*=3.1 and 2.7 Hz, H-12), 2.66 (dd, *J*=3.1 and 2.7 Hz, H-14), 2.00 (s, C*H*$_3$COO–), 1.07 (s, Me-25), 1.03 (s, Me-24), 0.86 (s, Me-21), 0.82 (s, Me-23 and Me-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9 (s, CH_3*C*OO–), 168 (s, C-19), 154 (s, C-17), 138.3 (d, C-15), 133.1 (s, C-18), 118.7 (d, C-16), 95.4 (d, C-20), 72.3 (d, C-12), 56.7 (d, C-5), 53.7 (d, C-14), 52.0 (d, C-9), 41.9 (t, C-3), 40.8 (t, C-7), 39.9 (s, C-13), 39.6 (t, C-1), 37.0 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, Me-21), 21.3 (2×q, Me-22 and *C*H_3COO–), 21.2 (t, C-11), 18.8 (q, Me-24), 18.4 (t, C-2), 17.8 (t, C-6), 17.2 (q, Me-25), 15.8 (q, Me-23). Treatment of **10** with Ac_2O/Py and subsequent HPLC purification (hexane/EtOAc, 75:25) yielded compound **10a** (1.2 mg).

3.2.6.1. 20-*O*-Acetylhyatolide E (10a). Amorphous solid; $[\alpha]_D$ +46.0 (*c* 0.1, $CHCl_3$); IR (film) 2930, 1771, 1740, 1240, 1209 cm^{-1}; UV (MeOH) 294 (ε 8785) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 6.83 (1H, s, H-20), 6.41 (1H, dd, *J*=9.7 and 2.7 Hz, H-15), 6.19 (1H, dd, *J*=9.7 and 3.3 Hz, H-16), 5.47 (1H, dd, *J*=3.0 and 2.4 Hz, H-12), 2.68 (1H, dd, *J*=3.3 and 2.7 Hz, H-14), 2.17 (3H, s, C*H*$_3$COO-20), 2.00 (3H, s, C*H*$_3$COO-12), 2.08 (1H, ddd, *J*=14.9, 3.0, and 2.7 Hz, H-11α),1.94 (1H, dddd, *J*=12.5, 3.3, and 3.3 Hz, H-7β), 1.64 (1H, ddd, *J*=14.9, 13.4, and 2.4 Hz, H-11β), 1.60 (3H, m, H-1β, H-2, and H6α), 1.45 (2H, m, H-2 and H-6β), 1.37 (1H, m, H-3β), 1.24 (1H, dd, *J*=13.4 and 2.7 Hz, H-9), 1.13 (1H, ddd, *J*=13.3, 13.3, and 3.4 Hz, H-3α), 1.09 (3H, s, H-25), 1.04 (3H, s, H-24), 1.03 (1H, m, H-7α), 0.86 (1H, m, H-5), 0.86 (3H, s, H-21), 0.61 (1H, m, H-1α), 0.83 (3H, s, H-23), 0.82 (3H, s, H-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.7 (s, CH_3*C*OO-12), 169.4 (s, CH_3*C*OO-20), 167.8 (s, C-19), 152.5 (s, C-17), 138.8 (d, C-15), 133.5 (s, C-18), 118.3 (d, C-16), 91.2 (d, C-20), 72.2 (d, C-12), 56.7 (d, C-5), 53.7 (d, C-14), 52.0 (d, C-9), 41.9 (t, C-3), 40.8 (t, C-7), 40.1 (s, C-13), 39.6 (t, C-1), 37.1 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, C-21), 21.3 (q, C-22), 21.2 (t, C-11), 21.2 (q, *C*H_3COO-12), 20.8 (q, *C*H_3COO-20), 18.8 (q, C-24), 18.4 (t, C-2), 17.7 (t, C-6), 17.1 (q, C-25), 15.8 (q, C-23); EIMS (70 eV) *m*/*z* (rel int.) 485 ($[M+H]^+$, 1), 424 (6), 409 (22), 381 (47), 365 (97), 349 (48), 241 (59), 226 (100); HRCIMS (+) Obsd *m*/*z*=485.2935 $(M+H)^+$, $C_{29}H_{41}O_6$ requires *m*/*z*=418.2903.

3.2.7. Hyatelactam (11). Amorphous solid; $[\alpha]_D$ +56.0 (*c* 0.1, $CHCl_3$); IR (film) 3240, 2926, 1732, 1694, 1242 cm^{-1}; UV (MeOH) 217 (ε 7900), 278 (ε 2500) nm; ^1H and ^{13}C NMR (in $CDCl_3$, Table 3); ^1H NMR (600 MHz, $(CD_3)_2CO$) δ (selected data) 6.93 (1H, br s, NH), 6.21 (1H, dd, *J*=9.6 and 3.1 Hz, H-16), 5.99 (1H, dd, *J*=9.6 and 2.5 Hz, H-15), 5.04 (1H, dd, *J*=3.3 and 2.2 Hz, H-12), 3.96 (1H, d, *J*=20.0 Hz, H-19), 3.84 (1H, d, *J*=20.0 Hz, H-19), 2.69 (1H, br s, H-14), 2.07 (3H, s, C*H*$_3$COO–), 1.91 (1H, ddd, *J*=14.8, 3.3, and 3.0 Hz, H-11α), 1.77 (1H, ddd, *J*=14.8, 13.0, and 2.2 Hz, H-11β), 1.10 (3H, s, H-24), 1.07 (3H, s, H-25), 0.88 (3H, s, H-23), 0.86 (3H, s, H-21), 0.84 (3H, s, H-22); EIMS (70 eV) *m*/*z* (rel int.) 425 ($[M]^+$, 15), 365 (29), 350 (79), 228 (77), 214 (100), 173 (93), 148 (84); HRCIMS (+) Obsd *m*/*z*=426.3013 $(M+H)^+$, $C_{27}H_{40}NO_3$ requires *m*/*z*=426.3008.

3.2.8. 12-*O*-Deacetyl-19-*epi*-scalarin (12). Amorphous solid; $[\alpha]_D$ +129.0 (*c* 0.1, $CHCl_3$); IR (film) 3479, 2931, 1747, 1693 cm^{-1}; UV (MeOH) 227 (ε 8864) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 6.83 (1H, ddd, *J*=3.8, 3.5, and 3.5 Hz, H-16), 5.57 (1H, d, *J*=6.7 Hz, H-19), 4.00 (1H, br s, H-12), 3.38 (1H, dddd, *J*=6.7, 5.0, 3.5, and 3.5 Hz, H-18), 2.30 (1H, dddd, *J*=20.1, 5.6, 3.8, and 3.8 Hz, H-15α), 2.18 (1H, dddd, *J*=20.1, 11.4, 5.0, and 3.2 Hz, H-15β), 1.82 (1H, ddd, *J*=14.6, 13.3, and 2.3 Hz, H-11β), 1.71 (1H, ddd, *J*=12.6, 3.2, and 3.2 Hz, H-7β), 1.66 (1H, m, H-1β), 1.64 (1H, m, H-2), 1.60 (1H, m, H-11α), 1.56 (1H, dd, *J*=11.4 and 5.6 Hz, H-14), 1.54 (1H, m, H-6α), 1.43 (1H, m, H-2), 1.38 (2H, m, H-3β and H-6β), 1.37 (1H, dd, *J*=13.3 and 1.8 Hz, H-9), 1.15 (1H, ddd, *J*=13.6, 13.6, and 4.1 Hz, H-3α), 0.99 (1H, ddd, *J*=12.6, 12.6, and 3.8 Hz, H-7α), 0.99 (3H, s, H-25), 0.94 (3H, s, H-24), 0.88 (1H, dd, *J*=12.6 and 2.3 Hz, H-5), 0.85 (3H, s, H-21), 0.85 (3H, s, H-23), 0.81 (3H, s, H-22), 0.79 (1H, ddd, *J*=13.1, 13.1, and 3.8 Hz, H-1α); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.3 (s, C-20), 135.5 (d, C-16), 127.1 (s, C-17), 106.6 (d, C-19), 72.3 (d, C-12), 56.5 (d, C-5), 51.6 (d, C-9), 50.8 (d, C-14), 46.6 (d, C-18), 42.0 (t, C-3), 41.7 (t, C-7), 40.1 (s, C-13), 39.9 (t, C-1), 37.9 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, C-21), 25.9 (t, C-11), 24.6 (t, C-15), 21.3 (q, C-22), 18.5 (t, C-2), 18.0 (t, C-6), 16.4 (q, C-23), 16.1 (q, C-24), 15.3 (q, C-25); EIMS (70 eV) *m*/*z* (rel int.) 403 ($[M+H]^+$, 9), 386 (28), 384 (85), 366 (35), 356 (94), 351 (43), 341 (36), 205 (51), 191 (96), 69 (100); HRCIMS (+) Obsd *m*/*z*=384.2673 $(M-H_2O)^+$, $C_{25}H_{36}O_3$ requires *m*/*z*=384.2664; Obsd *m*/*z*=367.2629 $(M+H-2H_2O)^+$, $C_{25}H_{35}O_2$ requires *m*/*z*=367.2637.

3.2.9. 12-*O*-Deacetylnorscalaral B (13). Amorphous solid; $[\alpha]_D$ +30 (*c* 0.1, $CHCl_3$); IR (film) 3703, 3457, 2927, 1668 cm^{-1}; UV (MeOH) 222 (ε 10386) nm; ^1H and ^{13}C NMR (Table 3); EIMS (70 eV) *m*/*z* (rel int.) 358 ($[M]^+$, 8), 356 (29), 346 (44), 342 (22), 338 (16), 324 (11), 235 (46), 217 (47), 206 (23), 192 (93), 136 (100); HRCIMS (+) Obsd *m*/*z*=374.2819 $(M)^+$, $C_{24}H_{38}O_3$ requires *m*/*z*=374.2821.

3.3. Cytotoxicity assays

Compounds **3**, **4a**, **5a**, **6**, **7**, **8a**, and **11–13** were tested against the human tumor cell lines MDA-MB-231 (breast carcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Cytotoxicity assays were performed by PharmaMar. A colorimetric type of assay using sulforhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability following the method described in the literature.[18]

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References and notes

1. (a) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. J. *Nat. Prod. Rep.* **2006**, *23*, 26–78 and previous reviews of this series; (b) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1–48 and previous reviews of this series.
2. (a) Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. *Tetrahedron* **1972**, *28*, 5993–5997; (b) Cimino, G.; De Stefano, S.; Minale, L.; Trivellone, E. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1587–1593.
3. (a) Cafieri, F.; De Napoli, L.; Fattorusso, E.; Santacroce, C.; Sica, D. *Gazz. Chim. Ital.* **1977**, *107*, 71–74; (b) Cimino, G.; Cafieri, F.; De Napoli, L.; Fattorusso, E. *Tetrahedron Lett.* **1978**, 2041–2044.
4. (a) Youssef, D. T. A.; Shaala, L. A.; Emara, S. *J. Nat. Prod.* **2005**, *68*, 1782–1784; (b) Wonganuchitmeta, S.; Yuenyongsawad, S.; Keawpradub, N.; Plubrukarn, A. *J. Nat. Prod.* **2004**, *67*, 1767–1770; (c) El Sayed, K. A.; Bartyzel, P.; Shen, X.; Perry, T. L.; Zjawiony, J. K.; Hamann, M. T. *Tetrahedron* **2000**, *56*, 949–953.
5. (a) Pettit, G. R.; Tan, R.; Cichacz, Z. A. *J. Nat. Prod.* **2005**, *68*, 1253–1255; (b) Tsukamoto, S.; Miura, S.; van Soest, R. W. M.; Ohta, T. *J. Nat. Prod.* **2003**, *66*, 438–440; (c) Miyaoka, H.; Nishijima, S.; Mitome, H.; Yamada, Y. *J. Nat. Prod.* **2000**, *63*, 1369–1372; (d) Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Hoard, M. S.; Melody, N.; Pettit, R. K. *J. Nat. Prod.* **1998**, *61*, 13–16.
6. Cambie, R. C.; Craw, P. A.; Stone, M. J.; Bergquist, P. R. *J. Nat. Prod.* **1988**, *51*, 293–297.
7. Karuso, P.; Cambie, R. C.; Bowden, B. F.; Bergquist, P. R. *J. Nat. Prod.* **1989**, *52*, 289–293.
8. (a) Talpir, R.; Rudi, A.; Kashman, Y.; Loya, Y.; Hizi, A. *Tetrahedron* **1994**, *50*, 4179–4184; (b) Poigny, S.; Huor, T.; Guyot, M.; Samadi, M. *J. Org. Chem.* **1999**, *64*, 9318–9320.
9. Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J. *J. Org. Chem.* **1988**, *53*, 3644–3646.
10. Rudi, A.; Benayahu, Y.; Kashman, Y. *Org. Lett.* **2004**, *6*, 4013–4016.
11. Rueda, A.; Zubía, E.; Ortega, M. J.; Carballo, J. L.; Salvá, J. *J. Org. Chem.* **1997**, *62*, 1481–1485.
12. Cimino, G.; Fontana, A.; Giménez, F.; Marin, A.; Mollo, E.; Trivellone, E.; Zubía, E. *Experientia* **1993**, *49*, 582–586.
13. Lu, Q.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 195–198.
14. Venkateswarlu, Y.; Biabani, M. A. F.; Rao, T. P. *Indian J. Chem.* **1995**, *34B*, 563–564.
15. Cimino, G.; De Rosa, S.; De Stefano, S. *Experientia* **1981**, *37*, 214–216.
16. Carté, B.; Kernan, M. R.; Barrabee, E. B.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 3528–3532.
17. Cafieri, F.; De Napoli, L.; Fattorusso, E.; Santacroce, C.; Sica, D. *Tetrahedron Lett.* **1977**, 477–480.
18. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.