Marine Natural Products. XX.[1] Bioactive Scalarane-Type Bishomosesterterpenes from the Okinawan Marine Sponge *Phyllospongia foliascens*

Isao KITAGAWA,*[,a] Motomasa KOBAYASHI,[a] Nam Kyung LEE,[a] Yoshiaki OYAMA[b] and Yoshimasa KYOGOKU[c]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1–6, Yamada-oka, Suita, Osaka 565, Japan, Suntory Institute for Biomedical Research,[b] Shimamoto-cho, Mishima-gun, Osaka 618, Japan and Institute for Protein Research, Osaka University,[c] 3–2, Yamada-oka, Suita, Osaka 565, Japan.
Received February 10, 1989

Two new scalarane-type bishomosesterterpenes, dehydrofoliaspongin (17) and phyllofoliaspongin (18), were isolated together with foliaspongin (4) and scalardysin-B (15) from the Okinawan marine sponge *Phyllospongia foliascens*. On the basis of chemical and physicochemical evidence including the X-ray crystallographic analysis of a methylated derivative (16) of 15, the structures of 17 and 18 were determined and the previously proposed structure (3) of foliaspongin was revised to 4, having a 4β-ethyl moiety. Phyllofoliaspongin (18) showed cytotoxic, anti-thrombocyte, and vasodilative activities.

Keywords marine sponge; *Phyllospongia foliascens*; scalarane-type bishomosesterterpene; foliaspongin; dehydrofoliaspongin; phyllofoliaspongin; sesterterpene ^{13}C-NMR; sesterterpene X-ray analysis

In recent years, a number of scalarane-type sesterterpenes (with C_{25}, C_{26}, and C_{27}) have been isolated from various marine sponges, *e.g. Cacospongia mollior*,[2a,b] *Heteronema erecta*,[2c] *Spongia nitens*,[2d–f] *Dysidea herbacea*,[2g] *Phyllospongia radiata*,[2h] *P. dendyi*,[2h] *P. foliascens*,[2h] *Spongia idia*,[2i] *Cacospongia scalaris*,[2j] *Lendenfeldia* sp.,[2k] *Hyrtios erecta*,[2l] *Dictyoceratida* sp.,[2m] and *Halichondria* sp.[2m] Among these species, the Australian marine sponge *Phyllospongia foliascens* was shown to characteristically produce C_{27} scalarane-type sesterterpenes (*i.e.* bishomosesterterpenes **5**, **6**, and **7**), and their chemical structures having a 4α-ethyl moiety were proposed with reference to the X-ray crystallographic analysis of a 12-dehydro derivative (**9**) of a C_{27} scalarane-type sesterterpene **8**, which had been isolated from another species, *P. radiata*.[2h]

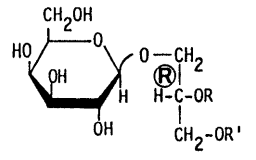
While monitoring biological activities, we isolated two glycolipids from the water-soluble portion of the Okinawan marine sponge *Phyllospongia foliascens* (PALLAS) (Spongiidae). They were a galactolipid designated as M-5 (**1**)[3] exhibiting anti-inflammatory activity and a sulfonoglycolipid designated as M-6 (**2**)[3] which showed anti-complement fixation activity. We also isolated a new anti-inflammatory scalarane-type bishomosesterterpene named foliaspongin from the lipid-soluble portion of the same marine sponge.[4] The chemical structure **3** with a 4α-ethyl moiety was proposed for foliaspongin,[4] refering to the reported structures of scalarane-type bishomosesterterpenes (**5**, **6**, and **7**) which were isolated from the above-mentioned Australian marine sponge of the same species, *Phyllospongia foliascens*.[2h]

Afterwards, two scalarane-type bishomosesterterpenes having a 4β-ethyl moiety were isolated, *i.e.* **10**[5] from the New Guinean marine sponge *Carteriospongia* (=*Phyllospongia*) *foliascens* and **12**[6] from a marine sponge of *Carteriospongia* sp. collected in Fiji. Their structures were independently elucidated by means of the X-ray crystallographic analysis of **11** (the methyl ester of **10**) and **12**. Furthermore, it was suggested[5b] that the structure **3** proposed for foliaspongin should be revised to **4** having a 4β-ethyl moiety based on a detailed comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **11** and foliaspongin.

During the course of continuing studies in search of new bioactive marine natural products,[1] we have reinvestigated in more detail the chemical constituents in the lipid-soluble portion of the Okinawan marine sponge *Phyllospongia foliascens*. We have found two new bishomosesterterpenes named dehydrofoliaspongin (**17**) and phyllofoliaspongin (**18**) in addition to hitherto isolated foliaspongin (**4**) and scalardysin-B (**15**). Since the 4α-ethyl configuration of foliaspongin (as proposed in **3**)[4] lacked definite proof as mentioned above, we attempted an X-ray crystallographic analysis of foliaspongin or its derivatives. However, we could not prepare a crystalline sample of foliaspongin suitable for an X-ray analysis, so we carried out an X-ray crystallographic analysis of **16** which was prepared from scalardysin-B (**15**),[2g,7] a co-occurring bishomosesterterpene with foliaspongin. Based on the established structure of scalardysin-B (**15**) and a detailed comparison of the ^{13}C-NMR data, we conclude that the chemical structure of foliaspongin should be revised from **3** to **4**, having a 4β-ethyl moiety. This paper presents a full account of the structure elucidation of dehydrofoliaspongin (**17**) and phyllofoliaspongin (**18**), and also of the structure revision of foliaspongin to **4**.

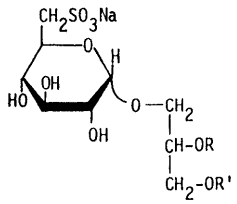
The acetone extract of the marine sponge, which was collected in July at Kohama-jima, Okinawa Prefecture, was partitioned into a mixture of ethyl acetate and water. The ethyl acetate-soluble portion was subjected to repeated silica gel column chromatography to provide two new scalarane-type bishomosesterterpenes named dehydrofoliaspongin (**17**) and phyllofoliaspongin (**18**), together with two known furano-diterpenes, dihydrofurospongin-2 (**13**)[8] and furospongin-1 (**14**),[9] and two scalarane-type bishomosesterterpenes, foliaspongin (**4**) and scalardysin-B (**15**).[2g,7]

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of dehydrofoliaspongin (**17**) showed the presence of a methyl ketone moiety (δ2.09, 3H, s) and an aldehyde group (δ 10.10, 1H, br s). It also showed signals ascribable to protons in ring D [δ5.56 (1H, m, 16β-H), 3.13 (1H, dd, J=12, 3Hz, 17β-H), 3.25 (1H, d, J=12 Hz, 18α-H)] and in the 3-hydroxypentanoyl residue [δ2.30 (2H, m, 2′-H_2), 3.77 (1H, m, 3′-H)]. The presence of the 3-hydroxy-pentanoyl residue was further substantiated by sodium methoxide–



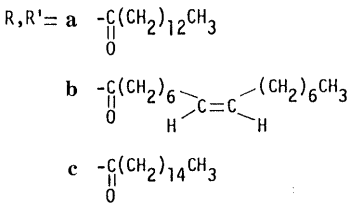
M-5 (1)

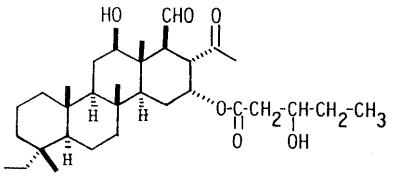
(a : b : c = 1 : 10 : 10)



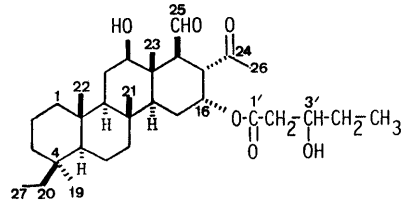
M-6 (2)

(b : c = 1 : 2)

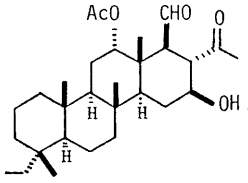




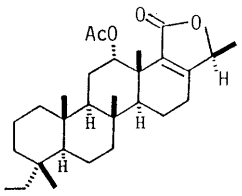
3



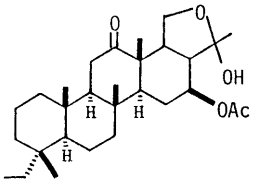
foliaspongin (4)



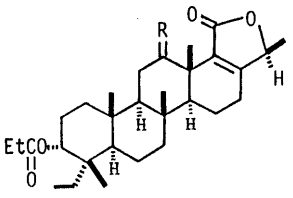
5



6

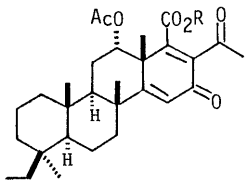


7



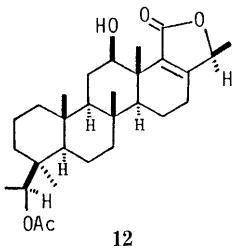
8 : R = α-OH, β-H

9 : R = O

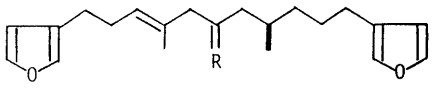


10 : R = H

11 : R = CH_3

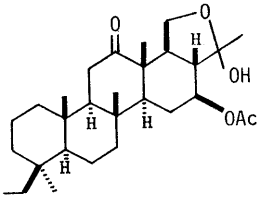


12

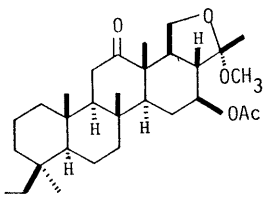


dihydrofurospongin-2 (13) : R= O

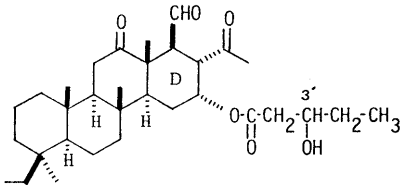
furospongin-1 (14) : R= α-OH, β-H



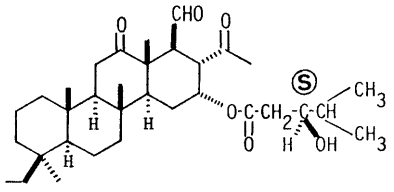
scalardysin-B (15)



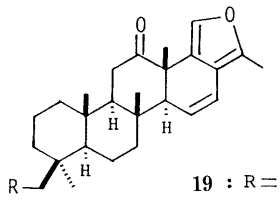
16



dehydrofoliaspongin (17)

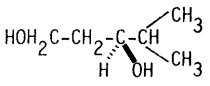


phyllofoliaspongin (18)



19 : R = CH_3

20 : R = H



21

Chart 1

methanol treatment of **17**, which yielded methyl 3-hydroxypentanoate.

In order to elucidate the carbon skeleton, dehydrofoliaspongin (**17**) was subjected to pyrolysis[2h,4] to afford a furano ketone **19**. The ultraviolet (UV) and ^{1}H-NMR spectra of the furano compound **19** were very similar to those of **20**,[2h] which possessed a 4,4-dimethyl moiety. However, the electron-impact mass spectrum (EI-MS) of **19** gave the molecular ion peak at *m/z* 394 and an ion peak at *m/z* 205 derivable from the A/B ring (through C_8-C_{14} and C_9-C_{11} fissions), both of which were 14 mass units larger than the corresponding ion peaks (*m/z* 380, 191) observed

TABLE I. ^{13}C-NMR Data for **4**, **15**, **17** and **18**

Carbon	4	15	17	18
1	40.2	39.8	39.7	39.6
2	18.3[a]	18.1[a]	18.1[a]	18.2
3	24.5	24.5	24.4	24.6
4	36.1	36.1	36.1	36.3
5	51.5[d]	50.9	51.4[d]	51.6[d]
6	18.0[a]	17.9[a]	17.8[a]	18.2
7	41.7	41.9	41.5	41.7
8	37.5[b]	37.7[b]	37.4[b]	37.6[b]
9	58.2[c]	61.2[c]	60.9[c]	61.1[c]
10	36.9[b]	38.2[b]	38.2[b]	38.3[b]
11	28.9	34.8	34.8	35.0
12	79.7	214.6	214.1	214.1
13	45.5	51.2	54.8	54.9
14	50.5[d]	46.9	52.5[d]	52.3[d]
15	27.5	26.3	25.1	25.3
16	68.9	72.8	68.4	68.5
17	58.5[c]	58.4[c]	58.5[c]	58.7[c]
18	58.7[c]	58.6[c]	52.2[d]	52.6[d]
19	28.5[e]	28.5	28.3	28.6
20	36.6	36.5	36.4	36.6
21	17.0[f]	16.8[d]	16.8[e]	16.9[e]
22	17.1[f]	16.3[d]	16.4[e]	16.5[e]
23	9.9[g]	13.6	14.4	14.6
24	208.6	101.8	206.7	206.7
25	204.1	67.3	204.1	204.3
26	25.1[e]	16.3	28.3	28.6
27	8.6	8.6	8.5	8.7
1′	171.9	170.5	171.7	172.4
2′	42.0	21.1	39.7	39.6
3′	69.7		69.6	73.3
4′	29.7		29.6	33.5
5′	10.1[g]		9.8	17.7
4′-CH_3				18.6

a—*g*) These assignments may be interchanged.

in the EI-MS of **20**.[2h] In addition, the ^{13}C-NMR spectrum of dehydrofoliaspongin (**17**) was very similar (*e.g.* δ_c 8.5 for C-27) to that of foliaspongin (**4**) except for the signals due to the 12-carbonyl carbon (δ_c 214.1) and its neighboring carbons (Table I).

Based on the above-mentioned evidence, dehydrofoliaspongin (**17**) has been shown to be the 12-dehydro derivative of foliaspongin (**4**).

The ^{1}H-NMR spectrum of phyllofoliaspongin (**18**) showed the presence of a methyl ketone moiety (δ 2.09, 3H, s) and an aldehyde group (δ 10.10, 1H, br s) as in dehydrofoliaspongin (**17**). It also showed signals attributable to the ring D protons [δ 5.56 (1H, m, 16β-H), 3.12 (1H, dd, J=12, 3Hz, 17β-H), 3.26 (1H, d, J=12 Hz, 18α-H)] which were very similar to those observed in the ^{1}H-NMR spectrum of **17**. In order to correlate the carbon skeleton of phyllofoliaspongin (**18**) with that of dehydrofoliaspongin (**17**), **18** was subjected to pyrolysis[2a,4] to afford a furano ketone which was identical with **19** obtained above from **17**. Thus, it was presumed that phyllofoliaspongin (**18**) possessed the same carbon skeleton as dehydrofoliaspongin (**17**) but differed in its organic acid residue from **17**. Lithium aluminum hydride reduction of **18** afforded 4-methylpentane-1,3-diol (**21**), which was identical with a racemic sample synthesized by hydroboration–oxidation of 4-methyl-1,3-pentadiene. In addition, the ^{1}H-NMR and ^{13}C-NMR spectra of **18** showed the presence of a 3-hydroxy-4-methylpentanoyl residue [δ 2.29 (2H, m, 2′-H_2), 3.61 (1H, m, 3′-H); δ_c 172.4 (C-1′), 73.3 (C-3′)], so that phyllofoliaspongin (**18**) was presumed to be an analogue of dehydrofoliaspongin (**17**) esterified with a 3-hydroxy-4-methylpentanoyl residue at the 16α-hydroxyl moiety.

In order to determine the absolute configuration at C-3

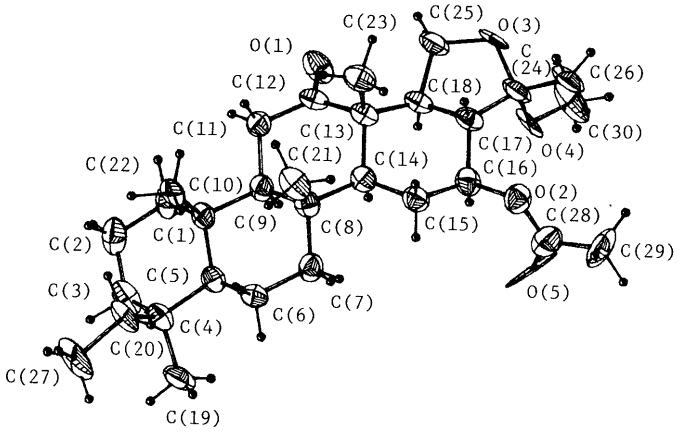


Fig. 1

TABLE II. Atomic Coordinates and Thermal Parameters with e.s.d.'s in Parentheses

	x	y	z	B_{eq}/B (Å^2)
O(1)	0.1028 (1)	0.7743 (5)	0.4894 (1)	4.6
O(2)	0.4236 (1)	0.5501 (4)	0.8434 (2)	3.9
O(3)	0.3895 (1)	0.6789 (5)	0.5420 (2)	4.9
O(4)	0.4018 (1)	0.3648 (5)	0.6273 (2)	4.8
O(5)	0.3933 (2)	0.2206 (6)	0.8759 (2)	9.5
C(1)	−0.1499 (2)	0.7565 (7)	0.6338 (2)	4.1
C(2)	−0.2407 (2)	0.7467 (8)	0.6583 (2)	4.9
C(3)	−0.2393 (2)	0.6031 (7)	0.7412 (3)	4.6
C(4)	−0.1679 (2)	0.6604 (6)	0.8324 (2)	3.7
C(5)	−0.0764 (2)	0.6773 (6)	0.8040 (2)	3.2
C(6)	0.0042 (2)	0.7147 (6)	0.8872 (2)	3.6
C(7)	0.0920 (2)	0.6726 (6)	0.8596 (2)	3.5
C(8)	0.1042 (2)	0.8083 (5)	0.7760 (2)	3.2
C(9)	0.0175 (2)	0.7789 (5)	0.6939 (2)	3.1
C(10)	−0.0736 (2)	0.8237 (6)	0.7192 (2)	3.3
C(11)	0.0265 (2)	0.8904 (6)	0.6031 (2)	3.9
C(12)	0.1077 (2)	0.8217 (5)	0.5711 (2)	3.3
C(13)	0.1970 (2)	0.8211 (5)	0.6446 (2)	3.2
C(14)	0.1824 (2)	0.7169 (5)	0.7375 (2)	3.0
C(15)	0.2720 (2)	0.6926 (6)	0.8127 (2)	3.6
C(16)	0.3383 (2)	0.5668 (6)	0.7733 (2)	3.4
C(17)	0.3542 (2)	0.6750 (6)	0.6872 (2)	3.5
C(18)	0.2655 (2)	0.6819 (6)	0.6122 (2)	3.3
C(19)	−0.1627 (2)	0.4770 (7)	0.9011 (3)	4.8
C(20)	−0.1926 (2)	0.8622 (7)	0.8786 (2)	4.6
C(21)	0.1228 (2)	1.0349 (6)	0.8112 (2)	4.1
C(22)	−0.0851 (2)	1.0594 (6)	0.7361 (2)	4.1
C(23)	0.2289 (2)	1.0530 (6)	0.6546 (2)	4.1
C(24)	0.4174 (2)	0.5846 (7)	0.6331 (2)	4.2
C(25)	0.2955 (2)	0.7309 (7)	0.5213 (2)	4.2
C(26)	0.5158 (2)	0.6371 (9)	0.6719 (3)	5.9
C(27)	−0.2760 (3)	0.8483 (9)	0.9185 (3)	6.8
C(28)	0.4442 (2)	0.3634 (8)	0.8852 (3)	5.3
C(29)	0.5396 (3)	0.3613 (9)	0.9432 (3)	6.6
C(30)	0.4520 (3)	0.2494 (9)	0.5741 (3)	6.9
C(5)-H	−0.066 (2)	0.522 (5)	0.778 (2)	1.0
C(9)-H	0.019 (2)	0.618 (5)	0.680 (2)	0.7
C(14)-H	0.164 (2)	0.562 (5)	0.719 (2)	0.6
C(16)-H	0.315 (2)	0.419 (5)	0.759 (2)	0.0
C(17)-H	0.372 (2)	0.826 (5)	0.704 (2)	0.1
C(18)-H	0.238 (2)	0.534 (5)	0.605 (2)	0.4

TABLE III. Bond Distances (Å) and Angles (°) with Their e.s.d.'s in Parentheses

Bond distances (Å)			
O(1)–C(12)	1.213 (5)	C(8)–C(9)	1.572 (5)
O(2)–C(16)	1.459 (5)	C(8)–C(14)	1.564 (5)
O(2)–C(28)	1.342 (6)	C(8)–C(21)	1.540 (5)
O(3)–C(24)	1.425 (5)	C(9)–C(10)	1.565 (5)
O(3)–C(25)	1.447 (5)	C(9)–C(11)	1.539 (5)
O(4)–C(24)	1.424 (5)	C(10)–C(22)	1.544 (5)
O(4)–C(30)	1.428 (7)	C(11)–C(12)	1.506 (5)
O(5)–C(28)	1.191 (6)	C(12)–C(13)	1.525 (5)
		C(13)–C(14)	1.573 (5)
C(1)–C(2)	1.530 (7)	C(13)–C(18)	1.541 (5)
C(1)–C(10)	1.549 (6)	C(13)–C(23)	1.557 (5)
C(2)–C(3)	1.514 (7)	C(14)–C(15)	1.549 (5)
C(3)–C(4)	1.548 (6)	C(15)–C(16)	1.520 (6)
C(4)–C(5)	1.570 (5)	C(16)–C(17)	1.504 (5)
C(4)–C(19)	1.531 (6)	C(17)–C(18)	1.530 (5)
C(4)–C(20)	1.546 (6)	C(17)–C(24)	1.511 (6)
C(5)–C(6)	1.528 (5)	C(18)–C(25)	1.538 (6)
C(5)–C(10)	1.559 (5)	C(20)–C(27)	1.540 (7)
C(6)–C(7)	1.530 (6)	C(24)–C(26)	1.523 (7)
C(7)–C(8)	1.543 (5)	C(28)–C(29)	1.508 (7)
Bond angles (°)			
C(2)–C(1)–C(10)	112.5 (4)	C(12)–C(13)–C(14)	108.0 (3)
C(1)–C(2)–C(3)	112.2 (4)	C(12)–C(13)–C(18)	110.8 (3)
C(2)–C(3)–C(4)	114.1 (4)	C(12)–C(13)–C(23)	105.9 (3)
C(3)–C(4)–C(5)	106.7 (3)	C(14)–C(13)–C(18)	105.9 (3)
C(3)–C(4)–C(19)	107.0 (3)	C(14)–C(13)–C(23)	115.6 (3)
C(3)–C(4)–C(20)	111.8 (3)	C(18)–C(13)–C(23)	110.7 (3)
C(5)–C(4)–C(19)	108.6 (3)	C(8)–C(14)–C(13)	115.9 (3)
C(5)–C(4)–C(20)	113.0 (3)	C(8)–C(14)–C(15)	114.7 (3)
C(19)–C(4)–C(20)	109.4 (3)	C(13)–C(14)–C(15)	111.1 (3)
C(4)–C(5)–C(6)	114.3 (3)	C(14)–C(15)–C(16)	110.7 (3)
C(4)–C(5)–C(10)	116.8 (3)	C(15)–C(16)–C(17)	109.5 (3)
C(6)–C(5)–C(10)	111.1 (3)	C(15)–C(16)–O(2)	110.4 (3)
C(5)–C(6)–C(7)	111.4 (3)	C(17)–C(16)–O(2)	108.3 (3)
C(6)–C(7)–C(8)	113.3 (3)	C(16)–C(17)–C(18)	108.0 (3)
C(7)–C(8)–C(9)	106.8 (3)	C(16)–C(17)–C(24)	121.2 (3)
C(7)–C(8)–C(14)	109.1 (3)	C(18)–C(17)–C(24)	102.6 (3)
C(7)–C(8)–C(21)	108.3 (3)	C(13)–C(18)–C(17)	111.0 (3)
C(9)–C(8)–C(14)	105.6 (3)	C(13)–C(18)–C(25)	121.3 (3)
C(9)–C(8)–C(21)	114.9 (3)	C(17)–C(18)–C(25)	102.4 (3)
C(14)–C(8)–C(21)	111.9 (3)	C(4)–C(20)–C(27)	115.8 (4)
C(8)–C(9)–C(10)	116.4 (3)	C(17)–C(24)–C(26)	115.7 (4)
C(8)–C(9)–C(11)	111.1 (3)	C(17)–C(24)–O(3)	103.7 (3)
C(10)–C(9)–C(11)	113.2 (3)	C(17)–C(24)–O(4)	106.6 (3)
C(1)–C(10)–C(5)	107.9 (3)	C(26)–C(24)–O(3)	107.5 (4)
C(1)–C(10)–C(9)	108.1 (3)	C(26)–C(24)–O(4)	112.2 (4)
C(1)–C(10)–C(22)	107.8 (3)	O(3)–C(24)–O(4)	110.8 (3)
C(5)–C(10)–C(9)	106.1 (3)	C(18)–C(25)–O(3)	105.8 (3)
C(5)–C(10)–C(22)	115.6 (3)	C(29)–C(28)–O(2)	110.7 (4)
C(9)–C(10)–C(22)	111.2 (3)	C(29)–C(28)–O(5)	125.7 (5)
C(9)–C(11)–C(12)	112.9 (3)	O(2)–C(28)–O(5)	123.6 (5)
C(11)–C(12)–C(13)	117.4 (3)	C(16)–O(2)–C(28)	117.1 (3)
C(11)–C(12)–O(1)	121.4 (3)	C(24)–O(3)–C(25)	110.5 (3)
C(13)–C(12)–O(1)	121.1 (3)	C(24)–O(4)–C(30)	115.7 (4)

of the 3-hydroxy-4-methylpentanoyl residue, we applied Horeau's method[10] to phyllofoliaspongin (**18**). The recovered α-phenylbutyric acid showed $[\alpha]_D$ −4° ($c=0.4$, benzene), so that the 3′*S* configuration in phyllofoliaspongin (**18**) has been determined.

The ^{13}C-NMR spectra of foliaspongin (**4**), scalardysin-B (**15**), dehydrofoliaspongin (**17**), and phyllofoliaspongin (**18**) were examined in detail and the signals of all carbons were assigned as shown in Table I.[4b] The signals due to the A/B ring carbons of **4**, **17**, and **18** appeared at chemical shifts very similar to those of scalardysin-B (**15**), and thus these scalarane-type bishomosesterterpenes are presumed to possess the same A/B ring structures as **15**.

Finally, in order to determine the configuration of the 4-ethyl moiety in these scalarane-type bishomosesterterpenes, we carried out an X-ray crystallographic analysis of a methylated derivative (**16**) which was obtained by treatment of scalardysin-B (**15**) with hot aqueous methanol. As illustrated by a stereoview in Fig. 1, the structure having a 4β-ethyl moiety has been elucidated for **16** and consequently for scalardysin-B (**15**). Based on the accumulated evidence mentioned above, the structure of foliaspongin has been revised from **3** to **4** with a 4β-ethyl moiety and the structures of dehydrofoliaspongin and phyllofoliaspongin have been determined as **17** and **18**, respectively.[11]

Phyllofoliaspongin (**18**) was found to exhibit various pharmacological activities. Thus, it showed a growth-inhibitory effect against P-388 leukemia cells (1 − T/C 84% at 5 μg/ml) and an inhibitory effect on adenosine diphosphate (ADP) ($IC_{50}=2.3$ μg/ml)- and collagen ($IC_{50}=0.6$ μg/ml)-induced aggregation of rabbit platelets *in vitro*. It also showed a vasodilative action (effect on norepinephrine-induced contraction in isolated rabbit femoral arteries: $EC_{50}=1.8$ μg/ml).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1] Computations were performed on a PANAFACOM U-1200 II (Rigaku RASA-5RP system).

Isolation of Scalardysin-B (15), Dehydrofoliaspongin (17), and Phyllofoliaspongin (18) The fresh sponges (*Phyllospongia foliascens*, 10 kg) were extracted three times with acetone (10 l each) at room temperature (for 15 h each). After removal of the solvent from the combined extract under reduced pressure, the residue was partitioned into an AcOEt–H_2O mixture to afford 65 g of AcOEt-soluble material. This AcOEt-soluble portion (5 g) was further fractionated by centrifugal chromatography (*n*-hexane–AcOEt) and then purified successively by repeated silica gel column chromatography (*n*-hexane–AcOEt, benzene–acetone as eluants) to furnish dihydrofurospongin-2 (**13**) (65 mg), furospongin-1 (**14**) (105 mg), and a sesterterpene fraction (517 mg). Purification of the combined sesterterpene fraction obtained from the AcOEt-soluble portion (3.7 g) by Lobar column chromatography (LiChroprep SiO_2 60, benzene–acetone) afforded dehydrofoliaspongin (**17**) (150 mg), foliaspongin (**4**) (100 mg), scalardysin-B (**15**) (300 mg), and phyllofoliaspongin (**18**) (150 mg). Dihydrofurospongin-2 (**13**): Colorless oil. UV λ_{max}^{hexane} nm (ε): 220 (sh) (9000). IR ν_{max}^{film} cm^{-1}: 1704 (CO). ^{1}H-NMR (90 MHz, CCl_4) δ: 7.17 (2H, br s), 7.05 (2H, br s), 6.09 (2H, br s), 5.15 (1H, t, $J=6$ Hz), 2.86 (2H, s), 2.37 (4H, t, $J=6$ Hz), 1.46 (3H, br s), 0.83 (3H, d, $J=7$ Hz). EI-MS *m/z* (%): 328 (M^+, 5.3). Furospongin-1 (**14**): Colorless oil. UV λ_{max}^{hexane} nm (ε): 220 (sh) (10500). IR ν_{max}^{film} cm^{-1}: 3455. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 7.32 (2H, br s), 7.19 (2H, br s), 6.25 (2H, br s), 5.25 (1H, t, $J=7$ Hz), 3.70 (1H, m), 2.39 (4H, t, $J=6$ Hz), 1.60 (3H, br s), 0.90 (3H, d, $J=6.5$ Hz). EI-MS *m/z* (%): 330 (M^+, 4.1). Dehydrofoliaspongin (**17**): Colorless glass. $[\alpha]_D^{20}$ +39° ($c=3.7$, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 10.10 (1H, br s), 5.56 (1H, m), 3.77 (1H, m), 3.25 (1H, d, $J=12$ Hz), 3.13 (1H, dd, $J=12$, 3 Hz), 2.52 (1H, t, $J=13.5$ Hz), 2.19 (2H, m), 2.09 (3H, s), 1.05 (3H, s), 0.72—0.93 (methyl signals). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δ_c: as shown in Table I. EI-MS *m/z* (%): 530 (M^+, 1.6), 412 ($M^+ - C_5H_{10}O_3$, 7), 205 (A/B ring, 51), 165 (D ring, 100). High-resolution MS Found: 530.358. Calcd for $C_{32}H_{50}O_6$: 530.361. Scalardysin-B (**15**): Colorless needles, mp 175—177 °C (CH_3CN–H_2O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 4.75 (1H, dt, $J=5$, 10.5 Hz), 4.32 (1H, dd, $J=9$, 8.5 Hz), 3.51 (1H, dd, $J=9.5$, 9 Hz), 1.97, 1.36, 1.04, 0.94, 0.78, 0.71 (each 3H, s), 0.65 (3H, t, $J=7.5$ Hz). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δ_c: as shown in Table I. EI-MS *m/z* (%): 474 (M^+, 1.0), 205 (A/B ring, 70). Phyllofoliaspongin (**18**): Colorless glass, $[\alpha]_D^{20}$ +40° ($c=0.9$, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 10.10 (1H, br s), 5.56 (1H, m), 3.61 (1H, m), 3.26 (1H, d, $J=12$ Hz), 3.12 (1H, dd, $J=12$, 3 Hz), 2.52 (1H, t, $J=13.5$ Hz), 2.29 (2H, m), 2.09, 1.05 (both, 3H, s), 0.79—0.93 (methyl signals). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δ_c: as shown in Table I. EI-MS *m/z* (%): 544 (M^+, 0.5), 501 ($M^+ - COCH_3$, 1), 472 ($M^+ - CHO - COCH_3$, 2),

412 ($M^+ - C_6H_{12}O_3$, 8), 205 (A/B ring, 49), 165 (D ring, 92). High-resolution MS Found: 544.374. Calcd for $C_{33}H_{52}O_6$: 544.376.

Alkaline Treatment of Dehydrofoliaspongin (17) A solution of **17** (15 mg) in dry MeOH (2 ml) was treated with 4 N NaOMe–MeOH (0.1 ml) and the whole mixture was stirred under an N_2 atmosphere at 25 °C for 1 h. The reaction mixture was partitioned into AcOEt–H_2O, and the AcOEt phase was separated and washed repeatedly with saturated aqueous NaCl, then dried over $MgSO_4$. The residue, obtained after removal of the solvent under reduced pressure, was analyzed by gas–liquid chromatography combined with mass spectrometry (GC-MS) (PEG-HT capillary column 40 m; column temperature 120 °C→220 °C) to identify methyl 3-hydroxypentanoate.

Pyrolysis of Dehydrofoliaspongin (17) Compound **17** (20 mg) was heated at 240 °C under an N_2 atmosphere for 3 min. Purification of the product by silica gel column chromatography (*n*-hexane–AcOEt) furnished **19** (6 mg). **19**: $[\alpha]_D^{20}$ −61° ($c=0.12$, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 223 (10900), 230 (11500), 240 (12300). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 7.59 (1H, s, 25-H), 6.47 (1H, dd, $J=9.5$, 2.5 Hz, 16-H), 5.73 (1H, dd, $J=9.5$, 2.5 Hz, 15-H), 2.27 (3H, s, 24-CH_3). EI-MS *m/z* (%): 394 (M^+, 40), 205 (A/B ring, 17), 146 (D/E ring, 100). High-resolution MS Found: 394.287. Calcd for $C_{27}H_{38}O_2$: 394.287.

Pyrolysis of Phyllofoliaspongin (18) Compound **18** (20 mg) was heated at 240 °C under an N_2 atmosphere for 3 min. Purification of the product by silica gel column chromatography furnished **19** (4 mg), which was identical with a sample obtained above from dehydrofoliaspongin (**17**) by comparisons of $[\alpha]_D$, UV, ^{1}H-NMR, and MS data.

$LiAlH_4$ Reduction of Phyllofoliaspongin (18) A solution of **18** (10 mg) in dry ether (5 ml) was treated with $LiAlH_4$ (25 mg) and the whole mixture was heated under reflux for 5 h with stirring. After treating the reaction mixture with aqueous AcOEt, the clear supernatant was taken and concentrated for GC-MS analysis (SE-30 capillary column, 50 m; column temperature 145 °C). The product was identical with authentic 4-methylpentane-1,3-diol (racemic).

Hydroboration–Oxidation of 4-Methyl-1,3-pentadiene A solution of 4-methyl-1,3-pentadiene (320 mg) in tetrahydrofuran (5 ml) was treated with $NaBH_4$ (304 mg) at 25 °C under an N_2 atmosphere and the whole mixture was treated dropwise with a solution of BF_3-etherate (1.1 ml) in tetrahydrofuran (3 ml) over a period of 10 min, then stirred for a further 30 min at room temperature (25 °C). Water (5 ml) was added, and the reaction mixture was made alkaline by addition of 2N aqueous NaOH (10 ml). Next, 30% aqueous H_2O_2 (5 ml) was added, and the whole was kept stirring at 25 °C for a further 1 h. The reaction mixture was partitioned into an AcOEt–H_2O mixture and the AcOEt phase was separated and washed with saturated aqueous NaCl, then dried over $MgSO_4$. The residue (440 mg), obtained after removal of the solvent under reduced pressure, was purified by silica gel column chromatography ($CHCl_3$–AcOEt) to furnish 4-methylpentane-1,3-diol (racemic, **21**) (260 mg). Colorless oil. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.84 (2H, t, $J=5.5$ Hz), 3.60 (1H, dd, $J=12.5$, 5.5 Hz), 1.68 (total 3H, m), 0.93 (3H, d, $J=6.5$ Hz), 0.91 (3H, d, $J=6.5$ Hz). ^{13}C-NMR (22.5 MHz, $CDCl_3$) δ_c: 77.1 (d), 62.0 (t), 35.2 (t), 34.1 (d), 18.4 (q), 17.6 (q). EI-MS *m/z* (%): 119 ($M^+ + H$, 0.1), 100 ($M^+ - H_2O$, 26), 75 ($M^+ - C_3H_7$, 100).

Application of Horeau's Method to Phyllofoliaspongin (18) A solution of **18** (40 mg) in pyridine (1 ml) was treated with (±)-α-phenylbutyric anhydride (40 mg) and the whole mixture was stirred under an N_2 atmosphere at 20 °C for 2 d. The reaction mixture was then partitioned into an AcOEt–saturated aqueous $NaHCO_3$ mixture and the AcOEt phase was separated and washed with saturated aqueous NaCl, then dried over $MgSO_4$. The product, obtained after removal of the solvent under reduced pressure, was purified by silica gel column chromatography (*n*-hexane–AcOEt) to furnish an ester (40 mg) and **18** (recovered, 6 mg). The aqueous $NaHCO_3$ phase was acidified with aqueous 2 N HCl and the whole was extracted with AcOEt. Work-up of the AcOEt in the usual manner afforded the recovered acid, which was purified by silica gel column chromatography (*n*-hexane–AcOEt) to furnish α-phenylbutyric acid (32 mg) of $[\alpha]_D^{20}$ −4° ($c=0.4$, benzene). The ester, colorless powder. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 7.27 (5H, m, phenyl), 5.6 (total 1H, m, 16β-H), 5.0 (total 1H, m, 3′-H).

Conversion from 15 to 16 A solution of **15** (100 mg) in MeOH–H_2O (10 : 1, 5 ml) was heated at 60 °C on a water-bath for 10 min. Evaporation of the solvent under reduced pressure yielded a residue which was purified by silica gel column chromatography with benzene–acetone to furnish **16** (65 mg) and **15** (recovered, 18 mg). Recrystallization from CH_3CN–H_2O (10 : 1) gave a pure sample of **16** as colorless needles, mp 220 °C. Chemical ionization MS [CI (C_4H_{10})-MS] *m/z*: 489 $(M+H)^+$. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 4.92 (1H, m), 4.26 (1H, dd, $J=8.5$, 8.5 Hz), 3.64 (1H, dd, $J=9.0$, 9.0 Hz), 3.19, 2.04, 1.37, 1.13, 0.87, 0.80 (each 3H, s), 0.73 (3H, t, $J=7.5$ Hz). EI-MS *m/z* (%): 473 ($M^+ - CH_3$, 6), 457 ($M^+ - CH_3O$, 6), 205 (A/B ring, 54). High-resolution MS Found: 473.324, 457.333, 205.197. Calcd for $C_{29}H_{45}O_5$: 473.321. $C_{29}H_{45}O_4$: 457.332. $C_{15}H_{25}$: 205.198.

X-Ray Analysis of 16 The crystals were recrystallized from CH_3CN–H_2O: $C_{30}H_{48}O_5$, needles, monoclinic, space group $P2_1$, $a=15.434(1)$, $b=6.3910(8)$, $c=14.571(2)$ Å, $\beta=104.173$ (9)°, $V=1393.5(2)$ Å^3, $z=2$, $D_c=1.17$ g/cm^3, $\mu=5.773$ cm^{-1}. The X-ray diffraction intensity data from the crystal (0.1 × 0.05 × 0.5 mm) were obtained on a Rigaku AFC diffractometer equipped with a rotating anode X-ray generator (55 kV- 200 mA), using graphite-monochromated CuK_α radiation ($\lambda=1.5418$ Å). A total of 2490 independent reflections with $2\theta \leqq 126°$ were collected in the $\omega/2\theta$ scanning mode. The structure was solved by the direct method using MULTAN 84 (Main *et al.*, 1984). H atoms were located from difference Fourier syntheses. The refinement was carried out by the block-diagonal least-squares method with anisotropic thermal parameters for non-H atoms and with isotropic thermal parameters for H atoms. The *R* factor was reduced to 0.037 using 2241 reflections with $|F_o| > 3\sigma(F_o)$. The atomic parameters, bond distances and angles are given in Tables II and III.

Acknowledgement The authors are grateful to Dr. H. Kikuchi, Fujisawa Pharmaceutical Co., Ltd., for testing the biological activities. They are also indebted to the Ministry of Education, Science, and Culture of Japan for financial support (Grant No. 62470137).

References and Notes

1) Part XIX: I. Kitagawa, M. Kobayashi, B. W. Son, S. Suzuki and Y. Kyogoku, *Chem. Pharm. Bull.*, **37**, 1230 (1989).
2) *a*) G. Cimino, S. De Stefano and L. Minale, *Experientia*, **30**, 846 (1974); *b*) G. Cimino, F. Cafieri, L. De Napoli and E. Fattorusso, *Tetrahedron Lett.*, **1978**, 2041; *c*) R. Kazlauskas, P. T. Murphy, R. J. Quinn and R. J. Wells, *ibid.*, **1976**, 2631; *d*) G. Cimino, S. De Stefano, L. Minale and E. Trivellone, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1587; *e*) G. Cimino, S. De Stefano and A. Di Luccia, *Experientia*, **35**, 1277 (1979); *f*) G. Cimino, S. De Rosa and S. De Stefano, *ibid.*, **37**, 214 (1981); *g*) Y. Kashman and M. Zviely, *Tetrahedron Lett.*, **1979**, 3879; *h*) R. Kazlauskas, P. T. Murphy, R. J. Wells and J. J. Daly, *Aust. J. Chem.*, **33**, 1783 (1980); *i*) R. P. Walker, J. E. Thompson and D. J. Faulkner, *J. Org. Chem.*, **45**, 4976 (1980); *j*) F. Yasuda and H. Tada, *Experientia*, **37**, 110 (1981); *k*) R. Kazlauskas, P. T. Murphy and R. J. Wells, *Aust. J. Chem.*, **35**, 51 (1982); *l*) P. Crews and P. Bescansa, *J. Nat. Prod.*, **49**, 1041 (1986); *m*) M. Nakagawa, Y. Hamamoto, M. Ishihama, S. Hamasaki and M. Endo, *Tetrahedron Lett.*, **28**, 431 (1987).
3) H. Kikuchi, Y. Tsukitani, T. Manda, T. Fujii, H. Nakanishi, M. Kobayashi and I. Kitagawa, *Chem. Pharm. Bull.*, **30**, 3544 (1982).
4) *a*) H. Kikuchi, Y. Tsukitani, I. Shimizu, M. Kobayashi and I. Kitagawa, *Chem. Pharm. Bull.*, **29**, 1492 (1981); *b*) *Idem, ibid.*, **31**, 552 (1983).
5) *a*) J. P. Declercq, M. Van Meerssche, J. C. Braekman and D. Daloze, *Acta Crystallogr., Sect. C*, **41**, 1222 (1985); *b*) J. C. Braekman, D. Daloze, M. Kaisin and B. Moussiaux, *Tetrahedron*, **41**, 4603 (1985).
6) K. D. Croft, E. L. Ghisalberti, B. W. Skelton and A. H. White, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 155.
7) Kashman and Zviely[2g] isolated a scalarane-type sesterterpene (named scalardysin-B) which seemed to be identical with another scalarane-type sesterterpene (**7**) isolated shortly afterwards from Australian *Phyllospongia foliascens* by an Australian group.[2h] However, the 4-ethyl configuration of scalardysin-B was not defined by Kashman and Zviely[2g] while that of **7** was proposed as α.[2h]
8) G. Cimino, S. De Stefano, L. Minale and E. Fattorusso, *Tetrahedron*, **28**, 267 (1972).
9) G. Cimino, S. De Stefano, L. Minale and E. Fattorusso, *Tetrahedron*, **27**, 4673 (1971).
10) A. Horeau, *Tetrahedron Lett.*, **1961**, 506.
11) The absolute stereostructures of **17** and **18** are depicted here according to those previously proved for other scalarane-type sesterterpenes.[2d]