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Phyllolactones A–E: new bishomoscalarane sesterterpenes from the marine sponge *Phyllospongia lamellosa*

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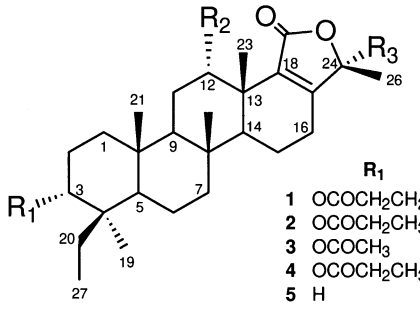
Abstract—Five new bishomoscalarane sesterterpenes, phyllolactones A–E (**1**–**5**), have been isolated from the marine sponge *Phyllospongia lamellosa*. The structures were elucidated by 1D and 2D ^{1}H and ^{13}C NMR, unambiguous assignments of overlapping ^{1}H and ^{13}C resonances were made by analysis of an HSQC–TOCSY spectrum, and relative stereochemistry established by analysis of coupling constants and ROESY and NOESY spectra. A summary of previously reported carbolactone-containing bishomoscalarane structures and the range of ^{13}C chemical shifts arising from the various oxy substituents at C-3, C-12, C-16, C-20 and C-24 is included. Phyllolactones A–E modestly inhibit HIV-1 envelope-mediated fusion in vitro with IC_{50}s of ~2 μM, and show negligible cytotoxicity toward the cell lines used in the fusion assay (BS-C-1 and NIH 3T3). Published by Elsevier Science Ltd.

1. Introduction

Marine invertebrates have proven to be a prolific and valuable source of novel secondary metabolites, many of which are limited to organisms of a particular order or family.[1] Thousands of marine extracts have been tested in a variety of biological assays ranging from those of a biomedical nature where assays target a particular cell type or pathogen associated with a given disease (such as cancer,[2a] inflammation[2b] and AIDS[2c]), to those of an ecological nature where assays are designed to uncover the role of these molecules in their natural marine environment (e.g. chemical defense[2d]). Consequently, in addition to the array of novel carbon skeletons produced by marine invertebrates, it has been amply demonstrated that many marine natural products also exhibit biological activities that are of potential value from the perspective of drug discovery.[2a–c] As part of an effort to identify inhibitors of viral entry of human immunodeficiency virus (HIV), we have tested a number of marine extracts. In this paper, we report the isolation and structure elucidation of a series of five new carbolactone-containing bishomoscalarane sesterterpenes (**1**–**5**) from the marine sponge *Phyllospongia lamellosa*, and report on their inhibition of HIV-1 envelope-mediated fusion in vitro.



	R_1	R_2	R_3
1	$OCOCH_2CH_2CH_3$	OH	H
2	$OCOCH_2CH_3$	OH	H
3	$OCOCH_3$	OH	H
4	$OCOCH_2CH_3$	OAc	H
5	H	OH	OH

A number of related carbolactone-containing bishomoscalaranes have been reported and are currently limited to three other sponge genera belonging to the order Dictyoceratida, namely *Carteriospongia*,[3–5] *Phyllospongia*,[6–13] and *Strepsichordaia*.[14] To facilitate comparison of these structures we have presented a summary of published carbolactone-containing bishomoscalarane sesterterpenes illustrating the location and composition of the various oxy-substituents found on the sesterterpene skeleton. A table correlating the carbon chemical shifts of the A-, C- and D-ring carbons as a function of the oxy-substituent present (or absent in the case of a methylene) at C-3, C-12 or C-16, respectively, is included.

2. Results

The crude organic extract of the marine sponge *P. lamellosa* was chosen for investigation since it inhibited HIV-1 envelope-mediated fusion at a concentration of less than

Keywords: marine metabolites; terpenes; biologically active metabolites.

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Table 1. ^{1}H and ^{13}C NMR data (ppm) for **2** in CD_3OD

Atom	δ_C	δ_H	Mult	J (Hz)	HMBC	NOEs and ROEs
1	34.3	1.20_{ax}	m		C-2, C-21	$H\text{-}2_{eq}$, $H\text{-}9_{ax}$
		1.44_{eq}	m			$H\text{-}2_{ax}$, $H\text{-}2_{eq}$
2	23.4	1.66_{ax}	m		C-1, C-3	$H\text{-}1_{eq}$, $H\text{-}3_{eq}$, CH_3-21
		1.89_{eq}	m		C-1, C-3	$H\text{-}1_{ax}$, $H\text{-}1_{eq}$, $H\text{-}3_{eq}$
3	76.3	4.64_{eq}	t	2.4	C-1, C-2, C-4, C-5, C-6, C-1′	$H\text{-}2_{ax}$, $H\text{-}2_{eq}$, CH_3-19, CH_3-27
4	39.8					
5	52.6	1.45_{ax}	m		C-3, C-4, C-6, C-9	$H\text{-}6_{eq}$, $H\text{-}7_{ax}$, $H\text{-}9_{ax}$, CH_3-19
6	18.6	1.40_{ax}	m		C-5, C-10	CH_3-21, 22
		1.60_{eq}	m			$H\text{-}5_{ax}$, $H\text{-}7_{ax}$
7	42.9	1.10_{ax}	m		C-6, C-8, C-9	$H\text{-}5_{ax}$, $H\text{-}6_{eq}$, $H\text{-}9_{ax}$, $H\text{-}14_{ax}$
		1.88_{eq}	m		C-5, C-6, C-8	CH_3-22
8	38.3					
9	53.2	1.58_{ax}	m		C-8, C-10, C-11, C-21	$H\text{-}1_{ax}$, $H\text{-}5_{ax}$, $H\text{-}7_{ax}$
10	38.9					
11	25.6	1.67_{ax}	m		C-8, C-9, C-12, C-13	$H\text{-}12_{eq}$, CH_3-21, CH_3-22, CH_3-23
		1.76_{eq}	m			
12	71.7	4.56_{eq}	t	2.4	C-9, C-13, C-14, C-23	$H\text{-}11_{ax}$, CH_3-23
13	41.1					
14	51.0	1.64_{ax}	m		C-8, C-13, C-15, C-16	$H\text{-}7_{ax}$, $H\text{-}15_{eq}$, $H\text{-}16_{ax}$
15	18.0	1.54_{ax}	m			$H\text{-}16_{eq}$, CH_3-22, CH_3-23
		1.88_{eq}	m		C-13, C-14	$H\text{-}14_{ax}$, $H\text{-}16_{ax}$
16	25.4	2.24_{ax}	m		C-14, C-15, C-17, C-18	$H\text{-}14_{ax}$, $H\text{-}15_{eq}$
		2.40_{eq}	dd	4.5, 18.9	C-17, C-18	CH_3-26
17	167.3					
18	134.4					
19	17.9	0.88	s		C-3, C-4, C-5, C-20	$H\text{-}3_{eq}$, $H\text{-}5_{ax}$
20	32.8	1.33	m		C-3, C-4, C-5, C-19	
		1.44	m		C-3	
21	17.2	0.94	s		C-1, C-5, C-9, C-10	$H\text{-}2_{ax}$, $H\text{-}6_{ax}$, $H\text{-}11_{ax}$
22	17.9	0.94	s		C-7, C-8, C-14	$H\text{-}6_{ax}$, $H\text{-}7_{eq}$, $H\text{-}11_{ax}$, $H\text{-}15_{ax}$, CH_3-23
23	22.1	1.14	s		C-12, C-13, C-14, C-18	$H\text{-}11_{ax}$, $H\text{-}12_{eq}$, $H\text{-}15_{ax}$, CH_3-22
24	79.8	4.84_{ax}	q	6.9	C-17, C-18	
25	174.9					
26	18.7	1.34_{eq}	d	6.9	C-17, C-24	$H\text{-}16_{eq}$
27	7.8	0.71	t	7.5	C-4, C-20	$H\text{-}3_{eq}$
1′	174.4					
2′	29.1	2.30	q	7.5	C-1′	
3′	9.8	1.11	t	7.5	C-1′, C-2′	

Spectra were recorded at 500 MHz, 300 K, and referenced to residual CD_3OD signal (δ_H 3.31, δ_C 49.1).

20 μg/mL. Two grams of crude extract were subjected to bioassay-guided fractionation and purification using column chromatography (Si gel) followed by reversed-phase HPLC, which resulted in the isolation of compounds **1**–**5**. The molecular formulae of **1**–**5** were established by high resolution FAB mass spectrometry as $C_{31}H_{48}O_5$, $C_{30}H_{46}O_5$, $C_{29}H_{44}O_5$, $C_{32}H_{48}O_6$ and $C_{27}H_{42}O_4$, respectively, which indicated that **1**–**5** are homologs. A preliminary analysis of the ^{1}H and ^{13}C spectral data suggested that **1**–**3** differed only in the length of an alkyl chain, while compounds **4** and **5** differed in the oxidation states of other parts of the molecule. Since compound **2** was isolated in relative abundance (ca. 40 mg), we determined the structure of this compound first, followed by elucidation of the structures of compounds **1** and **3**–**5**.

The ^{13}C NMR spectrum of **2** contained 30 signals, as expected from the molecular formula, and a DEPT spectrum showed the presence of seven methyl, nine methylene, six methine, and eight quarternary carbons. Thus, compound **2** has one exchangeable proton, and as indicated by the molecular formula, eight degrees of unsaturation. Since the ^{13}C NMR spectrum shows the presence of two ester carbonyls (δ_C 174.4 and 174.9) and one double bond (δ_C 134.4 and 167.3) the compound must contain five rings. Integration of the ^{1}H NMR spectrum of **2** confirmed the presence of seven methyl signals which include four methyl singlets at δ_H 0.88, 0.94, 0.94 and 1.14; one methyl doublet at δ_H 1.34; and two methyl triplets at δ_H 0.71 and 1.11 (Table 1). The presence of only four methyl singlets in addition to several methyl doublets and triplets suggested that **2** was a 20,24-bishomoscalarane sesterterpene (using the numbering of Kazlauskas et al.[7]); and the IR spectrum and carbon signals at δ_C 174.9 (s), 134.4 (s), 167.3 (s), and 79.8 (d) indicated the presence of a 2,3,4-trisubstituted butenolide moiety. While the ^{1}H and ^{13}C chemical shifts of **2** (Table 1) showed similarities to the NMR spectral data of related compounds from the sponge *Phyllospongia foliascens*[9–11] there were many differences that could not be accounted for by merely changing the already reported oxysubstitutents at C-12, C-16, C-20 or C-24. Thus complete ^{1}H and ^{13}C assignments of **2** were made by interpretation of HSQC and HMBC spectra, and overlapping methylene signals were unambiguously assigned from an HSQC–TOCSY spectrum (Table 1).

Several correlations in the HMBC spectrum confirmed that **2** was a bishomoscalarane sesterterpene. These included all

Table 2. ^{1}H NMR data (ppm) for compounds **1** and **3**–**5** in CD_3OD

^{1}H	**1**			**3**			**4**			**5**		
	δ_H	Mult	J (Hz)	δ_H	Mult	J (Hz)	δ_H	Mult	J (Hz)	δ_H	Mult	J (Hz)
1_{ax}	1.20	m								0.88	m	
1_{eq}	1.44	m								1.64	m	
2_{ax}	1.66	m								1.40	m	
2_{eq}	1.89	m								1.40	m	
3_{ax}										0.90	m	
3_{eq}	4.64	t	2.4	4.62	t	2.4	4.63	t	2.4	1.70	m	
5_{ax}	1.45	m								0.97	m	
6_{ax}	1.40	m								1.37	m	
6_{eq}	1.60	m								1.57	m	
7_{ax}	1.10	m								1.03	m	
7_{eq}	1.88	m								1.86	m	
9_{ax}	1.58	m								1.50	m	
11_{ax}	1.67	m								1.67	m	
11_{eq}	1.76	m								1.76	m	
12_{eq}	4.56	t	2.4	4.56	t	3	5.48	t	2.4	4.52	t	2.3
14_{ax}	1.64	m								1.56	m	
15_{ax}	1.54	m								1.54	m	
15_{eq}	1.88	m								1.89	m	
16_{ax}	2.24	m		2.24	m		2.24	m		2.20	m	
16_{eq}	2.40	dd	4.5, 18.9	2.40	dd	4.5, 18.9	2.45	dd	4.6, 18.7	2.35	dd	4.6, 18.7
19	0.88	s		0.88	s		0.88	s		0.82	s	
20_{ax}	1.33	m								1.21	m	
20_{eq}	1.44	m								1.63	m	
21	0.94	s		0.95	s		0.95	s		0.90	s	
22	0.94	s		0.94	s		0.97	s		0.92	s	
23	1.14	s		1.14	s		1.19	s		1.14	s	
24_{ax}	4.84	q	6.9	4.84	q	6.9	4.84	q	6.9			
26_{eq}	1.34	d	6.9	1.34	d	6.9	1.35	d	6.9	1.51	s	
27	0.71	t	7.5	0.71	t	7.5	0.70	t	7.5	0.77	t	7.5
2′	2.26	t	7.5	2.00	s		2.31	q	7.5			
3′	1.35	m					1.13	t	7.5			
	1.60	m										
4′	0.95	t	7.5									
OAc							2.00	s				

Spectra were recorded at 300 or 500 MHz at 300 K and referenced to residual CD_3OD signal (δ_H 3.31).

possible two-bond and three-bond correlations from the methyl protons of CH_3-19, CH_3-21, CH_3-22 and CH_3-23 to their respective ring carbons (Table 1); and from CH_3-26 to C-17 and C-24, carbons that correspond to C-3 and C-4 of the 2,3,4-trisubstituted butenolide ring. The HSQC spectrum indicated that the downfield shifted methine protons at δ_H 4.56 and 4.64 were attached to oxygen-bearing carbons (δ_C 71.7 and 76.3, respectively); and were assigned as H-12 and H-3, respectively, due to HMBC correlations from δ_H 4.56 to C-9, C-13, C-14 and C-23; and from δ_H 4.64 to C-1, C-2, C-4, C-5, C-6 and C-1′ (Table 1). The chemical shifts for the 2,3,4-trisubstituted butenolide moiety were comparable to those observed in other butenolide-containing sesterterpenes, and HMBC correlations to C-17 (δ_C 167.3) from H-16_{ax}, H-16_{eq}, H-24 and CH_3-26, in combination with the correlation from CH_3-23 to C-18 (δ_C 134.4) confirmed the location of the lactone. The remaining signals in the NMR spectra of **2** suggested the presence of a propanoate ester [δ_H 2.30 (2H, q, J=7.5 Hz, H-2′) and 1.11 (3H, t, J=7.5 Hz, H-3′)], and HMBC correlations from H-3 and H-2′ to the ester carbonyl at δ_C 174.4 required attachment at C3.

The relative configuration of **2** was determined from the combined analysis of the NOESY and ROESY spectra and proton–proton coupling constants (Table 1). The small J_{H-H} values of 2.4 Hz for H-3 and H-12 suggested equatorial positions for these hydrogens. An all-*trans* A-B-C-D ring system for **2** was established from the NOESY and ROESY spectra including correlations from H-1_{ax} to H-9_{ax} (H-5_{ax} is degenerate with H-1_{eq}); H-5_{ax} to H-7_{ax} and H-9_{ax}; H-7_{ax} to H-14_{ax}; H-6_{ax} to CH_3-21 and CH_3-22; H-11_{ax} to CH_3-21, CH_3-22 and CH_3-23; H-15_{ax} to CH_3-22 and CH_3-23; and CH_3-22 to CH_3-23. Stereospecific assignments for the CH_2 protons of C-1, C-2, C-6, C-7, C-11, C-15 and C-16 were evident from the δ_H values, and were confirmed by NOEs between geminal axial and equatorial protons (Table 1). (For example, in the NOESY spectrum H-1_{ax} was correlated only to the equatorial H-2 proton, while H-1_{eq} was correlated to both H-2_{ax} and H-2_{eq}.) The relative configuration of the cyclohexene, or D, ring was deduced from the NOESY spectrum which showed correlations from H-15_{eq} to H-14_{ax} and H-16_{ax}; and H-15_{ax} to H-16_{eq}, CH_3-22 and CH_3-23. Based on the above, H-3 was assigned the β configuration due to the observation of strong NOEs from H-3 to H-2_{ax}, H-2_{eq}, CH_3-19, and CH_3-27. Similarly, a strong NOE was observed between H-12 and CH_3-23 in the NOESY spectrum supporting the β configuration for H-12. Last, a NOESY correlation between CH_3-26 and H-16_{eq} indicated the β-configuration for CH_3-26. Thus, the structure of **2** was assigned as 3α-yl-propanoate-12α-hydroxy-20,24-dimethyl-25-norscalar-17-ene-18,24-carbolactone, and given the trivial name phyllolactone B.

Table 3. ^{13}C NMR data (ppm) for **1** and **3–5** in CD_3OD

^{13}C	**1**	**3**	**4**	**5**
1	34.4 t	34.3 t	34.6 t	41.0 t
2	23.4 t	23.4 t	23.2 t	19.1 t
3	76.3 d	76.7 d	76.0 d	37.9 t
4	39.8 s	39.8 s	39.8 s	37.4 s
5	52.7 d	52.2 d	52.6 d	60.0 d
6	18.6 t	18.6 t	18.6 t	19.0 t
7	42.9 t	42.9 t	42.9 t	42.9 t
8	38.3 s	38.3 s	38.2 s	38.4 s
9	53.2 d	53.2 d	54.5 d	53.4 d
10	38.9 s	38.9 s	38.8 s	38.9 s
11	25.6 t	25.6 t	21.2 t	25.4 t
12	71.3 d	71.3 d	76.1 d	71.8 d
13	41.1 s	41.1 s	41.0 s	41.5 s
14	51.0 d	51.0 d	52.7d	50.8 d
15	18.1 t	18.0 t	18.0 t	17.4 t
16	25.4 t	25.4 t	25.4 t	24.0 t
17	167.3 s	167.3 s	167.7 s	166.0 s
18	134.4 s	134.4 s	133.3 s	136.0 s
19	17.9 q	18.0 q	18.0 q	28.8 q
20	32.7 t	32.7 t	32.7 t	25.2 t
21	17.2 q	17.2 q	16.9 q	17.5 q
22	17.9 q	18.0 q	17.9 q	17.8 q
23	22.1 q	22.1 q	22.0 q	21.6 q
24	79.8 d	79.8 d	80.0 d	106.8 s
25	174.9 s	174.9 s	174.9 s	174.0 s
26	18.7 q	18.8 q	18.8 q	23.3 q
27	7.8 q	7.8 q	7.7 q	8.7 q
1′	174.4 s	172.6 s	174.4 s	
2′	37.8 t	21.4 q	29.2 t	
3′	19.8 t		9.9 q	
4′	14.2 q			
OAc			171.9 s	
CH_3			21.8 q	

Spectra were recorded at 75 or 125 MHz at 300 K and referenced to residual CD_3OD (δ_C 49.1).

The molecular formula of compound **1** indicated that it differed from phyllolactone B by the addition of 14 mass units. The ^{13}C NMR spectrum showed the absence of signals for C-2′ and C-3′ (δ_C 29.1 and 9.8) and the appearance of three new signals at δ_C 14.2, 19.8 and 37.8 (Table 3), indicating the presence of a butanoate ester at C-3. Similarly for compound **3**, the molecular formula indicated loss of a methylene group relative to phyllolactone B, and the NMR spectra revealed the presence of an acetate group with carbons at δ_C 172.6 and 21.4 (Table 3) along with a methyl singlet at δ_H 2.00 (Table 2). As observed for phyllolactone B, specific rotations for compounds **1** and **3** were positive, and coupling constants and chemical shifts related to stereocenters at C-3, C-12 and C-24 were nearly identical to those for phyllolactone B. Thus compounds **1** and **3** were assigned the chemical names of 3α-yl butanoate-12α-hydroxy-20,24-dimethyl-25-norscalar-17-ene-18,24-carbolactone and 3α-acetoxy-12α-hydroxy-20,24-dimethyl-25-norscalar-17-ene-18,24-carbolactone, respectively, and given the trivial names phyllolactones A and C.

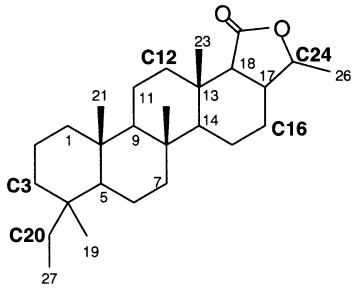
The molecular formula of compound **4** ($C_{32}H_{48}O_6$) which differs by 42 amu (C_2H_2O) from phyllolactone B, indicated the presence of one additional acetate group; and signals in the ^1H and ^{13}C NMR spectra at δ_C 171.9, 21.8 (Table 3) and δ_H 2.00 (Table 2) supported this notion. The downfield shift of C-12 from δ_C 71.3 to 76.1, in addition to an HMBC correlation from H-12 to the new acetate carbonyl at δ_C 171.9 established that the acetate is located at C-12. The same coupling constant of 2.4 Hz for H-12 as observed in the related compounds suggested that the β configuration is preserved at this center. Thus compound **4** was assigned as 3α-yl propanoate-12α-acetoxy-20,24-dimethyl-25-norscalar-17-ene-18,24-carbolactone and given the trivial name phyllolactone D.

The last sesterterpene of the series was isolated in very small quantities (~0.5 mg) as a colorless oil and has the molecular formula $C_{27}H_{42}O_4$. Comparison of the 1D spectra of **5** with those of phyllolactones A–D showed several large differences. Signals representing the alkyl esters were clearly absent as were signals at δ_H 4.84 (H-24), δ_C 76.3 (C-3) and 79.8 (C-24). The absence of the signal at δ_H 4.84, the appearance of a ketal carbon at δ_C 106.8, and replacement of the methyl doublet at δ_H 1.34 (C-26) by a methyl singlet at δ_H 1.51 indicated that H-24 had been replaced by a hydroxyl group, and was confirmed by HMBC correlations from CH_3-26 (δ_H 1.51) to δ_C 166.0 (C-17) and 106.8 (C-24). The β configuration for CH_3-26 was assigned on the basis of an NOE between protons at δ_H 1.51 and 2.35, corresponding to CH_3-26 and H-16_{eq}, respectively. Additional NOESY correlations establishing an all-*trans* A-B-C-D ring system for **5** included those corresponding to H-1_{ax} to H-5_{ax} and H-9_{ax}; H-7_{ax} to H-9_{ax}; and CH_3-23 to CH_3-21 and CH_3-22. Thus all of the *Phyllospongia* compounds reported here possess an all-*trans* A-B-C-D ring system in which CH_3-26 is β-oriented. The structure of compound **5** is described as 12α,24α-dihydroxy-20-methyl-25-norscalar-17-ene-18,24-carbolactone, and assigned the trivial name phyllolactone E.

3. Discussion

Bishomoscalarane sesterterpenes are characterized by methylation at C-20 and C-24[7] and are thus far limited to sponges of the order Dictyoceratida. Although many bishomoscalaranes have been reported, they are far less common than the C_{26} homoscalaranes. A prevalent feature of the bishomoscalaranes is the presence of a γ-lactone ring fused at ring D of the sesterterpene skeleton such that C17 and C18 are located in the α and β positions of the lactone ring. Dehydration of this bond yields the α,β-unsaturated lactone ring, which is the more common form within this class. In order to facilitate comparison of the γ-lactone-containing 20,24-bishomoscalaranes in particular, Table 4 summarizes the oxy-substituents and their respective locations for compounds of this type reported to date.[15] Several generalizations can be made by comparing these structures: in addition to the oxidation and carboxylation that give rise to the γ-lactone ring, compounds of this class contain between one and three oxy-substituents which include oxo-, hydroxy-, and alkyl ester functionalities located at C-3, C-12, C-16, C-20 and C-24 as can be seen in Table 4. As of yet there are no examples of carbolactone-containing bishomosesterterpenes devoid of additional oxy-substituents at these centers. Oxidation at C-12 occurs often in the bishomoscalaranes, while oxidation at C-3 and at C-24 of the γ-lactone ring is rare. (This observation also holds for both the homoscalarane sesterterpenes and the ring-opened 24-keto, 25-aldehyde-containing bishomoscalarane sesterterpenes.) Both α and β configurations have

Table 4. Oxy-substituents at C-3, C-12, C-16, C-20 and C-24 oxidized bishomoscalarane



Trivial names[a,b]		Oxy-substituents[c]					CH_3 orientation[d]	
		C-3	**C-12**	**C-16**	**C-20**	**C-24**	**C-19**	**C-26**
18,24-Carbolactones								
Braekman[4]			α-Acetate	β-Hydroxy				
Phyllofolactone[9]	A		Keto	β-Hydroxy			α	β
17-ene-18,24-Carbolactones								
Barron[5]			α-Acetate					
Croft[3]			β-Hydroxy		(S)-Acetate		α	β
Honulactones[14]	A		α-3-Hydroxybutanoate		Cyclopropyl	β-Methyl	α	β
	B		α-3-Hydroxybutanoate		Cyclopropyl	α-Methyl	α	α
	C		α-3-Hydroxybutanoate		β-Acetate	β-Methyl	α	β
	D		α-3-Hydroxybutanoate		β-Acetate	α-Methyl	α	α
	E		α-3-Hydroxypentanoate		Cyclopropyl	β-Methyl	α	β
	F		α-3-Hydroxypentanoate		Cyclopropyl	α-Methyl	α	α
	G		α-3-Hydroxybutanoate	α-Hydroxy	Cyclopropyl	β-Methyl	α	β
	H		α-3-Hydroxybutanoate	α-Hydroxy	Cyclopropyl	β-Methyl	α	α
	I		α-3-Hydroxypentanoate		β-Acetate	β-Methyl	α	β
	J		α-3-Hydroxypentanoate		β-Acetate	α-Methyl	α	α
	K		α-3-Hydroxybutanoate		β-Propanoate	β-Methyl	α	β
	L		α-3-Hydroxybutanoate		β-Propanoate	α-Methyl	α	α
Phyllactones[10]	A		β-3-Hydroxypentanoate	α-Hydroxy		α-Methyl	α	α
	B		β-3-Hydroxypentanoate	α-Hydroxy		β-Methyl	α	β
	C		β-3-Acetylpentanoate	α-Acetate		β-Methyl	α	β
	D,E		β-3-Hydroxypentanoate	15-ene		Hydroxy	α	
	F,G		β-3-Hydroxy-5-methylpentanoate	15-ene		Hydroxy	α	
Phyllofolactones[9]	B		α-Hydroxy				α	β
	C		Keto				α	β
Phyllolactones	A	α-Butanoate	α-Hydroxy				α	β
	B	α-Propanoate	α-Hydroxy				α	β
	C	α-Acetate	α-Hydroxy				α	β
	D	α-Propanoate	α-Acetate				α	β
	E		α-Hydroxy			α-Hydroxy	α	β
Reddy[12]			β-Hydroxy					

[a] If not named corresponding author is listed.
[b] In alphabetical order.
[c] Hydrogen is present if blank.
[d] Relative configuration of CH_3-19 (CH_2-19 for honulactones that contain the cyclopropyl ring at C4) and CH_3-26; if blank configuration was not determined.

been reported for each of these oxygen-bearing stereocenters, as well as for the carbons bearing the additional methyl groups, namely C-20 and C-24. The recently described honulactones A–L are the first examples of bishomoscalaranes possessing a cyclopropyl ring (at C-19-C-20).

Cruz and coworkers have suggested that the scalarane sesterterpenes may be a useful chemotaxonomic marker to identify sponges of the order Dictyoceratida.[16] Phyllolactones A–E were isolated from a crude extract of *P. lamellosa* and thereby endorse the suggested Dictyoceratid chemotaxonomy. Phyllolactones A–D differ from previously reported carbolactone-containing bishomoscalaranes as they possess oxy-substituents at C-3.[17] We have summarized the known structures of this class of compounds in Table 4. In addition we have consolidated the ^{13}C NMR data for the C-3 oxidized phyllolactones (Tables 1 and 3) and the previously reported C-12, C-16, C-20 and C-24 oxidized bishomoscalaranes in Table 5. Together the information provided in these tables should assist natural products chemists to more rapidly identify by NMR new sesterterpenes of this still relatively rare class.

The crude extract from *P. lamellosa* was tested in a vaccinia virus-based reporter gene assay that faithfully reproduces the sequence of events which leads to HIV-1 fusion or viral entry.[18] Phyllolactones A–D were found to be responsible for the inhibition of HIV-1 fusion exhibited by the crude organic extract of *P. lamellosa* with IC_{50} values of ~2.0 μM (values ranged from 1.5 to 2.2 μM). (Phyllolactone E was not tested due to lack of material.) Cell

Table 5. ^{13}C NMR chemical shifts of carbolactone-containing bishomoscalaranes

			A ring carbons			
Substituent	1	2	3	4	5	10
C-3=CH_2[3–5,9–11,13]	39.7–40.5	17.9–18.3	36.4–37.9	36.0–37.4	58.5–60.0	36.7–38.9
C-3=CHOCOR[a]	34.4–34.6	23.2–23.5	76.0–76.7	39.8	52.6	38.9
			C ring carbons[a]			
Substituent	8	9	11	12	13	14
C-12=CH-α-OH[9,a]	37.5–38.3	52.4–53.2	24.3–25.6	70.1–71.3	40.3–41.1	49.9–51.0
C-12=CH-β-OCOR[10]	37.0–37.6	58.0–58.2	24.3–25.1	75.1–76.1	41.1–42.4	50.1– 57.2
C-12=CH-α-OCOR[14]	36.7–37.8	51.3–53.7	21.0	74.2–74.8	38.3–39.0	45.9–51.2
C-12=C=O						
17-saturated[9]	38.6	51.5	35.0	212.4	50.0	50.0
C-12=C=O 17-*ene*[13]	38.4	64.5	35.1	201.8	51.0	58.7
			D ring carbons with lactone fused (C17–18 saturated)			
Substituent	16	17	18	24	25	26
C-16=CH-β-OH[9]	72.0	59.2	64.2	79.4	172.4	20.1
C-16=CH-β-OCOR[4,b]	73.8	52.4	58.6	73.6	172.8	16.5
			D ring carbons with α,β-unsaturated—lactone fused (C17-*ene*)			
Substituent	16	17	18	24	25	26
C-16=CH_2[9,13,14,a]	24.0–25.4	164.1–167.7	131.2–134.4	77.5–80.0	170.5–174.9	18.4–18.8
C-16=CH-α-OH-α-C26[14]	62.9	160.8	135.5	78.9	170.8	19.8
C-16=CH-α-OH, β-C26[14]	61.5	162.0	135.5	76.6	170.8	18.0
C-16=CH-α-OCOR[10]	63.3	160.1	139.0	75.8	169.3	18.3
C-16=15-*ene*[10,11]	119.1–119.6	158.0–158.6	132.0–132.1	102.8–103	167.9–168.6	24.1
			D ring carbons α,β-unsaturated—lactone fused (C17-*ene*)			
Substituent	16	17	18	24	25	26
C-24=CH-β-CH_3[a]	25.4	167.3–167.7	133.3–134.4	79.8–80.0	174.9	18.7–18.8
C-24=C-α-OH-β-CH_3[a]	24.0	166.0	136.0	106.8	174.0	23.3

[a] This paper.
[b] ^{13}C Chemical shifts were reported for the C-16 β-Acetate.[4]

types used in the fusion assay include BS-C-1s for the HIV-1 envelope-expressing effector population and NIH 3T3s for the receptor-bearing target cell population. Since the fusion assay is complete in 2.5 h, we viewed it as unlikely that inhibitory effects are due to potent cytotoxicity. However, we tested phyllolactones A–D for cytotoxic[19] and lytic[20] effects towards the cell lines used in the fusion assay, namely BS-C-1s and NIH 3T3s, and found them to be inactive at 20 μg/mL. Phyllolactones A–D provide one of the few examples of small-molecules that inhibit HIV-1 envelope-mediated fusion in vitro.[21]

4. Experimental

4.1. General procedures

UV and IR spectra were obtained with a Beckman DU-600 spectrophotometer and a Bio-Rad FTS-45 FT-IR spectrophotometer, and optical rotations were measured on a Perkin–Elmer 341 polarimeter in $CHCl_3$ and CH_3OH. NMR experiments were recorded on a Bruker DMX500 spectrometer equipped with an *xyz*-shielded gradient triple resonance probe. FABMS was performed on a Jeol-SX102 and CIMS on a Finnigan 4500. Reversed-phase HPLC was carried out on a GBC system equipped with a photodiode array detector using a Waters μBondapak C_{18} column (7.8×300 mm^2) run with a flow rate of 3 mL/min, and detected at 210 nm.

4.2. Collection

P. lamellosa (Q66C0102) was collected in December 1987 in the Indo-West Pacific, immediately frozen and shipped to NCI. Following aqueous extraction of the frozen sponge at 4°C, the material was lyopholized and extracted successively with CH_2Cl_2–MeOH (1:1) and MeOH. The combined organic extracts were evaporated in vacuo and stored at −30°C. Voucher specimens were deposited at the Queensland Museum, Brisbane and the Smithsonian Sorting Center, Suitland, MD.

4.3. Isolation

A crude organic extract (2.0 g) derived from 750 g wet weight of the initial collection (provided by the NCI Natural Products Open Repository) was partitioned with petroleum ether to give 0.54 g of extract, followed by partitioning with ethyl acetate to give 0.63 g of extract. Both the petroleum ether- and EtOAc-soluble extracts inhibited HIV envelope-mediated fusion at <20 μg/mL. The ethyl acetate extract was purified using Si gel column chromatography eluting with a toluene to acetone gradient in steps of 5% followed by a final MeOH wash. Active fractions which eluted with 10–30% acetone were repurified using the same conditions. Final purification of the active components was accomplished by reversed-phase HPLC eluting with 80% aqueous CH_3CN in 0.05% TFA to give compound **1** (t_R 11.8 min; 6.4 mg; 0.00085% wet weight), **2** (t_R 11.65 min; 40 mg; 0.0053% of wet weight) and **3** (t_R 14.2 min; 5.2 mg; 0.00069% of wet weight). The petroleum-ether soluble extract was also initially purified using Si gel eluting with an equivalent toluene–acetone stepwise gradient to give two major sesterterpene-containing fractions eluting with 5–10% acetone. One fraction was rechromatographed on Si gel eluting with 20:1 toluene–acetone to give a pure sample of **4** (4.0 mg; 0.00053% of wet weight), and the second fraction was further purified by RP-HPLC (eluting with 75% aq. CH_3CN in 0.05 % TFA) to yield a pure sample of **5** (t_R 16.8 min; 0.8 mg; 0.0001% of wet weight).

4.3.1. Phyllolactone A (1). White powder; $[\alpha]_D^{20}=+9.5°$ (*c* 0.254, MeOH); UV (MeOH) λ_{max} 215 nm (ϵ 5100); IR (film) ν_{max} 3486, 2942, 2874, 1728, 1674, 1386, 1324, 1196 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 2; ^{13}C NMR (75 or 125 MHz, CD_3OD) see Table 3; FABMS (Pos.) *m/z* 501 [$(M+H)^+$], 443, 413, 395; HRFABMS (NBA) *m/z* 501.3571 [$(M+H)^+$], $C_{31}H_{49}O_5$ requires *m/z* 501.3567 ($\Delta-0.4$ mmu).

4.3.2. Phyllolactone B (2). White powder; $[\alpha]_D^{20}=+10.6°$ (*c* 0.09, MeOH); UV (MeOH) λ_{max} 203 nm (ϵ 6179); IR (film) ν_{max} 3510, 2935, 1730, 1666, 1384, 1323, 1202 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 1; ^{13}C NMR (75 or 125 MHz, CD_3OD) see Table 1; CIMS *m/z* 486 (M^-), 407, 274, 232, 145, 116, 74; HRFABMS (NBA) *m/z* 487.3395 [$(M+H)^+$], $C_{30}H_{47}O_5$ requires *m/z* 487.3411 ($\Delta-1.6$ mmu).

4.3.3. Phyllolactone C (3). White powder; $[\alpha]_D^{20}=+8.6°$ (*c* 0.14, MeOH); UV (MeOH) λ_{max} 216 nm (ϵ 8425); IR (film) ν_{max} 3470, 2940, 2880, 1736, 1666, 1370, 1200 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 2; ^{13}C NMR (75 or 125 MHz, CD_3OD) see Table 3; LRFABMS *m/z* 473 [$(M+H)^+$], 395, 307, 289, 154, 107; HRFABMS (NBA) *m/z* 473.3264 [$(M+H)^+$], $C_{29}H_{45}O_5$ requires *m/z* 473.3255 ($\Delta+0.9$ mmu).

4.3.4. Phyllolactone D (4). Amorphous solid; $[\alpha]_D^{20}=+7.2°$ (*c* 0.08, MeOH); UV (MeOH) λ_{max} 215 nm (ϵ 5500); IR (film) ν_{max} 2932, 2857, 1726, 1675, 1200 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 2; ^{13}C NMR (75 or 125 MHz, CD_3OD) see Table 3; LRFABMS *m/z* 529 [$(M+H)^+$], 469, 395, 154, 137; HRFABMS (NBA) *m/z* 529.3537 [$(M+H)^+$], $C_{32}H_{49}O_6$ requires *m/z* 529.3516 ($\Delta+2.1$ mmu).

4.3.5. Phyllolactone E (5). Colorless oil; $[\alpha]_D^{20}=+3.0°$ (*c* 0.02, MeOH); UV (MeOH) λ_{max} (ϵ) 214 nm (6050); IR ν_{max} (film) 3400, 2945, 1722, 1691, 1202 cm^{-1}; ^1H- and ^{13}C NMR data (see Tables 2 and 3); FABMS *m/z* 431 [$(M+H)^+$], 413, 307, 154, 136; HRFABMS *m/z* 431.3157 [$(M+H)^+$], $C_{27}H_{43}O_4$ requires 431.3150 ($\Delta+0.7$ mmu).

4.4. Bioassays

Cell fusion assays were conducted as previously described[18] using soluble CD4 and recombinant vaccinia viruses expressing genes for HIV-1 Env, CXCR4 (Fusin[22]), T7 polymerase and β-galactosidase (βGal). BS-C-1 cells were used for effector cells which express HIV-1 Env and T7 polymerase, and NIH 3T3 cells were used for targets which express CXCR4 and βGal. For inhibition studies extracts or pure compounds were added to effector cells in a 96-well plate just prior to addition of CD4-containing target cells for a final volume of 200 μL per well containing 1×10^5 cells of each population. Following 2.5 h incubation, βGal activity of cell lysates was measured (A_{570}, Molecular Devices 96-well spectrophotometer) upon addition of chlorophenol-red-β-D-galactopyranoside (CPRG). Cytotoxicity assays[19] using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St Louis) and cell lysis assays using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride[20] (INT; Cytotoxicity Detection Kit, Roche Diagnostics, GmbH) were performed in triplicate following 24 h incubations in the presence (or absence) of inhibitor for both BS-C-1 and NIH 3T3 cells as previously described.[19,20]

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