Brominated Unsaturated Fatty Acids from Marine Sponge Collected in Papua New Guinea

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New brominated fatty acids (3, 5—8, 10) and new sterol esters (14—16) have been isolated from an unidentified marine sponge collected in Papua New Guinea. Their structures were determined on the basis of their spectroscopic data. A major component of the marine sponge (1) was tested for activities against *Arutemia salina* and some fungi.

Key words brominated fatty acid; marine sponge; Papua New Guinea

The brominated fatty acids and sterol esters have been attracting the interests of organic chemists and biochemists because of their unique chemical structures such as increased chain length, unusual unsaturation patterns, and bromine and because of their functions as secondary metabolites. During our investigation of components of the hexane extract of the marine sponge we have isolated six new brominated fatty acids (**3**, **5**—**8**, **10**) and three new sterol esters (**14**—**16**) together with known related metabolites (**1**, **2**, **4**, **9**, **11**—**13**). This paper deals with the isolation and structure elucidation of the new compounds and bioactivities of a major component of the marine sponge. Considering these components, the sponge might belong to the genus *Xestospongia*.

The hexane extract of the freeze-dried sponge was subjected to flash column chromatography eluting with exponentially gradient from hexane : EtOAc (95 : 5) to EtOAc, and three acidic fractions and a low polarity fraction were obtained. Three acidic fractions constituted of mostly 18-bromooctadeca-9*E*,17*E*-diene-7,15-diynoic acid (**1a**), which was deduced by the ^{1}H-NMR spectrum. They were methylated with $TMSCHN_2$, respectively to give the corresponding methyl esters. Methylated fractions were subjected to flash column chromatography eluting with hexane–EtOAc (9 : 1) and further separated by recycle HPLC with hexane–EtOAc to give twelve brominated fatty acid methyl esters (**1**—**12**). The low polarity fraction was subjected to flash column chromatography eluting with hexane–EtOAc (26 : 1) and further separated by recycle HPLC with hexane–EtOAc (24 : 1) to give four xestosterol esters of brominated acetylenic fatty acids (**13**—**16**).

The structures of known compounds (**1**, **2**, **4**, **9**, **11**—**13**) were determined by spectroscopic methods. Their NMR data were identical with reported data.

The molecular formula of new compound **3b** was established as $C_{19}H_{23}BrO_2$ by high resolution (HR)-EI-MS and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectra revealed signals of units ***a*** and ***b*** [δ_H 6.36 (d, 1H, J=14 Hz), 6.17 (dd, 1H, J=14, 2 Hz), 5.37 (br dd, 1H, J=16, 2 Hz), 5.96 (dt, 1H, J=16, 6.9 Hz), 5.99 (dt, 1H, J=15.6, 6.7 Hz), 5.51 (dquint, 1H, J=15.6, 1.8 Hz), 2.15 (td, 2H, J=6.9, 1.8 Hz); δ_C 79.7 (s), 85.4 (s), 89.4 (s), 91.0 (s)] and ***c*** [δ_H 3.38 (s, 3H), 2.06 (t, 2H, J=7.5 Hz), 1.49 (quint, 2H, J=7.5 Hz); δ_C 173.1 (s)] (Fig. 2). The large coupling constant (J=14 Hz) between H-17 and H-18 indicated 17*E*-geometry. H-17 was further cou-

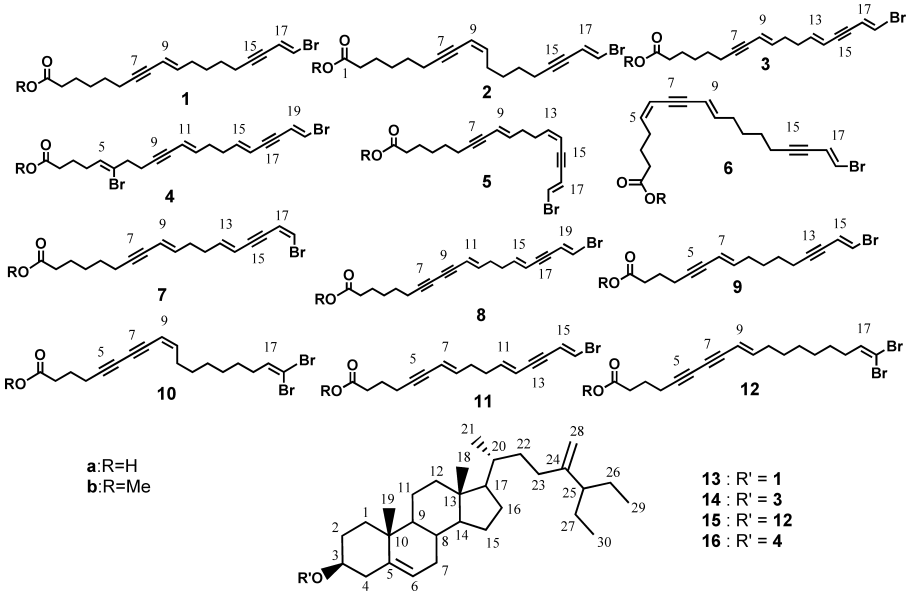

Fig. 1. Brominated Unsaturated Fatty Acids Isolated from a Marine Sponge

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pled with olefinic proton H-14 by 2 Hz, which is diagnostic for a dienyne system as unit ***a***. The ^{1}H–^{1}H COSY and HSQC experiments allowed the complete assignment of the protons and carbons (Tables 1a, b). The proton networks of the compound were deduced by the ^{1}H–^{1}H COSY spectrum. The HMBC spectrum allowed the complete connectivity of the carbons, which revealed the structure of **3b**. In the ^{1}H-NMR spectrum, H_2-11 and H_2-12 showed quite close chemical shift. H_2-11 is next to H_2-12 in the structure, which was revealed by interpretation of the HSQC and HMBC spectra (Fig. 3).

The molecular formula of **5b** was established as $C_{19}H_{23}BrO_2$ by HR-EI-MS. The ^{1}H-NMR and ^{1}H–^{1}H COSY spectra revealed signals of unit ***d*** [δ_H 6.34 (d, 1H, J=14 Hz), 6.13 (dd, 1H, J=14, 2 Hz), 5.41 (br d, 1H, J=11 Hz), 5.58 (dt, 1H, J=11, 7 Hz), 2.23 (br q, 2H, J=7 Hz)], unit ***b*** [δ_H 1.91 (br q, 2H, J=7 Hz), 6.09 (dt, 1H, J=15.7, 7 Hz), 5.58 (dd, 1H, J=15.7, 1.8 Hz), 2.14 (td, 2H, J=7, 1.8 Hz), 1.36 (m, 2H)] and unit ***c*** [δ_H 3.38 (s, 3H), 2.06 (t, 2H, J=7.5 Hz), 1.49 (quint, 2H, J=7.5 Hz), 1.25 (m, 2H)] (Fig. 2), and showed that the two allylic methylenes of units ***b*** and ***d*** [δ_H 2.23 (br q, 2H, J=7 Hz) and 1.91 (br q, 2H, J=7 Hz)] were adjacent. It follows that these three units are linked together, as shown in Fig. 1.

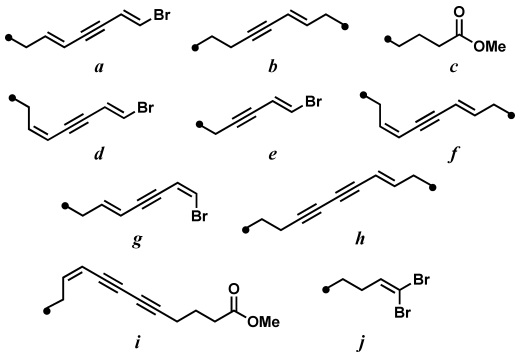

Fig. 2. Essential Substructures of the Brominated Unsaturated Fatty Acids Determined by NMR Spectroscopy

The MS data of **6b** was also established as $C_{19}H_{23}BrO_2$. The NMR data of **6b** suggested three units ***e*** and ***f*** [δ_H 6.38 (d, 1H, J=14 Hz), 6.11 (dt, 1H, J=14, 2.3 Hz), 1.91 (td, 2H, J=6.5, 2.3 Hz), 6.10 (dt, 1H, J=16, 7 Hz), 5.64 (br d, 1H, J=16 Hz), 5.65 (br d, 1H, J=11 Hz), 5.57 (dt, 1H, J=11, 7.3 Hz); δ_C 77.9 (s), 85.6 (s), {92.6 (s), observed by HMBC}, 93.0 (s)] and ***c*** [δ_H 3.38 (s, 3H), 2.14 (t, 2H, J=7.3 Hz), 1.66 (quint, 2H, J=7.3 Hz); δ_C 173.0 (s)] (Fig. 2), which were confirmed and linked together, as shown in Fig. 1, by interpretation of the ^{1}H–^{1}H COSY, HSQC, HMBC spectra.

The MS data of **7b** was also established as $C_{19}H_{23}BrO_2$. The ^{1}H-NMR and ^{1}H–^{1}H COSY spectra revealed signals of unit ***g*** [δ_H 6.52 (d, 1H, J=7.6 Hz), 6.40 (dd, 1H, J=7.6, 2 Hz), 5.67 (br d, 1H, J=16 Hz), 6.23 (dt, 1H, J=16, 6.8 Hz)], unit ***b*** [δ_H 6.01 (dt, 1H, J=16, 6.8 Hz), 5.48 (br d, 1H, J=16 Hz), 2.29 (td, 2H, J=7.1, 1.7 Hz)] and unit ***c*** [δ_H 3.66 (s, 3H), 2.32 (t, 2H, J=7.6 Hz), 1.64 (quint, 2H, J=7.6 Hz), 1.42 (quint, 2H, J=7.6 Hz)] (Fig. 2), and showed that unit ***g*** was coupled on with a 4H signal at δ_H 2.22, which was further coupled with an olefinic proton of unit ***b***. It follows that these three units are linked together, as shown in Fig. 1. The ^{1}H–^{1}H COSY and coupling constants of the protons allowed the assignment of the protons (Table 1a).

The MS data of **8b** was also established as $C_{21}H_{23}BrO_2$.

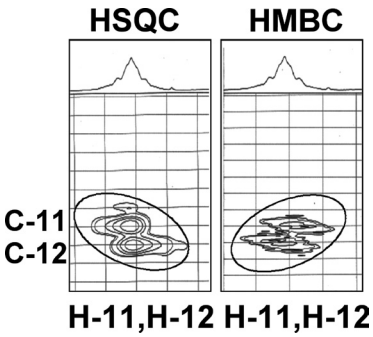

Fig. 3. HSQC and HMBC Crosspeaks of Compound **3b**

Table 1a. ^{1}H-NMR Chemical Shifts of Compound **3b**, **5b**—**8b**, and **10b** (**3b**, **5b**, **6b**, **8b**, **10b**, C_6D_6; **7b** $CDCl_3$, 400 MHz)

Position	3b	5b	6b	7b	8b	10b
OMe	3.38 s	3.38 s	3.38 s	3.66 s	3.39 s	3.31 s
1						
2	2.06 t (7.5)	2.06 t (7.5)	2.14 t (7.3)	2.32 t (7.6)	2.00 t (7.4)	2.08 t (7)
3	1.49 quint (7.5)	1.49 quint (7.5)	1.66 quint (7.3)	1.64 quint (7.6)	1.39 quint (7.4)	1.56 quint (7)
4	1.27 m	1.25 m	2.37 q (7.3)	1.42 quint (7.6)	1.13 m	1.99 br t (7)
5	1.36 m	1.36 m	5.57 dt (11, 7.3)	1.54—	1.19 m	
6	2.15 td (6.9, 1.8)	2.14 td (7, 1.8)	5.65 br d (11)	2.29 td (7.1, 1.7)	1.93 br t (7)	
7						
8						
9	5.51 dquint (15.6, 1.8)	5.58 dd (15.7, 1.8)	5.64 br d (16)	5.48 br d (16)		5.42 dq (10.7, 1.2)
10	5.99 dt (15.6, 6.7)	6.09 dt (15.7, 7)	6.10 dt (16, 7)	6.01 dt (16, 6.8)		5.74 dt (10.7, 7.5)
11	1.75 br s	1.91 br q (7)	1.77 br q (7)	2.22—	5.28 br d (16)	2.29 qd (7.5, 1.2)
12	1.75 br s	2.23 br q (7)	1.18 m	2.22—	5.96 dt (16, 6.8)	1.16—
13	5.96 dt (16, 6.9)	5.58 dt (11, 7)	1.18 m	6.23 dt (16, 6.8)	1.61—	1.02—
14	5.37 dd (16, 2)	5.41 br d (11)	1.91 td (6.5, 2.3)	5.67 br d (16)	1.61—	1.02—
15					5.87 dt (16, 6.8)	1.02—
16					5.33 dd (16, 2)	1.82 q (7.2)
17	6.17 dd (14, 2)	6.13 dd (14, 2)	6.11 dt (14, 2.3)	6.40 dd (7.6, 2)		6.10 t (7.2)
18	6.36 d (14)	6.34 d (14)	6.38 d (14)	6.52 d (7.6)		
19					6.18 dd (14, 2)	
20					6.38 d (14)	

Table 1b. ^{13}C-NMR Chemical Shifts of Compounds **3b**, **6b**, and **8b** (C_6D_6, 75 MHz)

Position	3b	6b	8b
OMe	50.9	50.9	50.9
1	173.1	173.0	173.1
2	33.9	33.2	33.7
3	24.7	24.3	24.5
4	28.5	29.8	28.3
5	28.7	141.9	28.0
6	19.5	110.7	19.4
7	89.4	85.6	84.1
8	79.7	93.0	66.5
9	111.8	110.9	74.7
10	141.2	143.7	74.2
11	32.0	32.6	110.1
12	32.5	28.0[a)]	146.1
13	144.3	27.8[a)]	32.0
14	110.4	19.3	32.0
15	91.0	92.6	143.9
16	85.4	77.9	110.5
17	118.1	118.4	90.9
18	118.0	117.4	85.5
19			118.0
20			118.1

a): Permutable.

The UV data suggested that **8b** had an enediyne system (see Experimental). The ^{1}H- and ^{13}C-NMR spectra revealed the signals of units ***a*** and ***h*** [δ_H 6.38 (d, 1H, J=14 Hz), 6.18 (dd, 1H, J=14, 2 Hz), 5.33 (dd, 1H, J=16, 2 Hz), 5.87 (dt, 1H, J=16, 6.8 Hz), 5.96 (dt, 1H, J=16, 6.8 Hz), 5.28 (br d, 1H, J=16 Hz), 1.93 (br t, 2H, J=7 Hz); δ_C 66.5 (s), 74.2 (s), 74.7 (s), 84.1 (s), 85.5 (s), 90.9 (s)] and ***c*** [δ_H 3.39 (s, 3H), 2.00 (t, 2H, J=7.4 Hz), 1.39 (quint, 2H, J=7.4 Hz); δ_C 173.1 (s)] (Fig. 2), which were confirmed and linked together, as shown in Fig. 1, by interpretation of the ^{1}H–^{1}H COSY, HSQC, HMBC spectra.

The MS data of **10b** was also established as $C_{19}H_{24}Br_2O_2$. The UV data suggested that **10b** had an enediyne system (see Experimental). The ^{1}H-NMR and ^{1}H–^{1}H COSY spectra revealed the signals of unit ***i*** [δ_H 3.31 (s, 3H), 2.08 (t, 2H, J=7 Hz), 1.56 (quint, 2H, J=7 Hz), 1.99 (br t, 2H, J=7 Hz), 5.42 (dq, 1H, J=10.7, 1.2 Hz), 5.74 (dt, 1H, J=10.7, 7.5 Hz), 2.29 (qd, 2H, J=7.5, 1.2 Hz)] as the enediyne system, unit ***j*** [δ_H 6.10 (t, 1H, J=7.2 Hz), 1.82 (q, 2H, J=7.2 Hz)] (Fig. 2), and showed that unit ***j*** was coupled with a methylene in the 8H multiplet region at δ_H 1.02, which was further coupled with an allylic methylene in unit ***i***. It follows that these units are linked together, as shown in Fig. 1. The ^{1}H–^{1}H COSY and coupling constants of the protons allowed the assignment of the all protons (Table 1a).

The molecular formula of **14** was established as $C_{48}H_{69}BrO_2$ by HR-FAB-MS and ^{13}C-NMR data. Its formula indicated a loss of two protons compared with that of **13**. The ^{13}C-NMR spectrum showed 48 carbon resonances, in which 30 were assigned to a steroid part and 18 as the fatty acid part, suggesting that **13** and **14** had a similar structure. The steroid part was the known compound xestosterol, which had previously been isolated from a sponge and its structure proposed using NMR and partial synthesis.[1)] The major difference between esters **13** and **14** was the presence of two olefinic carbons [δ_C 144.4 (d), 110.4 (d)] along with the absence of two methylene carbons in **14**. Therefore, the acid moiety was a new brominated fatty acid (**3**). Hence, **14** is a novel ester, with the linkage of a new acetylenic ester portion linked to the xestosterol system.

The ^{1}H–^{1}H COSY and HSQC experiments allowed the complete assignment of the protons and carbons (Table 2). The HMBC spectrum allowed the complete connectivity of the carbons, which revealed the planar structure of **14** (Table 2). The ^{13}C-NMR data of the xestosterol system of **14** exactly accord with one of **13**. And, the optical rotation data of **14** showed the same sign as that of **13** (see Experimental). Therefore, the absolute stereostructure of the xestosterol system of **14** was deduced to be the same as that of **13**.

The molecular formula of **15** was established as $C_{48}H_{70}Br_2O_2$ by HR-atmospheric pressure chemical ionization (APCI)-MS and ^{13}C-NMR data. The UV data suggested that **15** had an enediyne system (see Experimental). The ^{1}H-NMR spectrum showed the presence of olefinic protons characteristic of xestosterol [δ_H 4.91 (br s, 1H), 5.04 (br s, 1H), 4.90 (m, 1H), 5.40 (br s, 1H)] and olefinic protons contained in fatty acid **12** [δ_H 6.10 (t, 1H, J=7 Hz), 6.17 (dt, 1H, J=16, 7.2 Hz), 5.42 (br d, 1H, J=16 Hz)] (Table 2). The fragment analysis of the HR-APCI-MS suggested that **15** consisted of the xestosterol system (*m*/*z*: 409.3832 for $C_{30}H_{49}$) and **12** (*m*/*z*: 429.0056 for $C_{18}H_{21}O_2Br_2+H_2$) as the fatty acid part. And, the ^{13}C-NMR spectrum showed 48 carbon resonances, of which 30 were assigned to the xestosterol system and 18 as the fatty acid part, suggesting that **15** was the novel xestosterol ester of **12**.

The planar structure of **15** was revealed by interpretation of the ^{1}H–^{1}H COSY, HSQC and HMBC spectra (Table 2). The absolute stereostructure of the xestosterol system of **15** was identified as that of **13** in the same way that the absolute stereostructure of **14** was determined.

The molecular formula of **16** was established as $C_{50}H_{70}Br_2O_2$ by HR-APCI-MS and ^{13}C-NMR data. The ^{1}H-NMR spectrum showed the presence of olefinic protons had a characteristic of xestosterol [δ_H 4.92 (br s, 1H), 5.03 (br s, 1H), 4.94 (m, 1H), 5.44 (br s, 1H)] and olefinic protons indicative of fatty acid **4** [δ_H 6.40 (d, 1H, J=14 Hz), 6.20 (dd, 1H, J=14, 2 Hz), 5.42 (dt, 1H, J=17, 2 Hz), 5.99 (dt, 1H, J=17, 6 Hz), 5.99 (dt, 1H, J=16, 6 Hz), 5.50 (dt, 1H, J=16, 1.7 Hz), 5.88 (t, 1H, J=7.7 Hz)] (Table 2). The fragment analysis of the HR-APCI-MS suggested that **16** had the xestosterol system (*m*/*z*: 409.3822 for $C_{30}H_{49}$). The ^{13}C-NMR spectrum showed 50 carbon resonances, of which 30 were assigned to the xestosterol system and 20 as the fatty acid part, suggesting that **16** was a novel xestosterol ester of **4**.

The planar structure of **16** was revealed by interpretation of the ^{1}H–^{1}H COSY, HSQC and HMBC spectra (Table 2). The absolute stereostructure of the xestosterol system of **16** was identified as that of **13** in the same way that the absolute stereostructure of **14** was determined.

A major component of the marine sponge (**1**) was tested for activities against *Arutemia salina* and some fungi. As a result, **1a** and **1b** have a little toxicity against *A. salina*. The case fatality rate exceeded 50% when the concentration of **1** had been above 100 ppm. Therefore, it was deduced that **1a** would not be a kind of antifeedant against the predators of the marine sponge. On the other hand, **1b** was found to possess weak inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus mutans* and

Table 2. ^{1}H- and ^{13}C-NMR Chemical Shifts and HMBC of Compounds **14**—**16** (C_6D_6, ^{1}H: 400 MHz, ^{13}C: 75 MHz)

Position	**14**				Position	**15**				Position	**16**			
	^{13}C δ	^{1}H δ	Multi, J in Hz	HMBC		^{13}C δ	^{1}H δ	Multi, J in Hz	HMBC		^{13}C δ	^{1}H δ	Multi, J in Hz	HMBC
1	37.2	1.07	—	2, 3, 9, 10, 19	1	37.2	1.04	—	2, 3	1	37.2	1.08	—	2, 3, 19
		1.72	—	2, 5, 19			1.70	—	3, 5, 10			1.74	—	2, 3, 5, 10, 19
2	28.3	1.69	—	1, 3	2	28.2	1.63	—	3	2	28.4	1.69	—	
		1.98	—				1.93	—				1.99	—	
3	73.8	4.97	m	1′	3	74.0	4.90	m	1′	3	74.0	4.94	m	1′
4	38.8	2.50	brt, 12	6	4	38.7	2.46	brt, 12		4	38.8	2.52	—	
		2.61	brdd, 12, 5	2, 3, 5, 6, 10			2.55	ddd, 12, 4.7, 1.8	2, 3, 5, 6, 10			2.61	ddd, 12.5, 5, 2	2, 3, 6, 10
5	139.8				5	139.8				5	139.8			
6	122.9	5.41	brs	4, 10	6	122.9	5.40	brs	4, 8, 10	6	123.1	5.44	brs	4, 8, 10
7	32.2	1.56	—	5, 6	7	32.2	1.53	—	6	7	32.2	1.57	—	
		1.95	—	5, 6, 9			1.94	—	6, 5			1.96	—	6, 9
8	32.1	1.44	—	7	8	32.1	1.45	—	7, 14	8	32.1	1.45	—	7
9	50.2	0.95	—	11, 19	9	50.3	0.93	—		9	50.3	0.96	—	12
10	36.8				10	36.8				10	36.8			
11	21.3	1.44	—	9, 12, 13	11	21.3	1.44	—	9, 10, 12, 13	11	21.3	1.44	—	9, 12, 13, 14
12	40.1	1.16	—	13, 18	12	40.1	1.16		18	12	40.1	1.18	—	
		2.05	—	9, 11, 13, 14, 18			2.04	—	9, 13, 14, 18			2.05	dt, 12, 3.4	9, 11, 13, 14, 18
13	42.5				13	42.6				13	42.6			
14	56.8	0.96	—	13, 15, 18	14	56.9	0.97	—	13, 15, 16, 18	14	56.9	0.98	—	
15	24.5	1.05	—	14	15	24.5	1.06	—	14	15	24.5	1.06	—	14
		1.57	—				1.58	—				1.58	—	
16	28.5	1.33	—		16	28.5	1.32	—		16	28.5	1.33	—	20
		1.91	—	13, 15, 17			1.91	—	13, 15, 17			1.91	—	13
17	56.3	1.16	—	12, 13, 18, 20	17	56.3	1.15	—	12, 13, 20, 21, 22	17	56.3	1.17	—	12, 13, 16, 18, 20
18	12.0	0.70	s	12, 13, 14, 17	18	12.0	0.70	s	12, 13, 14, 17	18	12.0	0.71	s	12, 14, 17
19	19.4	0.99	s	1, 5, 9, 10	19	19.4	0.98	s	1, 5, 9, 10	19	19.4	1.01	s	1, 5, 9, 10
20	36.2	1.49	—		20	36.1	1.49	—		20	36.1	1.51	—	
21	19.0	1.07	d, 6.4	17, 20, 22	21	19.0	1.06	d, 6.4	17, 20, 22	21	19.0	1.07	d, 6.6	17, 20, 22
22	34.9	1.34	—	21, 23	22	34.9	1.36	—		22	34.9	1.35	—	
		1.76	—	21, 23			1.76	—				1.76	—	
23	29.8	1.94	—		23	29.6	1.94	—	22, 24, 25, 28	23	29.9	1.94	—	25
		2.16	—				2.15	—	22, 24, 28			2.16	—	24, 28
24	152.4				24	152.4				24	152.4			
25	50.6	1.91	—	23, 24, 26, 27, 28, 29, 30	25	50.6	1.91	—	23, 26/27, 29/30	25	50.6	1.91	—	23, 24, 26/27, 28, 29/30
26	26.9	1.48	—	24, 25, 27, 29	26/27	26.8	1.47	—	24, 25, 26/27, 29/30	26/27	26.8	1.47	quint, 7.3	24, 25, 27/26, 29/30
27	26.8	1.48	—	26, 30	26/27	26.9	1.47	—	24, 25, 26/27, 29/30	26/27	26.9	1.47	quint, 7.3	24, 25, 27/26, 29/30
28	109.6	4.92	brs	23, 25	28	109.6	4.91	brs	23, 25	28	09.6	4.92	brs	23, 25
		5.05	brs	23, 25			5.04	brs	23, 25			5.03	brs	23, 25
29	12.2	0.96	—	26	29/30	12.2	0.96	t, 7.1	25, 26/27	29/30	12.2	0.96	t, 7.3	25, 26/27
30	12.3	0.97	—	25, 27	29/30	12.3	0.96	t, 7.1	25, 26/27	29/30	12.3	0.96	t, 7.3	25, 26/27
1′	172.4				1′	171.7				1′	172.1		—	
2′	34.6	2.21	—	1′, 3′, 4′	2′	33.3	2.25	t, 7.3	1′, 3′, 4′	2′	33.8	2.13	t, 7.3	1′, 3′, 4′
3′	24.9	1.61	—	1′, 2′, 4′, 5′	3′	23.8	1.68	—	1′, 2′, 4′, 5′	3′	24.7	1.59	—	1′, 2′, 4′, 5′
4′	28.6	1.37	—	2′, 3′, 5′, 6′	4′	19.0	2.09	brt, 7	2′, 3′, 5′, 6′, 7′, 8′	4′	29.1	1.89	—	2′, 3′, 5′, 6′
5′	28.8	1.42	—	3′, 4′, 6′, 7′	5′	82.9				5′	133.4	5.88	t, 7.7	3′, 4′, 6′, 7′
6′	19.5	2.19	—	5′, 7′, 8′, 9′, 10′	6′	67.1				6′	124.6			
7′	89.4				7′	74.2				7′	35.1	2.51	—	5′, 6′, 8′, 9′
8′	79.8				8′	74.8				8′	18.9	2.52	—	7′, 10′, 11′
9′	111.8	5.53	brd, 16	10′, 11′	9′	109.3	5.42	brd, 16	7′, 11′	9′	87.7			
10′	141.2	6.00	m	8′, 11′, 12′	10′	148.2	6.17	dt, 16, 7.2	8′, 11′, 12′	10′	80.4			
11′	32.0	1.79	brs	9′, 12′, 13′	11′	33.2	1.74	—	9′, 10′, 12′, 13′/14′	11′	111.5	5.50	dt, 16, 1.7	
12′	32.5	1.79	brs	10′, 11′, 13′, 14′, 15′	12′	28.4	1.02	—	13′/14′	12′	141.8	5.99	dt, 16, 6	10′, 13′, 14′
13′	144.4	5.98	m	11′, 12′, 15′	13′/14′	28.8	0.94	—		13′	32.0	1.81	brs	11′, 12′, 14′
14′	110.4	5.39	brd, 17	12′, 13′	13′/14′	28.8	0.94	—		14′	32.4	1.81	brs	13′, 15′, 16′
15′	91.0				15′	27.7	1.02	—		15′	144.3	5.99	dt, 17, 6	13′, 14′, 17′
16′	85.4				16′	33.0	1.81	q, 7	13′/14′, 15′, 17′, 18′	16′	110.4	5.42	dt, 17, 2	
17′	118.1	6.18	dd, 13.9, 2.2	13′, 15′, 18′	17′	139.0	6.10	t, 7	15′, 16′, 18′	17′	91.0			
18′	118.0	6.37	d, 13.9	15′, 16′, 17′	18′	89.1				18′	85.5			
										19′	118.1	6.20	dd, 14, 2	17′
										20′	118.0	6.40	d, 14	17′, 18′

S. sobrinus by disc diffusion method (the growth inhibition zone diameter: MRSA, 12 mm; *S. mutans*, 17 mm; *S. sobrinus*, 14 mm; applying 100 μg of **1b** to sterile filter paper discs of 6 mm in diameter; bacterial concentration: MRSA, 1.5×10^6 cfu/ml; *Streptococcus* sp., 1×10^7 cfu/ml).

Experimental

General Remarks ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL AL-300 (^{1}H at 300 and ^{13}C at 75 MHz), a BRUKER AVANCE-400 (^{1}H at 400 and ^{13}C at 100 MHz). IR spectra were measured on a PERKIN-ELEMER 1720 FT-IR spectrophotometer. UV spectra were measured on a BECKMANN DU-650 spectrophotometer. LR-EI-MS spectra were taken on a JEOL JMX-DX 303, and JEOL JMS-AM150. LR-CI and FAB-MS spectra were taken on a JEOL JMS-SX102A. HR-MS were taken under electron impact (EI) and fast atom bombardment (FAB) conditions using JEOL JMS-SX102A having a direct inlet system, and atmospheric pressure chemical ionization (APCI) conditions using Agilent 1100LC/MSD TOF having a direct inlet system. Optical rotations were determined on a JASCO DIP-370 polarmeter. For column chromatography, Kieselgel 60 (Merck) was used. For preparative TLC, Kieselgel 60 F_{254}, 0.5 mm and 1.0 mm (Merck) were used. HPLC was performed on a JAI LC-908 and TOSOH SD-8023 instruments with LiChrosorb Si 60 (7 μm, 250×25 mm i.d., Merck).

Extraction and Isolation The unidentified sponge, rich pink color with hint of white, was collected at Rasch Pass of Madang by Lamiue Udu 50 m deep in Papua New Guinea, and whole body was freeze-dried in 1992 and stored in a refrigerator (<−20 °C) until extraction. Then the freeze-dried sponge (534 g) was extracted successively with hexane, CH_2Cl_2 and EtOAc at room temperature. The hexane extract was concentrated and the extract was subjected to flash column chromatography eluting with exponentially gradient from hexane : EtOAc (95 : 5) to EtOAc, and three acidic fractions (Frs. 1—3) were obtained. Fractions 1—3 were methylated with $TMSCHN_2$, respectively to give the corresponding methyl esters. Methylated fraction 1 was subjected to flash column chromatography eluting with hexane–EtOAc (9 : 1) and further separated by recycle HPLC with hexane–EtOAc (95 : 5) to give **1b** (111.2 mg), **2b** (1.1 mg), **4b** (7.8 mg), a new brominated fatty acid methyl ester (**3b**, 4.3 mg) and fraction 1-a. Fraction 1-a was repeatedly separated by recycle HPLC [hexane–EtOAc (95 : 5)] to afford two new brominated fatty acids both as methyl esters (**5b**, 0.3 mg; **6b**, 2.8 mg). Methylated fraction 2 was subjected to flash column chromatography eluting with hexane–EtOAc (9 : 1) and further purified by recycle HPLC [hexane–EtOAc (23 : 2)] to afford **9b** (4.7 mg) and three new brominated fatty acid methyl esters (**7b**, 1.1 mg; **8b**, 2.4 mg; **10b**, 0.4 mg). Methylated fraction 3 was subjected to flash column chromatography eluting with hexane–EtOAc (9 : 1) and further separated by recycle HPLC [hexane–EtOAc (14 : 1)] to afford **11b** (10.5 mg) and **12b** (7.5 mg). The low polarity fraction was subjected to flash column chromatography eluting with hexane–EtOAc (26 : 1) and further separated by recycle HPLC with hexane–EtOAc (24 : 1) to give three fractions (Frs. 4-a—c). Each of fractions 4-a, b and c was repeatedly separated by recycle HPLC [hexane–EtOAc (24 : 1)] to afford **13** (18.8 mg) and three new xestosterol esters of brominated acetylenic fatty acids (**14**, 14.7 mg; **15**, 3.4 mg; **16**, 3.8 mg).

Methyl 18-Bromooctadeca-9*E*,17*E*-diene-7,15-diynoate (**1b**): Colorless oil; HR-APCI-MS *m/z*: 365.1106 $[M+H]^+$ (Δ −0.5 mmu) for $C_{19}H_{26}{}^{79}BrO_2$; ^{1}H-, ^{13}C-NMR data of compound **1a** were identical with published data.[2,3]

Methyl 18-Bromooctadeca-9*Z*,17*E*-diene-7,15-diynoate (**2b**): Colorless oil; LR-CI-MS *m/z*: 365 $[M+H]^+$ for $C_{19}H_{26}{}^{79}BrO_2$; ^{1}H-NMR data was identical with published data.[4]

Methyl 18-Bromooctadeca-9*E*,13*E*,17*E*-triene-7,15-diynoate (**3b**): Colorless amorphous; HR-EI-MS *m/z*: 362.0884 M^+ (Δ +0.2 mmu) for $C_{19}H_{23}{}^{79}BrO_2$; UV (hexane) λ_{max} (ε): 288 (16900), 273 (20800), 228 (20500); IR (NaCl) 2924 (s), 2851 (s), 2215 (w), 2191 (w), 1737 (s), 954 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR see the text.

Methyl 6,20-Dibromoeicosa-5*Z*,11*E*,15*E*,19*E*-tetraene-9,17-diynoate (**4b**): Colorless oil; LR-EI-MS *m/z*: 466 M^+ for $C_{21}H_{24}{}^{79}Br_2O_2$; ^{1}H-NMR (400 MHz in $CDCl_3$) δ: 2.31 (2H, t, *J*=7.4 Hz, H-2), 1.72 (2H, quint, *J*=7.4 Hz, H-3), 2.12 (2H, q, *J*=7.4 Hz, H-4), 5.90 (1H, t, *J*=7.4 Hz, H-5), 2.62 (2H, br t, *J*=6.8 Hz, H-7), 2.53 (2H, br t, *J*=6.8 Hz, H-8), 5.45 (1H, br d, *J*=16 Hz, H-11), 5.99 (1H, dt, *J*=16, 6.5 Hz, H-12), 2.19 (4H, br s, H-13, 14), 6.14 (1H, dt, *J*=16, 6.8 Hz, H-15), 5.55 (1H, br d, *J*=16 Hz, H-16), 6.29 (1H, dd, *J*=14, 2 Hz, H-19), 6.64 (1H, d, *J*=14 Hz, H-20), 3.67 (3H, s, OMe); ^{13}C-NMR data was identical with published data.[5]

Methyl 18-Bromooctadeca-9*E*,13*Z*,17*E*-triene-7,15-diynoate (**5b**): Colorless oil; HR-EI-MS *m/z*: 362.0857 $[M+H]^+$ (Δ −2.4 mmu) for $C_{19}H_{23}{}^{79}BrO_2$; UV (hexane) λ_{max} (ε): 287 (19400), 274 (24400), 229 (25900); IR (NaCl) 2922 (s), 2850 (m), 2217 (w), 2187 (w), 1738 (s), 955 (m) cm^{-1}; ^{1}H-NMR see the text.

Methyl 18-Bromooctadeca-5*Z*,9*E*,17*E*-triene-7,15-diynoate (**6b**): Colorless oil; HR-EI-MS *m/z*: 362.0863 M^+ (Δ −1.8 mmu) for $C_{19}H_{23}{}^{79}BrO_2$; UV (hexane) λ_{max} (ε): 280 (14100), 265 (18400), 252 (21300); IR (NaCl) 2928 (s), 2855 (m), 2216 (w), 2188 (w), 1738 (s), 955 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR see the text.

Methyl 18-Bromooctadeca-9*E*,13*E*,17*Z*-triene-7,15-diynoate (**7b**): Colorless oil; HR-APCI-MS *m/z*: 363.0931 $[M+H]^+$ (Δ −2.3177 mmu) for $C_{19}H_{24}{}^{79}BrO_2$; UV (hexane) λ_{max} (ε): 288 (14200), 273 (16800), 228 (16900); IR (NaCl) 2918 (s), 2849 (s), 2214 (w), 2193 (w), 1737 (s), 956 (m) cm^{-1}; ^{1}H-NMR see the text.

Methyl 20-Bromoeicosa-11*E*,15*E*,19*Z*-triene-7,9,17-triynoate (**8b**): Colorless amorphous; HR-EI-MS *m/z*: 386.0885 M^+ (Δ +0.4 mmu) for $C_{21}H_{23}{}^{79}BrO_2$; UV (hexane) λ_{max} (ε): 286 (31900), 269 (33400), 255 (21500), 242 (12800), 228 (12400); IR (NaCl) 2923 (s), 2852 (s), 2234 (w), 2192 (w), 2141 (w), 1733 (s), 954 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR see the text.

16-Bromohexadeca-7*E*,15*E*-diene-5,13-diynoate (**9b**): Colorless oil; LR-EI-MS *m/z*: 336 M^+ for $C_{17}H_{21}{}^{79}BrO_2$; ^{1}H-, ^{13}C-NMR data were identical with published data.[3]

Methyl 18,18-Dibromooctadeca-9*Z*,17-diene-5,7-diynoate (**10b**): Colorless oil; HR-APCI-MS *m/z*: 443.0219 $[M+H]^+$ (Δ +0.3210 mmu) for $C_{19}H_{25}{}^{79}Br_2O_2$; UV (hexane) λ_{max} (ε): 283 (4680), 267 (5940), 253 (4850), 240 (3990), 228 (3540); IR (NaCl) 2919 (s), 2849 (s), 2233 (w), 2217 (w), 1739 (s) cm^{-1}; ^{1}H-NMR see the text.

Methyl 16-Bromohexadeca-7*E*,11*E*,15*E*-triene-5,13-diynoate (**11b**): Colorless oil; HR-EI-MS *m/z*: 334.0563 M^+ (Δ −0.5 mmu) for $C_{17}H_{19}{}^{79}BrO_2$; UV (hexane) λ_{max} (ε): 289 (9740), 273 (11900), 229 (13400); IR (NaCl) 2925 (s), 2852 (s), 2215 (w), 2190 (w), 1737 (s), 1436 (m), 957 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR data were identical with published data.[3]

Methyl 18,18-Dibromooctadeca-9*E*,17-diene-5,7-diynoate (**12b**): Colorless oil; HR-EI-MS *m/z*: 442.0138 M^+ (Δ −0.5 mmu) for $C_{19}H_{24}{}^{79}Br_2O_2$; UV (hexane) λ_{max} (ε): 284 (15800), 268 (19400), 253 (13200), 241 (6900); IR (NaCl) 2927 (s), 2854 (s), 2233 (w), 2141 (w), 1738 (s), 953 (m), 796 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR data were identical with published data.[3]

Xestosterol Ester of 18-Bromooctadeca-9*E*,7*E*-diene-7,15-diynoic Acid (**13**): White powder; HR-FAB-MS (pos.) *m/z*: 781.4532 $[M+Na]^+$ (Δ −0.3 mmu) for $C_{48}H_{71}{}^{79}BrO_2Na$; $[\alpha]_D$ −7.1° (*c*=0.205 in CH_2Cl_2); ^{1}H-, ^{13}C-NMR data were identical with published data.[6]

Xestosterol Ester of 18-Bromooctadeca-9*E*,13*E*,17*E*-triene-7,15-diynoic Acid (**14**): Colorless amorphous; HR-FAB-MS *m/z*: 779.4417 $[M+Na]^+$ (Δ +3.8 mmu) for $C_{48}H_{69}{}^{79}BrO_2Na$; $[\alpha]_D$ −15.3° (*c*=0.3 in CH_2Cl_2); UV (hexane) λ_{max} (ε): 289 (19300), 274 (23400), 230 (21600), 216 (17400); IR (NaCl) 2934 (s), 2867 (s), 2212 (w), 2190 (w), 1734 (s), 1463 (m), 956 (m), 886 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR see the text.

Xestosterol Ester of 18,18-Dibromooctadeca-9*E*,17-diene-5,7-diynoic Acid (**15**): Colorless oil; HR-APCI-MS *m/z*: 837.3769 $[M+H]^+$ (Δ −4.6307 mmu) for $C_{48}H_{71}{}^{79}Br_2O_2$; $[\alpha]_D$ −8.4° (*c*=0.17 in CH_2Cl_2); UV (hexane) λ_{max} (ε): 284 (12300), 268 (15100), 253 (10900), 241 (7040), 229 (5270), 214 nm (43500); IR (NaCl) 2927 (s), 2854 (s), 2235 (w), 2140 (w), 1731 (s), 1463 (m), 952 (m), 887 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR see the text.

Xestosterol Ester of 6,20-Dibromoeicosa-5*Z*,11*E*,15*E*,19*E*-tetraene-9,17-diynoic Acid (**16**): Colorless oil; HR-APCI-MS *m/z*: 861.3815 $[M+H]^+$ (Δ −0.5307 mmu) for $C_{50}H_{71}{}^{79}Br_2O_2$; $[\alpha]_D$ −12.4° (*c*=0.19 in CH_2Cl_2); UV (hexane) λ_{max} (ε): 288 (14500), 273 (17600), 230 nm (16500); IR (NaCl) 2929 (s), 2867 (s), 2190 (w), 1731 (s), 1455 (m), 954 (m), 887 (m) cm^{-1}; ^{1}H-, ^{13}C-NMR see the text.

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References

1) Kokke W. C. M. C., Tarchini C., Stierle D. B., Djerassi C., *J. Org. Chem.*, **44**, 3385—3388 (1979).
2) Quinn R. J., Tucker D. J., *Tetrahedron Lett.*, **26**, 1671—1672 (1985).
3) Hirsh S., Carmely S., Kashman Y., *Tetrahedron*, **43**, 3257—3261 (1987).
4) Quinn R. J., Tucker D. J., *J. Nat. Prod.*, **54**, 290—294 (1991).
5) Brantley S. E., Molinski T. F., *Tetrahedron*, **51**, 7667—7672 (1995).
6) Pham N. B., Butler M. S., Hooper J. N. A., Moni R. W., Quinn R. J., *J. Nat. Prod.*, **62**, 1439—1442 (1999).