Additional Scalarane Sesterterpenes from the Sponge *Phyllospongia papyracea*

Hou-Jin Li,[†,‡] Taro Amagata,[†] Karen Tenney,[†] and Phillip Crews*[,†]

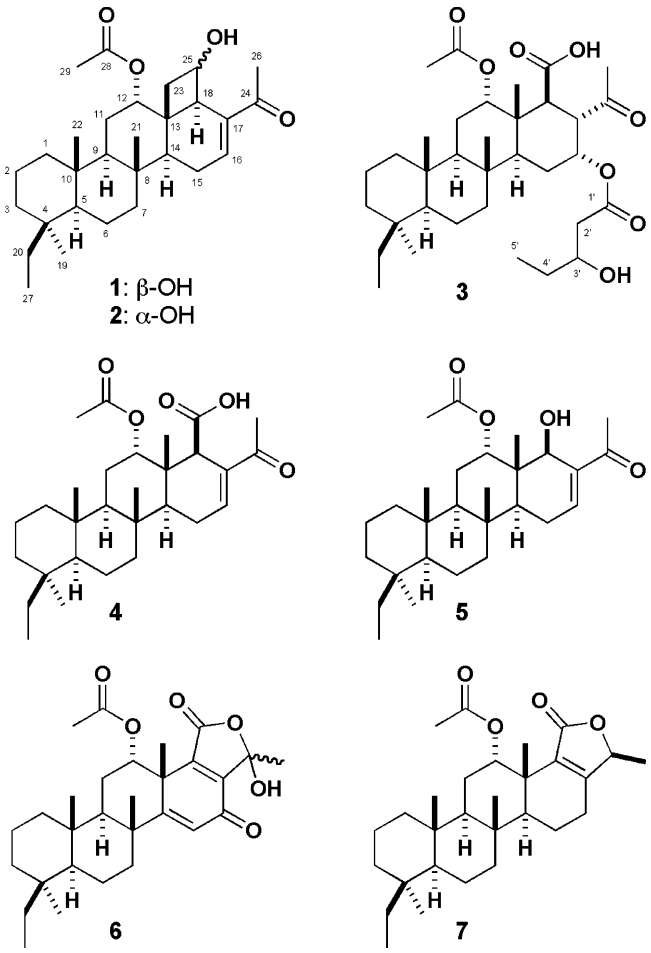
Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California Santa Cruz, Santa Cruz, California 95064, and School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, People's Republic of China

Received January 12, 2007

A chemical investigation of the marine sponge *Phyllospongia papyracea*, collected in Papua New Guinea, initiated by the screening result of a β-catenin/Tcf4 disruption assay afforded six new bishomoscalarane sesterterpenes containing two rare scalaranes with a cyclobutane ring in the molecule, together with one known scalarane sesterterpene. The structures of the new compounds were elucidated by 1D and 2D spectroscopic techniques. The compounds isolated in this study did not show activity against the β-catenin and Tcf4 complex.

There is growing interest in the evaluation of natural product repositories for small molecules that disrupt protein−protein interactions.[1] This strategy could emerge as an effective approach to discover additional molecular structures of value as anticancer therapeutic leads.[2] Many protein interfaces are characterized by flat and large interaction surfaces,[3] and there has been some success in identifying small molecules that can interfere with interprotein binding.[2,4,5] The oncology screening group at Novartis recently implemented an assay to identify antagonists of β-catenin and Tcf4 (T cell factor 4),[6] and in a proof of concept trial using a library of 7000 purified natural products, there were eight compounds identified demonstrating reproducible activity.[4] The relevance of this assay model lies in the circumstance that protein−protein interaction between β-catenin and Tcf4 is closely related to tumorgenesis and accumulation of β-catenin by disruption of the Wnt signaling pathway and/or mutation of APC protein is seen in most cases of colon cancer.[7,8] Our interest in further applying this assay model led to a survey of marine sponge extracts and was the stimulus for the project described in this account. Initial screening of a library of approximately 100 sponge crude extracts gave a hit showing dose-dependent inhibition of the β-catenin/Tcf4 interaction with an IC_{50} value of 9.3 μg/mL. The sponge had already been classified as *Phyllospongia papyracea* (coll no. 03552) of the Dictyoceratida order, and on the basis of the logic described next, it seemed likely to contain sesterterpenes.

The scalarane class of sesterterpenes is one major structural family obtained from Dictyoceratida sponges. As a quick overview, it is important to note that C_{25} scalarane sesterterpenes have been isolated from a variety of Dictyoceratida sponges (*Hyrtios electas*,[9] *Hyatella intestinalis*,[10] *Spongia* spp.,[11] *Phyllospongia dendyi*,[12] *Coscinoderma mathewsi*,[13] *Smenospongia* sp.,[14] and *Collospongia auris*[15]). In contrast, C_{26} homoscalaranes and C_{27} bishomoscalaranes appear to be produced by only a couple of genera of Dictyoceratida sponges (*Lendenfeldia* spp.,[16] *Phyllospongia* spp.,[17] previously identified as *Carteriospongia* spp.,[18] *Strepsichordaia* spp.,[19] and *Cacospongia scalaris*[20]) and by a single genera from the Dendroceratida sponge (*Dysidea* spp.[18b,21]). Especially relevant here is that the record from *Phyllospongia* shows only two types of molecular structures, scalarane sesterterpenes and polybrominated biphenyl ethers.[22] Discussed below is our isolation work that resulted in the characterization of six new sesterterpenes, **1**−**6**, together with one known bishomoscalarane sesterterpene, **7**.



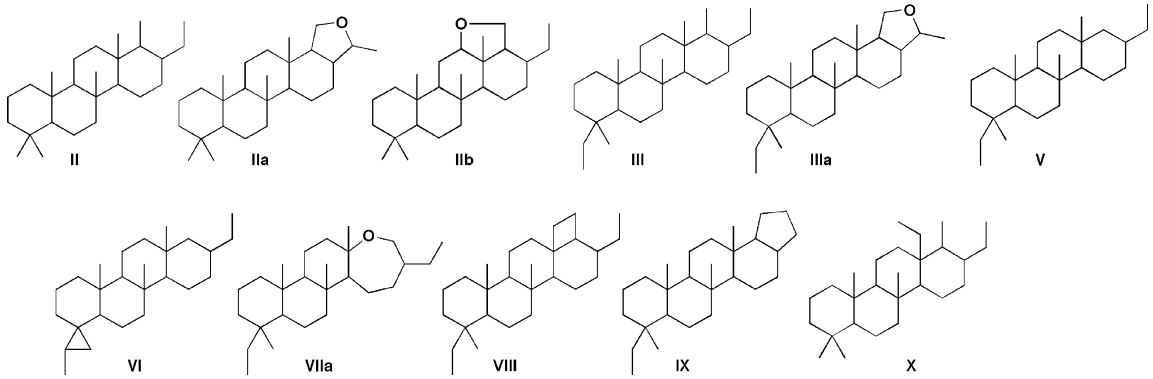
Results and Discussion

In view of the prior chemical literature on *Phyllospongia*, we were prepared to isolate both scalarane sesterterpenes and polybrominated biphenyl ethers. The ^1H NMR spectrum of the semipure methanol extract, obtained after partitioning with EtOAc and H_2O, showed many singlet methyl and complex methine and methylene signals from δ_H 0.5 to 2.5. Alternatively the ^{13}C and ^1H NMR spectra showed a lack of resonances for aromatic rings, and the LCMS analysis also indicated that halogenated compounds were not present. Therefore, the crude extract was assumed to be rich in scalarane-type compounds, especially 20,24-bishomoscalarane sesterterpenes because they are abundant among *Phyllospongia*-derived terpenoids.

* To whom correspondence should be addressed. Tel: (831) 459-2603. Fax: (831) 459-2935. E-mail: phil@chemistry.ucsc.edu.
† University of California Santa Cruz.
‡ Sun Yat-sen University.

10.1021/np070020f CCC: $37.00
Published on Web 04/25/2007

Table 1. Summary of C_{26} and C_{27} Homoscalarane Sesterterpene Frameworks: Structural Types[a] and Selected Examples



		C_{26} homoscalarane						C_{27} bishomoscalarane				
sponge	collection site	II	IIa	IIb	V	VI	VIIa	III	IIIa	VIII	IX	X
Lendenfeldia sp.	Madagascar[b]	×		×								
Phyllospongia madagascarensis	Madagascar[c]				×		×		×			
Phyllospongia sp.	Indonesia[d]	×	×		×			×	×			
	South China Sea[e]								×		×	
Carteriospongia sp.	Indonesia[f]								×			
Carteriospongia foliascens	New Guinea[g]				×			×	×	×		
Strepsichordaia aliena	Indonesia[h]					×			×			
Strepsichordaia lendenfeldi	Australia[i]				×			×		×		
Cacospongia scalaris	Tyrrhenian Sea[j]											×
Dysidea sp.	Solomon Islands[f]								×			

[a] The type IV homoscalarane in ref 16b was incorrectly drawn and should be revised to IIIa. [b]Chill, L.; Aknin, M.; Loya, S.; Hizi, A.; Kashman, Y. *Tetrahedron* **2004**, *60*, 10619−10626. [c]Ponomarenko, L. P.; Kalinovsky, A. I.; Stonik, V. A. *J. Nat. Prod.* **2004**, *67*, 1507−1510. [d]Roy, M. C.; Tanaka, J.; de Voogd, N.; Higa T. *J. Nat. Prod.* **2002**, *65*, 1838−1842. [e]Zeng, L.; Fu, X.; Su, J.; Pordesimo, E. O.; Traeger, S. C.; Schmitz, F. J. *J. Nat. Prod.* **1991**, *54*, 421−427. [f]Jaspars, M.; Jackson, E.; Lobkovsky, E.; Clardy, J.; Diaz, M. C.; Crews, P. *J. Nat. Prod.* **1997**, 556−561. [g]Braekman, J. C.; Daloze, D.; Kaisin M.; Moussiaux, B. *Tetrahedron* **1985**, *41*, 4603−4614. [h]Jiménez, J. I.; Yoshida, W. Y.; Scheuer, P. J.; Kelly M. *J. Nat. Prod.* **2000**, *63*, 1388−1392. [i]Jahn, T.; König, G. M.; Wright A. D. *J. Nat. Prod.* **1999**, *62*, 375−377. [j]De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C.; Tommonaro, G.; Zavodnik, N. *Tetrahedron* **1998**, *54*, 6185−6190.

In a previous report, we outlined an efficient approach to the dereplication of the scalarane class of sesterterpenoids.[16b] At that point five structural types were considered including (1) a normal C_{25} scalarane denoted as type I; (2) C_{26} homoscalaranes called types II, IV, and V; and (3) one C_{27} bishomoscalarane named type III. We now expand and slightly revise this overview by considering the 11 structural motifs shown in Table 1. These now include six C_{26} homoscalaranes and five C_{27} bishomoscalaranes divided among molecular structures with (a) tetracarbocyclics (types II, III, V, X), (b) tetracyclics with one oxo ring (type VIIa), (c) pentacarbocyclics (types VI, VIII, IX), and (d) pentacyclics with one oxo ring (types IIa, IIb, IIIa). Additional noteworthy patterns among this set include the following three factors. First, the A/B/C rings and their all-*trans* stereochemistry are highly conserved. Second, an oxygen functionality such as a hydroxy, ester, or ketone, is often present at C-12. Third, C-24 and C-25 are usually oxygen bearing. Finally, the most likely sites for structural diversity are in the D and/or E rings except for phyllolactones, which possess several different ester groups on C-3.[17c] Selected examples of the literature pertaining to these compound types as a function of the sponge source and structural class are shown in Table 1. These served as a database for further structure analysis in this project.

The first new compound characterized was **1**, of molecular formula $C_{29}H_{44}O_4$ (eight degrees of unsaturation). Subtracting the OAc group [δ_H 2.07 (H-29), δ_C 170.8 (C-28), 21.5 (C-29)] from this molecular formula indicated a bishomosesterterpene. Further evaluation of the ^{1}H and ^{13}C NMR data yielded the following diagnostic functional groups: (1) a trisubstituted double bond [δ_H 7.18 (H-16), δ_C 142.6 (C-16), 137.3 (C-17)]; (2) a carbonyl carbon [δ_C 202.1 (C-24)]; (3) three singlet methyls [δ_H 0.82 (H-19), 0.78 (C-21), 0.84 (C-22), δ_C 28.6 (C-19), 14.9 (C-21), 17.1 (C-22)]; and (4) one triplet methyl (C-27) (Tables 2 and 3), which suggested

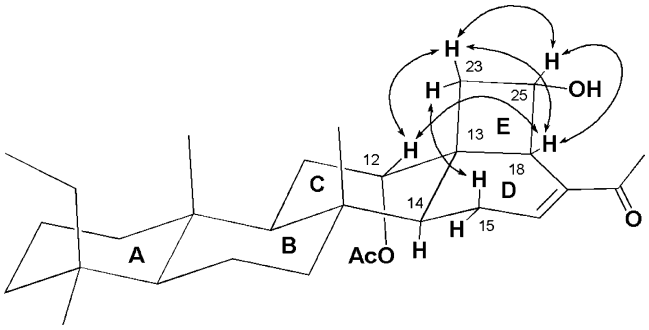


Figure 1. Selected key ROESY correlations for **1**.

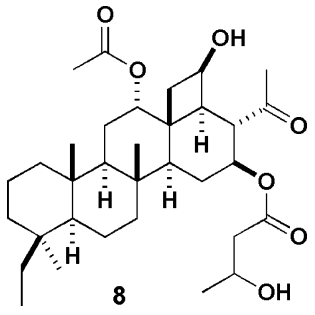
a type VIII ring system. The presence of these methyl groups assigned at C-19, C-21, and C-22 plus the signals for the acetyl group were reminiscent of signature resonances for the known bishomoscalarane sesterterpene **8**.[19b] The major NMR spectral differences between **1** and **8** were the lack of an ester group on C-16 and the trisubstituted olefin was required at C-16−C-17. The new E ring as well as the remaining planar structure shown for **1** was supported by diagnostic chemical shifts and key HMBC correlations (H-16/C-18, H-16/C-24, H-25/C-17). The all-*trans* A/B/C rings of **1** were the same as in **8** on the basis of the parallel coupling constants (at H-5, H-9, H-14) and carbon NMR shifts for attached methyl groups (at C-4, C-8, C-10). The remaining relative configurations on the C/D/E rings were determined by ROESY correlations shown in Figure 1. The acetyl group on C-12 was assigned as α by the ROESY correlation between H-12/H-23α. In addition, ROESY correlations between H-18/H-23α, H-18/H-25, H-23α/H-25, H-12/H-18, and H-15ax/H-23β allowed assignment of a β-OH at C-25 for **1**, which was identical to the configuration in **8**. Thus, the structure of **1** was finalized as 12α-

Table 2. ^{13}C NMR Data for **1**−**6** in $CDCl_3$ (125.7 MHz)

position	1	2	3	4	5	6
1	40.4 (CH_2)	40.4 (CH_2)	40.2 (CH_2)	40.2 (CH_2)	40.2 (CH_2)	40.4 (CH_2)
2	18.3 (CH_2)	18.3 (CH_2)	18.3 (CH_2)	18.3 (CH_2)	18.3 (CH_2)	18.6 (CH_2)
3	36.7 (CH_2)	36.7 (CH_2)	36.7 (CH_2)	36.7 (CH_2)	36.8 (CH_2)	36.5 (CH_2)
4	36.2 (C)	36.2 (C)	36.2 (C)	36.2 (C)	36.2 (C)	36.2 (C)
5	58.7 (CH)	58.8 (CH)	58.5 (CH)	58.6 (CH)	58.7 (CH)	58.4 (CH)
6	18.1 (CH_2)	18.1 (CH_2)	18.1 (CH_2)	18.1 (CH_2)	18.0 (CH_2)	18.2 (CH_2)
7	40.8 (CH_2)	40.8 (CH_2)	41.7 (CH_2)	42.0 (CH_2)	41.7 (CH_2)	41.1 (CH_2)
8	37.9 (C)	37.9 (C)	37.7 (C)	38.0 (C)	37.5 (C)	42.2 (C)
9	53.1 (CH)	53.1 (CH)	52.8 (CH)	52.3 (CH)	52.6 (CH)	50.6 (CH)
10	37.0 (C)	37.0 (C)	37.0 (C)	36.9 (C)	37.0 (C)	38.2 (C)
11	22.2 (CH_2)	21.3 (CH_2)	21.9 (CH_2)	22.1 (CH_2)	22.2 (CH_2)	21.3[a] (CH_2)
						21.2[a]
12	76.2 (CH)	76.9 (CH)	75.7 (CH)	75.7 (CH)	73.8 (CH)	75.9[b] (CH)
						75.8[b]
13	36.9 (C)	40.3 (C)	40.4 (C)	39.0 (C)	40.1 (C)	44.3[c] (C)
						44.2[c]
14	43.2 (CH)	44.1 (CH)	46.4 (CH)	49.2 (CH)	47.7 (CH)	176.3 (C)
						176.1
15	23.1 (CH_2)	22.5 (CH_2)	25.5 (CH_2)	23.3 (CH_2)	24.4 (CH_2)	125.5 (CH)
16	142.6 (CH)	140.7 (CH)	68.6 (CH)	140.0 (CH)	142.2 (CH)	182.2 (C)
17	137.3 (C)	139.2 (C)	52.0 (CH)	138.0 (C)	140.1 (C)	150.0 (C)
18	43.1 (CH)	45.7 (CH)	45.6 (CH)	48.1 (CH)	70.0 (CH)	149.1[d] (C)
						149.0[d]
19	28.6 (CH_3)	28.6 (CH_3)	28.5 (CH_3)	28.6 (CH_3)	28.6 (CH_3)	28.6 (CH_3)
20	24.7 (CH_2)	24.7 (CH_2)	24.6 (CH_2)	24.6 (CH_2)	24.6 (CH_2)	24.7 (CH_2)
21	14.9 (CH_3)	15.3 (CH_3)	17.0 (CH_3)	15.8 (CH_3)	16.4 (CH_3)	22.9[e] (CH_3)
						22.8[e]
22	17.1 (CH_3)	17.2 (CH_3)	17.1 (CH_3)	16.9 (CH_3)	17.2 (CH_3)	17.0 (CH_3)
23	35.3 (CH_2)	34.0 (CH_2)	15.2 (CH_3)	17.2 (CH_3)	12.9 (CH_3)	25.8[f] (CH_3)
						25.7[f]
24	202.1 (C)	198.5 (C)	207.9 (C)	199.3 (C)	202.3 (C)	103.8 (C)
25	67.0 (CH)	73.4 (CH)	175.6 (C)	174.5 (C)		166.8[g] (C)
						166.7[g]
26	25.6 (CH_3)	25.4 (CH_3)	28.9 (CH_3)	25.5 (CH_3)	26.2 (CH_3)	25.0 (CH_3)
27	8.7 (CH_3)	8.7 (CH_3)	8.7 (CH_3)	8.8 (CH_3)	8.8 (CH_3)	8.8 (CH_3)
28	170.8 (C)	170.9 (C)	171.3 (C)	170.5 (C)	170.2 (C)	169.5[h] (C)
						169.1[h]
29	21.5 (CH_3)	21.6 (CH_3)	21.7 (CH_3)	21.6 (CH_3)	21.6 (CH_3)	21.0[i] (CH_3)
						20.9[i]
1′			172.2 (C)			
2′			41.7 (CH_2)			
3′			69.7 (CH)			
4′			29.7 (CH_2)			
5′			10.1 (CH_3)			

[a−i] Values with the same superscripts indicate the isomer signals.

acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25β-ol.



8

Compound **2**, an isomer of **1**, was analyzed next. The only new feature in its ^{13}C NMR spectrum was that C-25 was shifted 6.5 ppm downfield compared to that of **1**. Additional divergences (> ±0.25 ppm) were observed by ^1H NMR, especially for protons in the vicinity of the E ring, including H-12, H-18, H-23, and H-25. The most significant variation was for the oxymethine proton signal (H-25), which was shifted 1.0 ppm downfield and showed a new splitting pattern versus H-25 of **1**. This was consistent with the proposal that **2** was the 25-epimer of **1**. Further substantiation of the proposed C-25 α-OH group came from the observed ROESY correlation between H-23β and H-25. Therefore, compound **2** was assigned as 12α-acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25α-ol.

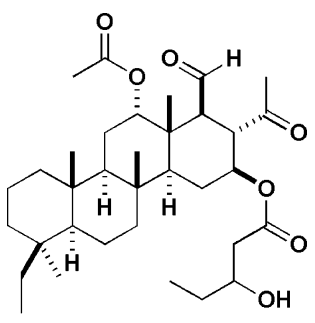
The characterization of **3**, whose molecular formula of $C_{34}H_{54}O_8$ required eight degrees of unsaturation, was slightly more complex. The functional groups consisted of an acetyl group (C-28/C-29), a methyl ketone (C-24/C-26), a carboxylic acid (C-25), and a 3-hydroxypentanoate group assembled by gCOSY correlations (C-1′−C-5′). Subtracting the carbon count of C_7 associated with the two ester groups was an important step, as it indicated **3** was a bishomosesterterpene with four carbocyclic rings. There were four additional singlet methyls (C-19, C-21, C-22, and C-23) and one ethyl group, diagnostic of a type III ring system. Further dereplication showed **3** had one more oxygen than the molecular formula of bishomoscalarane **9**.[19c] Side-by-side comparison of their NMR properties showed that this pair had identical all-*trans* A/B/C/D ring junctions and configurations at C-4 and C-12. Further, the major differences identified were the configurations of the ester groups at C-16, β for **9** and α for **3**, and that the β aldehyde of **9** was now a β carboxylic group in **3**. These latter structural features were affirmed by three key observations. (1) The 3-hydroxypentanoate, the methyl ketone, and the carboxylic acid were located on C-16, C-17, and C-18, respectively, on the basis of gCOSY correlations (H16/H17 and H-17/H-18) and gHMBC correlations (H-16/C-1′, H-17/C-24 and C-25, and H-18/C-24 and C-25). (2) The geometries of the substituents at C-17 and C-18 were determined by a combination of coupling constants and ROESY data. For example, the large coupling constant (J = 12.0 Hz) between H-17 and H-18 suggested that H-17 and H-18 existed in

Table 3. ^{1}H NMR Data for **1**−**6** in $CDCl_3$ (500 MHz, *J* values in Hz)

position	1	2	3	4	5	6
1ax	0.64 (td, 12.5, 4.0)	0.65 (td, 12.5, 3.5)	0.61 (td, 13.0, 3.5)	0.60 (td, 12.5, 3.5)	0.63 (td, 12.5, 4.0)	0.61 (td, 13.0, 4.0)
1eq	1.58 (m)	1.60 (m)	1.61 (m)	1.61 (m)	1.61 (m)	1.64 (m)
2ax	1.40 (m)	1.38 (m)	1.37 (m)	1.33 (m)	1.36 (m)	1.37 (m)
2eq	1.52 (m)	1.54 (m)	1.48 (m)	1.45 (m)	1.52 (m)	1.49 (m)
3ax	0.87 (ddd, 12.5, 12.0, 2.0)	0.86 (m)	0.86 (m)	0.89 (td, 13.5, 3.5)	0.88 (m)	0.84 (m)
3eq	1.67 (dt, 12.5, 2.5)	1.65 (m)	1.63 (m)	1.64 (m)	1.66 (m)	1.67 (m)
5	0.89 (dd, 12.0, 2.0)	0.88 (dd, 12.0, 2.0)	0.92 (dd, 13.0, 2.0)	0.90 (dd, 12.5, 2.0)	0.91 (dd, 12.5, 2.0)	0.90 (dd, 12.0, 2.0)
6ax	1.36 (m)	1.33 (m)	1.42 (m)	1.42 (m)	1.43 (td, 13.5, 3.0)	1.58 (m)
6eq	1.42 (m)	1.49 (dt, 13.5, 3.5)	1.55 (m)	1.56 (m)	1.58 (m)	1.72 (m)
7ax	1.06 (td, 12.5, 4.0)	1.05 (td, 12.5, 4.0)	0.88 (m)	0.98 (m)	0.99 (td, 12.5, 2.0)	1.60 (m)
7eq	1.73 (dt, 12.5, 3.0)	1.73 (dt, 13.5, 3.5)	1.69 (m)	1.78 (m)	1.73 (dt, 12.5, 3.0)	1.98 (m)
9	1.23 (dd, 13.0, 2.0)	1.22 (dd, 13.5, 2.0)	1.28 (d, 13.0)	1.28 (br d, 12.0)	1.26 (dd, 13.0, 1.5)	1.32 (m)
11ax	1.60 (m)	1.66 (m)	1.68 (m)	1.64 (m)	1.65 (m)	1.98 (m)
11eq	1.82 (ddd, 15.0, 3.0, 2.5)	1.85 (dt, 15.0, 3.0)	1.89 (m)	1.89 (br d, 14.5)	1.80 (ddd, 15.0, 3.0, 2.5)	2.15[a] (dt, 15.0, 3.0)
						2.17[a] (dt, 15.0, 3.0)
12	5.12 (dd, 2.5, 2.0)	5.66 (t, 3.0)	4.71 (br s)	4.85 (t, 2.0)	5.07 (t, 3.0)	5.76 (t, 3.0)
14	1.33 (dd, 11.5, 5.0)	1.31 (dd, 12.0, 4.5)	1.68 (m)	1.50 (m)	1.50 (m)	
15ax	2.24 (ddt, 19.0, 11.5, 2.5)	2.13 (ddt, 19.0, 12.5, 2.0)	1.67 (m)	2.27 (2H, m)	2.25 (2H, m)	6.360[b] (s)
15eq	2.41 (dt, 19.0, 5.5)	2.37 (ddd, 19.0, 6.5, 5.0)	1.92 (m)			6.364[b] (s)
16ax	7.18 (dd, 7.0, 2.0)	6.99 (dd, 7.0, 2.0)	5.64 (br s)	6.93 (td, 3.5, 2.5)	6.88 (td, 4.0, 1.5)	
16eq						
17			3.18 (dd, 12.0, 3.0)			
18	3.02 (d, 8.0)	2.72 (br s)	3.33 (d, 12.0)	3.86 (q, 2.5)	4.60 (dd, 4.0, 2.5)	
19	0.82 (s)	0.81 (s)	0.81 (s)	0.81 (s)	0.81 (s)	0.839[c] (s)
						0.841[c] (s)
20a	1.19 (dq, 15.0, 7.5)	1.16 (dq, 15.0, 7.5)	1.15 (dq, 15.0, 7.5)	1.16 (dq, 14.5, 7.5)	1.16 (dq, 15.0, 7.5)	1.20 (dq, 15.0, 7.5)
20b	1.53 (m)	1.55 (dq, 15.0, 7.5)	1.55 (m)	1.53 (dq, 14.5, 7.5)	1.52 (dq, 15.0, 7.5)	1.55 (dq, 15.0, 7.5)
21	0.78 (s)	0.78 (s)	0.85 (s)	0.98 (s)	0.96 (s)	1.306[d] (s)
						1.310[d] (s)
22	0.84 (s)	0.84 (s)	0.82 (s)	0.84 (s)	0.85 (s)	0.95 (s)
23a	1.86 (dd, 12.0, 8.0)	1.58 (m)	1.00 (s)	0.97 (s)	0.93 (s)	1.62 (s)
23b	2.00 (m)	2.28 (m)				1.65 (s)
24						
25	4.65 (qd, 8.0, 3.0)	3.63 (d, 7.0)				
26	2.37 (s)	2.31 (s)	2.20 (s)	2.31 (s)	2.32 (s)	1.85 (s)
						1.93 (s)
27	0.75 (t, 7.5)	0.75 (t, 7.5)	0.74 (t, 7.5)	0.75 (t, 7.5)	0.75 (s)	0.77 (t, 7.5)
29	2.07 (s)	2.07 (s)	2.17 (s)	2.14 (s)	2.11 (s)	1.86[e] (s)
						1.93[e] (s)
2′a			2.42 (dd, 15.0, 9.0)			
2′b			2.48 (dd, 15.0, 3.5)			
3′			3.89 (m)			
4′a			1.48 (m)			
4′b			1.52 (m)			
5			0.99 (t, 7.5)			
24-OH						3.60 (br s)
25-OH					4.15 (br s)	

[a−e] Values with the same superscripts indicate the isomer signals.

a *trans*-diaxial arrangement. In addition, ROESY correlations were observed between H-17/H-23 and H-18/H-14. (3) The small coupling constant between H-17 axial and H-16 (J = 3.0 Hz) showed equatorial placement for H-16. Therefore, compound **3** was elucidated as 12α-acetoxy-16α-(3′-hydroxypentanoyloxy)-20,24-dimethyl-24-oxoscalaran-25*β*-oic acid.



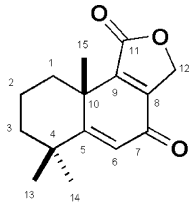
9

Closely related to **3** was the new compound **4** of molecular formula $C_{29}H_{44}O_5$ established by HRESIMS. Most of the ^{1}H and ^{13}C NMR resonances of this pair were similar. The major differences were that the signals of H-17 and of the 3-hydroxypentanoate in **3** were missing and there was a new $\Delta^{16,17}$ double bond. The relative configurations at all chiral carbons of **4** were deduced to be identical to those of **3** on the basis of the following two reasons: (a) the δ_C's for each of the four singlet (by ^{1}H NMR) methyls were identical, and (b) a strong ROESY correlation was seen between H-18 and H-14. Therefore, compound **4** was determined to be 12α-acetoxy-20,24-dimethyl-24-oxoscalar-16-en-25*β*-oic acid.

The structural theme seen above in the bishomosesterterpenes **3** and **4** was slightly altered in the homosesterterpene compound **5**. Subtracting the atom count of the C-12 OAc from the molecular formula $C_{28}H_{45}O_4$, established by HRESIMS, was the important first step. As evident from the following analysis, it was straightforward to conclude that the *β*-C-18 CO_2H of **4** was replaced by a *β* OH and that **5** possessed the type V carbon framework. The NMR

signals for the carboxylic acid at C-25 in **4** were missing but replaced by a new oxymethine (δ_C 70.0, δ_H 4.60, dd) with the expected associated HMBC correlations (H-14/C-18, H-18/C-17, H-18/C-13, and H-23/C18). The NMR data substantiated that the all-*trans* A/B/C/D ring, the β-ethyl group at C-4, and an α-C-12 OAc of **4** were also present in **5**. The ROESY correlation between H-18 and H-14 firmly established their relative stereochemistry. Thus, compound **5** was assigned as 25-nor-12α-acetoxy-20,24-dimethyl-24-oxoscalar-16-en-18β-ol.

A pentacyclic ring system was concluded to be present in compound **6**, but it was isolated as a 1:1 mixture of diastereomers. The molecular formula $C_{29}H_{40}O_6$, requiring 10 degrees of unsaturation, was seen to be consistent with the NMR data once it was recognized that pairs of signals were observed for many atoms (δ_C 20.9/21.0, 21.2/21.3, 22.8/22.9, 25.7/25.8, 44.2/44.3, 75.8/75.9, 149.0/149.1, 166.7/166.8, 169.1/169.5, δ_H 0.839/0.841, 1.306/1.310, 1.86/1.93, 2.15/2.17, 6.360/6.364). The signature peaks observed for the *trans* A/B/C rings of **1**−**5** were all located in **6**. An axial C-23 ring junction methyl was also assigned on the basis of its diagnostic upfield shift (δ_C 25.8/25.7). The functional groups were identified as an α C-12 acetyl group (C-28 and C-29), a cross-conjugated ketone (C-16), an unsaturated γ-lactone carbonyl (C-25), a trisubstituted olefin (C-14 and C-15), and a tetrasubstituted olefin (C-17 and C-18). A hemiketal quaternary carbon (δ 103.8) was located at C-24 to complete the furanone E-ring. This was concluded to be the diastereomeric center, and the regiochemistry shown was affirmed by HMBC correlations (H_3-26/C-17 and C-24). The overall data were consistent with those expected for a type IIIa molecular structure. The additional arguments in support of the structure shown here were as follows. The cross-conjugated ketone system and the lactone carbonyl of **6** possessed nearly identical NMR properties versus the similar residues of 7-ketoisodrimenin sesquiterpene **10** (C-10, δ_C 40.9; C-5, δ_C 177.0; C-6, δ_H 6.42, s, δ_C 124.8; C-7, δ_C 182.5; C-8, δ_C 149.6; C-9, δ_C 149.7; C-11, δ_C 170.7).[23] The remaining elements of the planar structure were confirmed by HMBC correlations (H-15/C-8 and C-17, H_3-23/C-12, C-13, C-14, and C-18). The final structure of **6** as 12α-acetoxy-20,24-dimethyl-16,24-dioxoscalara-14,17-dien-24-ol-25,24-olide represents a close analogue to that of known compound **7**, which was isolated and identified as 12α-acetoxy-20,24β-dimethylscalar-17-eno-25,24-lactone by comparison of the NMR data (Table S7) to the published data.[18a] It is important to note that the stereochemistry at C-24 (δ_C 77.8) of **7** was determined as β by reference to the diagnostic methyl shifts in other similar ring systems where a 24β-methyl has δ_C < 78.0, while the 24α-methyl has δ_C > 78.0.[19a]



10

Conclusions

The overview of Table 1 shows that homosesterterpenes and bishomosesterterpenes occur commonly among sponge genera including *Phyllospongia*, *Carteriospongia*, *Strepsichordaia*, *Lendenfeldia*, and occasionally *Dysidea* collected from several Indo-Pacific regions. The results of this study on *P. papyracea* collected in Papua New Guinea provide some interesting additions to this record. While no scalarane sesterterpenes were observed, the seven compounds isolated can be divided among homoscalarane (type V: **5**) and bishomoscalarane (type VIII: **1**, **2**, type III: **3**, **4**, and type IIIa: **6**, **7**) sesterterpenes. The metabolites **1** and **2** are unusual from a biosynthetic perspective and are also extremely rare. This framework has been observed only twice previously and in the following context. A Papua New Guinea sponge identified as *Carteriospongia foliascens* afforded almost identical results to those described herein with six compounds found including homoscalaranes (one type V) and bishomoscalaranes (two type III, two type IIIa, and one type VIII).[18c] A Great Barrier Reef, Australia, sponge classified as *Strepsichordaia lendenfeldi* also possessed a similar array of metabolites including homoscalaranes (eight type V) and bishomoscalaranes (one type III and one type VIII).[19b] These very similar metabolite profiles suggest the three organisms might be more closely related than implied by their current classifications into three distinct genera. Such an observation is reminiscent of a comment we made for several years about the difficulty in differentiating among morphologically similar sponges: *Phyllospongia*, *Carteriospongia*, and *Strepsichordaia*.[18b] As a final point, attempts to extend the bioactivities observed in the past for scalaranes[9b,16b,24,25] related to those isolated here were unsuccessful. None of the major components **1**−**7** exhibited activity in the β-catenin/Tcf4 PPI assay or in any other of a host of mechanism-based screens at Novartis.[6] Our search for the active minor constituents of this sponge continues.

Experimental Section

General Experimental Procedures. Melting points were measured on automated melting point apparatus EZ-Melt and are uncorrected. Optical rotations were acquired using a JASCO DPI-370 digital polarimeter. UV/vis spectra were recorded on a Perkin-Elmer Lambda 40 UV/visible spectrophotometer. The IR spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer model-337. NMR spectra were recorded on a Varian Inova-500 NMR spectrometer at 500 MHz (^{1}H) and 125.7 MHz (^{13}C), and $CDCl_3$ was used as an internal standard (δ_H 7.26 and δ_C 77.0). LCMS were performed with a Phenomenex C_{18} RP column (250 × 4.6 mm) using an ESITOFMS, a Waters 996 photodiode array detector, and a Sedex55 evaporative light scattering (ELS) detector. High-resolution mass measurements were obtained on a Mariner 5200 benchtop ESITOF mass spectrometer. HPLC was performed with a Phenomenex C_{18} RP column (5 μm) with a single wavelength (λ = 230 nm). Preparative thin-layer chromatographies were performed with Uniplate Si gel GF254 (20 × 20 cm).

Biological Material. The sponge was collected in Papua New Guinea (coll. no. 03552) using scuba at depths of 60 ft (9°14′948″ S, 150°46′808″ E). The sponge had a smooth surface, thin fan shape with the top surface brown and the underside purple. The oscules were barely visible. An underwater photo is in the Supporting Information (Figure S1). The sponge was identified as *Phyllospongia papyracea* (Esper, 1794) (order Dictyoceratida) by R. W. M. van Soest, University of Amsterdam. The voucher specimens were deposited in the marine natural products lab of UCSC and zoological museum of University of Amsterdam (ZMA museum number ZMAPOR19131).

Extraction and Isolation. The *P. papyracea* (coll. no. 03552, 0.9 kg, wet weight) was preserved by our standard procedure as described previously.[26] The organism was soaked in methanol three times, and the MeOH solution was evaporated under reduced pressure to give the MeOH extract (23.6 g). The methanol extract was further partitioned with EtOAc and water, and the organic layer was concentrated under reduced pressure to give the EtOAc extract (11.2 g). The EtOAc extract was fractionated by Si gel flash chromatography using an *n*-hexane−EtOAc−MeOH stepwise gradient to afford 25 fractions (coded F1−F25). The fractions coded F6−F8 were combined and further fractionated using preparative RP HPLC with a gradient of 40:60 up to 0:100 H_2O−MeOH to afford three fractions, F6H1−F6H3. The first fraction (F6H1) was further purified by repeated RP HPLC to yield **5** (5 mg). By using the same HPLC conditions, compound **6** (4 mg) was obtained from the second fraction (F6H2). The third fraction (F6H3) was subjected to RP HPLC to afford compound **7** (3 mg) and a semipure fraction. This semipure fraction was further purified by NP preparative TLC eluting with *n*-hexane−EtOAc (v/v 2:1) to give **1** (8 mg) and **2** (6 mg). Compound **4** (4 mg) was obtained from F16 of the Si gel column using RP HPLC (H_2O−MeOH, 60:40−0:100%). Compound **3** (82 mg) was purified from F20 of the Si gel column using a RP HPLC (H_2O−MeOH, 60:40−0:100%).

12α-Acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25β-ol (1): white solid; mp 174−175 °C; $[\alpha]^{24}_D$ +129 (*c* 0.37, MeOH); IR (CH_2Cl_2) ν_{max} 3447, 2958, 2931, 2871, 2847, 1731, 1663, 1463, 1444, 1386, 1371, 1352, 1245, 1135, 1023, 956, 735 cm^{-1}; UV (MeOH) λ_{max} 254 nm (log ϵ 3.23); ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 457.3332 $[M + H]^+$ (calcd for $C_{29}H_{45}O_4$, 457.3312).

12α-Acetoxy-13β,18β-cyclobutane-20,24-dimethyl-24-oxoscalar-16-en-25α-ol (2): white solid; mp 124−125 °C; $[\alpha]^{24}_D$ +46 (*c* 0.47, MeOH); IR (CH_2Cl_2) ν_{max} 3434, 2957, 2930, 2873, 2847, 1731, 1663, 1462, 1440, 1384, 1368, 1354, 1247, 1132, 1020, 1012, 959, 734 cm^{-1}; UV (MeOH) λ_{max} 257 nm (log ϵ 3.14); ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 479.3141 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_4Na$, 479.3131).

12α-Acetoxy-16α-(3′-hydroxypentanoyloxy)-20,24-dimethyl-24-oxoscalaran-25β-oic acid (3): white solid; mp 133−134 °C; $[\alpha]^{24}_D$ +65 (*c* 0.87, MeOH); IR (CH_2Cl_2) ν_{max} 3483, 2960, 2932, 2877, 2848, 1718, 1461, 1391, 1374, 1356, 1266, 1246, 1180, 1168, 1100, 1037, 1012, 981, 735 cm^{-1}; UV (MeOH) λ_{max} 230 nm (log ϵ 1.66); ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 613.3702 $[M + Na]^+$ (calcd for $C_{34}H_{54}O_8Na$, 613.3710).

12α-Acetoxy-20,24-dimethyl-24-oxoscalar-16-en-25β-oic acid (4): colorless oil; $[\alpha]^{24}_D$ −55 (*c* 0.67, MeOH); IR (CH_2Cl_2) ν_{max} 3498, 2957, 2928, 2874, 2848, 1736, 1724, 1671, 1460, 1425, 1388, 1375, 1274, 1259, 1241, 1200, 1035, 1003, 764, 749 cm^{-1}; UV (MeOH) λ_{max} 249 nm (log ϵ 3.06); ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 495.3056 $[M + H]^+$ (calcd for $C_{29}H_{44}O_5Na$ 495.3081).

25-Nor-12α-acetoxy-20,24-dimethyl-24-oxoscalar-16-en-18β-ol (5): colorless oil; $[\alpha]^{24}_D$ −95 (*c* 0.33, MeOH); IR (CH_2Cl_2) ν_{max} 3497, 2957, 2927, 2874, 2847, 1735, 1648, 1460, 1423, 1379, 1245, 1200, 1186, 1034, 1013, 975, 956, 764, 749 cm^{-1}; UV (MeOH) λ_{max} 252 nm (log ϵ 3.55); ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 445.3267 $[M + H]^+$ (calcd for $C_{28}H_{45}O_4$, 445.3312).

12α-Acetoxy-20,24-dimethyl-16,24-dioxoscalara-14,17-dien-24-ol-25,24-olide (6): white solid; mp 220−222 °C; $[\alpha]^{24}_D$ +93 (*c* 0.77, MeOH); IR (CH_2Cl_2) ν_{max} 3385, 2958, 2935, 2876, 2849, 1762, 1689, 1649, 1587, 1459, 1443, 1423, 1379, 1370, 1264, 1227, 1192, 1166, 1151, 1047, 1026, 1002, 973, 887, 735 cm^{-1}; UV(MeOH) λ_{max} 257 nm (log ϵ 3.51); ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 507.2728 $[M + Na]^+$ (calcd for $C_{29}H_{40}O_6Na$, 507.2717).

12α-Acetoxy-20,24β-dimethylscalar-17-eno-25,24-lactone (7): white solid; mp 188−190 °C; $[\alpha]^{24}_D$ +91 (*c* 0.31, MeOH); IR (CH_2Cl_2) ν_{max} 2960, 2929, 2874, 2847, 1747, 1673, 1462, 1442, 1386, 1373, 1320, 1240, 1203, 1067, 1037, 1026, 1003, 920, 787, 764 cm^{-1}; UV (MeOH) λ_{max} 251 nm (log ϵ 2.54); HRESITOFMS *m*/*z* 479.3111 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_4Na$, 479.3131). This compound was identified by comparison of the NMR data (Table S7) to the published values.[18a]

Acknowledgment. This work was supported by grants from the National Institutes of Health: RO1 CA 47135 and U19 CA 52955. We thank M. Leszczyniecka of Novartis Oncology for performing the *in vitro* biochemical β-catenine/Tcf4 interaction assay.

Supporting Information Available: The underwater photograph of the sponge *Phyllospongia papyracea* (collection number 03552), isolation scheme, ^1H and ^{13}C NMR spectra, and detailed NMR data of **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP070020F