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Antifungal Brominated C_{18} Acetylenic Acids from the Marine Sponge, *Petrosia volcano* Hoshino[1]

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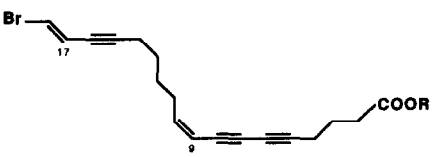
Abstract---New brominated C_{18} acetylenic acids and methyl esters (**3**, **5**, **9-10**, **12-17**) have been isolated from the marine sponge, *Petrosia volcano* Hoshino. Their structures were determined by spectroscopic methods as well as comparison of spectral data with those of known related metabolites. Both acetylenic acids and their methyl esters exhibited activity against the fungus *Mortierella ramannianus*.

Sponges of the orders Nepheliospongida and Haplosclerida often elaborate acetylenic metabolites including polyacetylenes,[2] acetylenic glyceryl ethers,[3] and brominated acetylenic acids,[4] which show a variety of biological activities, e.g. antimicrobial, cytotoxic, ichthyotoxic, H, K-ATPase inhibitory and HIV protease inhibitory. In the course of our studies on bioactive metabolites from Japanese marine invertebrates,[1] the marine sponge, *Petrosia volcano* Hoshino, collected off Hachijo-jima Island, showed antifungal activity. Bioassay-guided isolation afforded ten new brominated acetylenic acids (**3**, **5**, **9-10**, **12-17**), together with the known xestospongic acid (**1**)[4a] and 18-bromo-(9*E*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid (**7**).[4e, 4f] This paper deals with the isolation and structure elucidation of the new compounds.

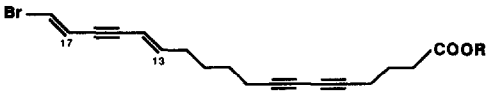
The ether and *n*-butanol soluble portions of the ethanol extract of the frozen sponge (800 g) were fractionated by silica gel, ODS flash, and ODS medium pressure column chromatographies, followed by repeated ODS HPLC to afford **1-17**: **1**, 5.3×10^{-3}%; **2**, 1.0×10^{-3}%; **3**, 2.8×10^{-4}%; **4**, 3.9×10^{-4}%; **5**, 2.8×10^{-3}%; **6**, 6.9×10^{-4}%; **7**, 5.5×10^{-4}%; **8**, 2.9×10^{-4}%; **9**, 1.1×10^{-4}% ; **10**, 3.6×10^{-3}%; **11**, 6.3×10^{-4}%; **12**, 5.4×10^{-4}%; **13**, 5.4×10^{-4}%; **14**, 8.9×10^{-4}%; **15**, 2.9×10^{-4}%; **16**, 5.5×10^{-4}%; **17**, 2.0×10^{-4}%, based on wet weight of sponge.[5] All compounds were antifungal against *Mortierella ramannianus*.

The major component in the antifungal fraction was identified as xestospongic acid (**1**).[6, 7] The ^1H and ^{13}C NMR spectra contained signals for a *trans*-disubstituted olefin adjacent to an acetylene, a diyne, three contiguous methylenes accommodated between a carboxyl and an acetylene groups, and a 1,6-disubstituted *n*-hexyl group. These NMR data together with an $(M-H)^-$ ion at *m/z* 347, 349 (1:1 ratio) in the negative FABMS allowed us to conclude that **1** was xestospongic acid. Incidentally, we also isolated xestospongic acid methyl ester (**2**) as an artifact. Compound **3** had the same molecular weight as **1**. Interpretation of the COSY spectrum indicated that **3** contained structural units *a* and

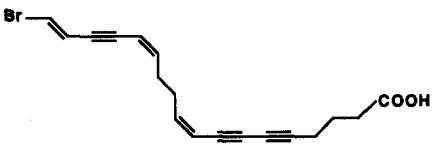
1, R=H; 17(*E*)
2, R=CH_3; 17(*E*)
3, R=H
4, R=CH_3



5, R=H
6, R=CH_3
7, R=H; 9(*E*)
8, R=CH_3: 9(*E*)
9, R=H; 17(*Z*)



10, R=H
11, R=CH_3
12, R=H; 13(*Z*)
13, R=H; 17(*Z*)



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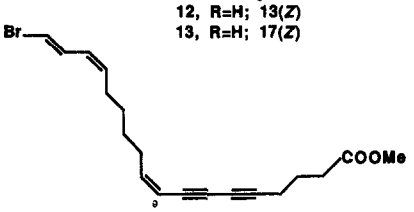
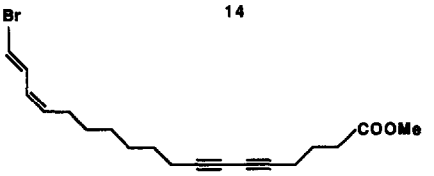


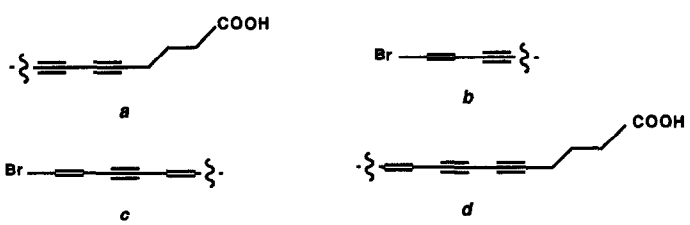
15
16, 9(*E*)



17

b in addition to a 1,6-disubstituted *n*-hexyl unit. *Z*-geometry of the C-17,18 double bond in **3** was secured by a coupling constant of J=7.3 Hz in contrast to J=14.0 Hz in **1**, thereby revealing that **3** was a 17Z-isomer of **1**.

Another major acid **5** had a molecular formula of $C_{18}H_{19}BrO_2$, which was established by FABMS and ^{13}C NMR data. The infrared spectrum contained an acetylenic band at 2200 cm^{-1} and a carboxylic band at 1700 cm^{-1}. The UV absorptions [λ_{max} 383 (7080), 267 (9330), 240 (25000), and 214 nm (ε 20100)] suggested the presence of both enyne and endiyne systems. The ^1H and ^{13}C NMR spectra revealed signals for unit *d* [δ_H 5.47 (d, 1H, J=10.2 Hz),



6.02 (dt, 1H, J=10.2, 7.3 Hz), 2.50 (t, 2H, J=7.2 Hz), 1.85 (tt, 2H, J=7.2, 7.0 Hz), 2.35 (t, 2H, J=7.0 Hz); δ_C 147.2, 108.5, 78.1, 66.2, 72.3, 18.4, 23.1, 32.6, 178.5] and unit *b* [δ_H 6.16 (dt, 1H, J=14.0, 2.4 Hz), 6.56 (d, 1H, J=14.0 Hz); δ_C 92.8, 83.1, 118.0, 117.0]. The large coupling constant (J=14.0 Hz) between H-17 and H-18 indicated

Table I. ^1H NMR spectral Data for **3, 5, 10, 14, 16** and **17***

C no	3(mult, J_{HH}, Hz)	5(mult, J_{HH}, Hz)
2	2.52 t, $J_{2,3}$=7.4	2.50 t, $J_{2,3}$=7.2
3	1.83 tt, $J_{2,3}$=7.4, $J_{3,4}$=6.6	1.85 tt $J_{2,3}$=7.2, $J_{3,4}$=7.0
4	2.35 t, $J_{3,4}$=6.6	2.34 t, $J_{3,4}$=7.0
9	2.25 t, $J_{9,10}$=6.7	5.47 d, $J_{9,10}$=10.2
10	1.52 m	6.02 dt, $J_{9,10}$=10.2, $J_{10,11}$=7.3
11	1.41 m	2.33 m
12	1.41 m	1.53 m
13	1.52 m	1.53 m
14	2.22 dt, $J_{13,14}$=7.0, $J_{17,14}$=2.2	2.27 dt, $J_{13,14}$=6.5, $J_{17,14}$=2.4
17	6.16 dt, $J_{17,18}$=7.3, $J_{14,17}$=2.2	6.16 dt, $J_{17,18}$=14.0, $J_{14,17}$=2.4
18	6.55 d, $J_{17,18}$=7.3	6.56 d, $J_{17,18}$=14.0
	10(mult, J_{HH}, Hz)	**14**(mult, J_{HH}, Hz)
2	2.50 t, $J_{2,3}$=7.3	2.40 m
3	1.85 tt $J_{2,3}$=7.3, $J_{3,4}$=7.0	1.80 tt, $J_{2,3}$=7.1 $J_{3,4}$=7.5
4	2.42 t, $J_{3,4}$=7.0	2.40 m
9	2.25 m	5.40 d, $J_{9,10}$=10.9
10	1.52 m	6.03 dt, $J_{9,10}$=10, 9, $J_{10,11}$=7.2
11	1.52 m	2.40 m
12	2.12 m	2.40 m
13	6.15 dt, $J_{13,14}$=15.8, $J_{13,12}$=6.5	5.95 dt, $J_{13,14}$=11.3, $J_{12,13}$=7.2
14	5.54 dd, $J_{13,14}$=15.8, $J_{17,14}$=2.3	5.56 dd, $J_{13,14}$=11.3, $J_{17,14}$=2.3
17	6.16 dd, $J_{17,18}$=14.0, $J_{14,17}$=2.3	6.33 dt, $J_{17,18}$=14.1, $J_{17,14}$=2.4
18	6.55 d, $J_{17,18}$=14.0	6.55 d, $J_{17,18}$=14.1
	16(mult, J_{HH}, Hz)	**17**(mult, J_{HH}, Hz)
2	2.38 t, $J_{2,3}$=7.3	2.38 t, $J_{2,3}$=7.4
3	1.84 tt, $J_{2,3}$=7.3, $J_{3,4}$=7.3	1.83 tt, J2,3=7.4 $J_{3,4}$=6.9
4	2.34 t, $J_{3,4}$=7.3	2.27 t, $J_{3,4}$=69
9	5.46 d, $J_{9,10}$=15.1	2.22 t, $J_{9,10}$=6.7
10	6.02 dt, $J_{9,10}$=15.1, $J_{10,11}$=7.3	1.48 m
11	2.11 m	1.38 m
12	1.38 m	1.38 m
13	1.38 m	1.48 m
14	2.11 m	2.13 dt, $J_{14,15}$=6.7 $J_{14,13}$=6.6
15	5.42 dt, $J_{15,16}$=10.4, $J_{14,15}$=7.5	5.41 dd, $J_{15,14}$=6.6, $J_{15,16}$=10.3
16	5.89 dd, $J_{15,16}$=10.4, $J_{16,17}$=11.4	5.85 dd, $J_{15,16}$=10.3, $J_{16,17}$=12.1
17	6.94 dd, $J_{17,18}$=13.6, $J_{16,17}$=11.4	6.87 dd, $J_{17,18}$=13.5, $J_{16,17}$=12.1
18	6.29 d, $J_{17,18}$=13.6	6.23 d, $J_{17,18}$=13.5
1'	3.65 s	3.61 s

* Solvent $CDCl_3$. Chemical shifts in ppm downfield from TMS as referenced to $CHCl_3$ at δ 7.2ppm

17*E*-geometry. H-17 was further coupled to H_2-14 by 2.4 Hz, which is diagnostic for an enyne system. Additional olefinic protons at δ 6.02 (dt, 1H, *J*=10.2, 7.3 Hz) and 5.47 (d, 1H, *J*=10.2 Hz) implied the presence of a *Z*-olefin linked to a 5,7-diyne. The NMR data for three contiguous methylene (C2-C4) protons were identical with those for **1**. The assignments of the ^1H NMR signals for the remaining C11-14 unit was unexceptional. The ^{13}C NMR data (Table II) supported the 18-bromo-(9*Z*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid structure for **5**. Compound **7** [negative FABMS, *m/z* 345/347, $(M-H)^-$], which had not been purified as the free carboxylic acid,[4e] could be isolated by

Table II ^{13}C NMR spectral Data for **1**, **5**, **10**, and **16**[a]

C.no	1	5	10	16
1	177.7	178.5	179.1	173.3
2	32.3	32.6	32.6	32.7
3	23.3	23.1	23.2	23.5
4	18.6	18.4	18.5	19.0
5	76.6	*b*	75.9	82.1
6	66.4	72.3	66.3	73.0
7	65.2	66.2	65.4	63.8
8	77.4	78.1	77.4	*b*
9	19.1	108.5	18.9	108.9
10	28.3	147.2	27.6	148.0
11	28.3	31.9	27.6	32.7[c]
12	28.3	29.4	29.7	28.7[d]
13	28.3	29.4	145.2	28.7[d]
14	19.4	19.2	109.6	33.0[c]
15	93.0	92.8	90.5	133.6
16	77.4	83.1	84.6	126.0
17	118.0	118.0	117.7	133.6
18	117.0	117.0	117.7	108.9
1'				51.6

[a]) In ppm at 75MHz, Solvent $CDCl_3$. Assigned on the basis of the comparison with the data of reported compounds. [b]) Not assignment due to overlap by $CDCl_3$ signal. [c,d]). May be exchanged.

ODS HPLC with aqueous MeOH containing 0.05% trifluoroacetic acid. The ^1H NMR spectrum of **7** was also superimposable on that of **8** except for the absence of an *O*-methyl group. The ^1H NMR data of **8** were identical with those reported in the literature.[4e, 4f] The third new compound **9**, having a molecular weight of 346, also had structural units *b* and *d* as revealed by interpretation of the COSY spectrum. The UV spectrum [λ_{max} 383 (6588), 267 (8510), 252 (7990) and 240 nm (ε 8190)] supported the presence of an endiyne unit. 9*Z*, 17*Z*-Geometry was assigned on the basis of $^1H,^1H$ coupling constants ($J_{9,10}$=10.9 Hz, $J_{17,18}$=7.3 Hz).

The acid **10**[7] had a molecular formula of $C_{18}H_{19}BrO_2$ as determined by FAB mass spectroscopy and NMR data, indicating one degree of unsaturation more than xestospongic acid (**1**). Fine UV bands (λ_{max} 287, 267, 227, 217 nm) were exceptional. The COSY spectrum allowed us to assign units *a* and *c* as well as four contiguous methylenes, which were also evident from the ^{13}C NMR data [δ 145.2, 109.6, 90.5, 84.6, 117.7 (2C), 29.7, 27.6 (2C), 18.9]. 13*E*,17*E*-Geometry was determined on the basis of $^1H,^1H$ coupling constant in the ^1H NMR data (15.8 Hz for $J_{13,14}$ and 14.1 Hz for $J_{17,18}$). Therefore, **10** was 18-bromo-(13*E*, 17*E*)-octadeca-13,17-diene-5,7,15-triynoic acid. Compounds **12** and **13**, possessing a molecular formula of $C_{18}H_{19}BrO_2$, had partial structures *a* and *c* linked via four contiguous methylenes as the case of **10**, thus indicating that **12** and **13** were double bond isomers of **10**. The 13*Z*, 17*E* geometry for **12** was secured by $^1H,^1H$ coupling constants ($J_{13,14}$=10.0 Hz, $J_{17,18}$=14.1 Hz), whereas **13** had 13*E*, 17*Z* geometry due to ^1H, ^1H coupling constants ($J_{13,14}$=15.8 Hz, $J_{17,18}$=7.3 Hz).

Compound **14**, the most highly unsaturated metabolite, had a molecular formula of $C_{18}H_{17}BrO_2$, which was 2 mass units less than **10**. The UV spectrum (λ_{max} 284, 267, 253, 240, 214 nm) of **14** was reminiscent of the presence of both endiyne and en-yne-ene systems. The COSY spectrum indicated that **14** had structural units *c* and *d*, which were

connected via two methylenes. 9Z, 13Z, 17*E*-Geometry was assigned on the basis of $^1H,^1H$ coupling constants ($J_{9,10}$=10.9 Hz, $J_{13,14}$=11.3 Hz, and $J_{17,18}$=14.1 Hz).

Ester **16**, which displayed an HPLC peak with the longest retention time had a molecular formula of $C_{19}H_{23}BrO_2$, thus indicating 8 degrees of unsaturation. The ^{13}C NMR spectrum showed the presence of only two acetylenic groups in sharp contrast with **1-14**. Mutually coupled ^1H NMR signals at δ 5.42 (1H, dt, *J*=10.4, 7.5 Hz), 5.89(1H, dd, *J*=10.4, 11.4 Hz), 6.94(1H, dd, *J*=13.6, 11.4 Hz), and 6.29(d, *J*=13.6 Hz) indicated that an (*E*)-vinyl bromide unit was connected to a Z-olefin, which was supported by the ^{13}C NMR data [δ 133.6 (2C), 126.0, and 108.9]. 9*E*-Geometry was indicated by a coupling constant of 15.8 Hz between H-9 and H-10. The other ester **15** was a double bond isomer of **16**. 9Z-Geometry was readily assigned by an $^1H,^1H$ coupling constant of 10.3 Hz.

The last compound **17** had a molecular weight 2 mass units larger than **15** and **16**. The UV spectrum [λ_{max} 245 nm (ε 5600)] indicated the presence of a diene, but not an endiyne system (Table I). ^1H NMR spectrum of ester **17** contained signals assignable to a (15Z,17*E*)-diene system, but no signals expected for the C-9 olefin as in **15**. Therefore, **17** was methyl 18-bromo-(15Z, 17*E*)-octadeca-15,17-diene-5,7-diynoate.

Methyl esters presumably were generated from corresponding free acids. In order to confirm this, we repeatedly injected pure xestospongic acid (**1**) into an ODS HPLC column (75% MeOH, 0.05% TFA). After three cycles of HPLC, approximately 30% of **1** was converted **2**, as revealed by TLC and 1H NMR spectrum.

EXPERIMENTAL SECTION

General---UV spectra were recorded on a Hitachi 330 spectrophotometer. Infrared spectra were measured with a JASCO IR-G infrared spectrometer. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AM600 or a Bruker AC300 NMR spectrometer in $CDCl_3$ at 300 K. FAB(with glycerol as a matrix), EI, and HREI mass spectra were measured on a JEOL JMX-SX102 mass spectrometer.

Isolation---Sponge samples were collected by Scuba at a depth of 15 m off Hachijo-jima Island, Japan, frozen immediately, and kept frozen at -20°C until processed. The frozen sponge (800 g) was homogenized and extracted with EtOH (3×3 L). The extract was concentrated and extracted with ether (3x0.6 L), followed by *n*-butanol (5x0.6 L). Both organic phases were combined and dried to give a brownish residue (7.02 g). This was subjected to flash chromatography on silica gel with MeOH-$CHCl_3$ systems. The antifungal fraction eluted with 2% MeOH/$CHCl_3$ gave an oily residue (1.45 g), which was further separated by ODS flash chromatography with 30, 50, 70, 90 and 100% MeOH. The active fractions eluted with 50% and 70% MeOH were combined with the active fraction eluted with 5% MeOH/$CHCl_3$, and evaporated to dryness to yield a brownish oil (998.7 mg). This was separated by medium pressure chromatography on ODS [ODS-AM 120-S50, YMC CO. LTD, 5x100 cm] with 75%MeOH containing 0.1% TFA to obtain **13** (4.3 mg), **14** (7.1 mg), **5** (22.1 mg), **10** (25.3 mg), and **1** (42.5 mg); the first, third and sixth fractions were further purified by reversed phase HPLC [Cosmosil ODS, 20x250 mm, flow rate 8 mL/min.; UV (244 nm) detection] with 70%, 60%, and 60% MeCN, respectively, to afford **9** (0.9 mg), **3** (2.2 mg), and **12** (1.6 mg). The last fraction (131.3 mg) was separated by HPLC on ODS with 75% MeOH containing 0.1% TFA to furnish **16** (4.4 mg), **6** (5.5 mg), **11** (5.0 mg), **2** (8.1 mg), and fraction containing **17** was repeatedly purified by ODS-HPLC with 60% MeCN to give pure **17** (1.6 mg). The fraction eluted from a silica gel flash column with 10% MeOH/$CHCl_3$ was subjected to reversed phase HPLC

with 75% MeOH containing 0.1% TFA, followed by 60% MeCN to yield **7** (4.4 mg), **8** (2.3 mg) and **15** (2.3 mg). The fraction (247.3 mg) eluted with 20% MeOH/$CHCl_3$ from a silica gel flash column was purified by ODS HPLC on Cosmosil 5C18-AR (75% MeOH containing 0.1% TFA and 70% MeCN) to furnish **4** (3.1 mg).

18-Bromo-(17*E*)-octadeca-17-ene-5,7,15-triynoic acid (**1**): colorless oil; FABMS (neg.) *m/z* 347/349 $(M-H)^-$; UV (MeOH) λ max 240 (9800), 215 (sh) nm (ε 3900); IR(film) 3550-3000, 2240, 1700, 1570, 1020, 915, 780, 730 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 2.52 (2H, t, *J*=7.4 Hz, H-2), 1.83 (2H, tt, *J*=7.4, 6.6 Hz, H-3), 2.35 (2H, t, *J*=6.6 Hz, H-4), 2.25 (2H, t, *J*=6.7 Hz, H-9), 1.52 (4H, m, H-10,13), 1.41 (4H, m, H-11,13), 2.22 (2H, dt, *J*=7.0, 2.2 Hz, H-14), 6.16 (1H, dt, *J*=14.0, 2.2 Hz, H-17), 6.55 (1H, d, *J*=14.0 Hz, H-18).

18-Bromo-(17*Z*)-octadeca-17-ene-5,7,15-triynoic acid (**3**): colorless oil; FABMS (neg.) *m/z* 347/349 $(M-H)^-$; UV (MeOH) λ max 245 sh (7900), 233 nm (ε 8300); IR (film) 2950, 2850, 2220, 1705, 1310 cm^{-1}; ^1H NMR see Table I.

Methyl 18-Bromo-(17*Z*)-octadeca-17-ene-5,7,15-triynoate (**4**): colorless oil; EIMS *m/z* 362/364 (M^+); HREIMS *m/z* 364.0882 (Δ 2.1 mmu) for $C_{19}H_{23}{}^{81}BrO_2$, 362.0905 (Δ 2.3 mmu) for $C_{19}H_{23}O^{79}BrO_2$; UV (MeOH) λ max 246 (8400), 233 nm (ε 9000); IR (film), 2950, 2850, 2230, 1740, 1430, 1310, 715 cm^{-1}; ^1H NMR (600 MHz, in $CDCl_3$) δ 2.41 (2H, t, *J*=7.4 Hz, H-2), 1.83 (2H, tt, *J*=7.4, 7.0 Hz, H-3), 2.33 (2H, t, *J*=7.0 Hz, H-4), 2.25 (2H, t, *J*=7.0 Hz, H-9), 1.53 (4H, m, H-10,13), 1.42 (4H, m, H-11,12), 2.38 (2H, dt, *J*=7.0, 2.1 Hz, H-14), 6.23 (1H, dt, *J*=7.6, 2.1 Hz, H-17), 6.43 (1H, d, *J*=7.6 Hz, H-18).

18-Bromo-(9*Z*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid (**5**): colorless oil; FABMS (neg.), *m/z* 345/347 $(M-H)^-$; UV (MeOH) λ max 283 (7100), 267 (9300), 240 (25000), 214 nm (ε 20100); IR (film), 3500-2400, 2230, 1700, 915, 8452 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables I and II.

Methyl 18-bromo (9*Z*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoate (**6**):[8] colorless oil; UV (MeOH) λ max 283 (6200), 267 (6800), 252 (8300), 240 (8500), 214 nm (ε 15900); IR (film), 2900, 2800, 2230, 1740, 1440, 920 cm^{-1}; ^1H NMR (600 MHz, in $CDCl_3$) δ 2.48 (2H, t, *J*=7.2 Hz, H-2), 1.83 (2H, tt, *J*=7.2, 7.3 Hz, H-3), 2.43 (2H, t, *J*=7.3 Hz, H-4), 5.47 (1H, d, *J*=10.2 Hz, H-9), 6.02 (1H, dt, *J*=10.2, 7.3 Hz, H-10), 2.32 (2H, m, H-11), 1.52 (4H, m, H-12,13), 2.28 (2H, dt, *J*=6.4, 2.2 Hz, H-14), 6.15 (1H, dt, *J*=14.0, 2.2 Hz, H-17), 6.55 (1H, d, *J*=14.0 Hz, H-18), 3.65 (3H, s, COOMe); ^{13}C NMR (75 MHz, in $CDCl_3$) δ 173.3 (C-1), 32.7 (C-2), 23.2 (C-3), 19.0 (C-4), 83.4 (C-5), 77.2 (C-6), 66.0 (C-7), 77.4 (C-8), 108.5 (C-9), 147.2 (C-10), 30.1 (C-11), 27.8 C-12), 27.7 (C-13), 19.2 (C-14), 92.8 (C-15), 77.4 (C-16), 117.9 (C-17), 117.0 (C-18), 51.6 (COO*Me*).

18-Bromo-(9*Z*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid (**7**): colorless oil; FABMS (neg.) *m/z* 345/347 $(M-H)^-$; UV (MeOH) λ max 283 (7800), 267 (12000), 252 (15800), 239 (15800), 214 nm (ε 26000); IR (film) 3500-2400, 2950, 2850, 2200, 1700, 1193, 920 cm^{-1}, ^1H NMR(600 MHz, in $CDCl_3$) δ 2.49 (2H, t, *J*=7.4 Hz, H-2), 1.83 (2H, tt, *J*=7.4, 6.9 Hz, H-3), 2.35 (2H, t, *J*=6.9 Hz, H-4), 5.47 (1H, d, *J*=15.8 Hz, H-9), 6.02 (1H, dt, *J*=15.8, 7.3 Hz, H-10), 2.13 (2H, m, H-11), 1.47 (4H, m, H-12,13), 2.27 (2H, dt, *J*=6.2, 2.0 Hz, H-14), 6.15 (1H, dt, *J*=14.1, 2.0 Hz, H-17), 6.55 (1H, d, *J*=14.1 Hz, H-18).

18-Bromo-(9*Z*, 17*Z*)-octadeca-9,17-diene-5,7,15-triynoic acid (**9**): colorless oil; FABMS (neg.) *m/z* 345/347 $(M-H)^-$; UV (MeOH) λ max 283 (6600), 267 (8500), 252 (8000), 240 nm (ε 8190); IR (film) 3500-2400, 2950, 2850, 2230, 1705 cm^{-1}; ^1H NMR (600 MHz, in $CDCl_3$) δ 2.50 (2H, t, *J*=7.3 Hz, H-2), 1.83 (2H, tt, *J*=7.3, 7.0

Hz, H-3), 2.41 (2H, t, *J*=7.0 Hz, H-4), 5.42 (1H, d, *J*=10.9 Hz, H-9), 5.98 (1H, dt, *J*=10.9, 7.5 Hz, H-10), 2.32 (2H, m, H-11), 1.48 (4H, m, H-12,13), 2.38 (2H, m, H-14), 6.23 (1H, dt, *J*=7.3, 2.0 Hz, H-17), 6.41 (1H, d, *J*=7.3 Hz, H-18).

18-Bromo-(13*E*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid (10): colorless oil; FABMS (neg.) *m/z* 345/347 $(M-H)^-$; UV (MeOH) λ max 287 (15500), 267 (17000), 227 (9800), 217 nm (ε 11800); IR (film) 3500-2400, 2200, 1700, 1555, 1190, 950, 910, 745 cm^{-1}; ^1H NMR and ^{13}C NMR see Table I and II.

Methyl 18-Bromo (13*E*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoate (11):[8] colorless oil; UV (MeOH) λ max 285 (3300)sh, 268 (4000), 247 nm (ε 4200); IR (film) 3500-3000, 2900, 2850, 2220, 1740, 1430, 1330, 1195, 915 cm^{-1}; ^1H NMR (300 MHz, in $CDCl_3$) δ 2.44 (2H, t, *J*=7.4 Hz, H-2), 1.83 (2H, tt, *J*=7.4, 6.9 Hz, H-3), 2.31(2H, t, *J*=6.9 Hz, H-4), 2.12 (2H, m, H-9), 1.50 (4H, m, H-10,11), 2.26 (2H, m, H-12), 6.14 (1H, dt, *J*=15.8, 7.2 Hz, H-13), 5.53 (1H, dd, *J*=15.8, 2.2 Hz, H-14), 6.28 (1H, dd, *J*=14.0, 2.2 Hz, H-17), 6.43 (1H, d, *J*=14.0 Hz, H-18), 3,67 (3H, s, COO*Me*).^{13}C NMR(75 MHz, in $CDCl_3$) δ 173.5 (C-1), 32.7 (C-2), 23.5 (C-3), 18.7 (C-4), 75.8 (C-5), 66.1 (C-6), 65.4 (C-7), 77.4 (C-8), 19.0 (C-9), 27.6 (C-10,11), 29.7 (C-12), 145.2 (C-13), 109.6 (C-14), 90.6 (C-15), 84.6 (C-16), 117.7 (C-17,18), 51.6 (COO*Me*).

18-Bromo-(13*Z*, 17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid (12): colorless oil; FABMS (neg.) *m/z* 345/347 $[M-H)^-$; UV (MeOH) λ max 283 (13100), 267 nm (ε 15100); IR (film) 3500-2400, 2950, 2850, 2200, 1700, 1457, 1210, 915, 710 cm^{-1}; ^1H NMR (300 MHz, in $CDCl_3$) δ 2.48 (2H, t, *J*=7.0 Hz, H-2), 1.83 (2H, tt, *J*=6.5, 7.0 Hz, H-3), 2.35 (2H, t, *J*=6.5 Hz, H-4), 2.28 (4H, m, H-9,12), 1.52 (4H, m, H-10,11), 5.90 (1H, dt, *J*=10.0, 7.1 Hz, H-13), 5.53 (1H, dd, *J*=10.0, 2.1 Hz, H-14), 6.29 (1H, dd, *J*=14.1, 2.1 Hz, H-17), 6.60 (1H, d, *J*=14.1 Hz, H-18).

18-Bromo-(13*E*, 17*Z*)-octadeca-9,17-diene-5,7,15-triynoic acid (13): colorless oil; FABMS (neg.) *m/z* 345/347 $(M-H)^-$; UV (MeOH) λ max 285 (4500), 270 (sh) nm (5400); IR (film) 3500-3000, 2900, 2855, 2200, 1700, 1410, 1310, 1250, 1050, 950, 835, 800 cm^{-1}; ^1H NMR (300 MHz, in $CDCl_3$) δ 2.47 (2H, t, *J*=7.4 Hz, H-2), 1.83 (2H, tt, *J*=7.4 Hz, 6.9, H-3), 2.33 (2H, t, *J*=6.9 Hz, H-4), 2.13 (2H, m, H-9), 1.52 (4H, m, H-10,11), 2.24 (2H, m, H-12), 6.22 (1H, dt, *J*=15.8, 7.8 Hz, H-13), 5.53 (1H, dd, *J*=15.8, 2.1 Hz, H-14), 6.28 (1H, dd, *J*=7.3, 2.1 Hz, H-17), 6.43 (1H, d, *J*=7.3 Hz, H-18).

18-Bromo-(9*Z*, 13*Z*, 17*E*)-octadeca-9,13,17-triene-5,7,15-triynoic acid (14): colorless oil; FABMS (neg.) *m/z* 343/345 $(M-H)^-$; UV (MeOH) λ max 284 (4600), 267 (6500), 253 sh (6300), 240 (6600), 214 nm (ε 9550); IR (film) 3500-2400, 2950, 2850, 2240, 1705, 1445, 1340, 1250, 917, 838, 802, 735 cm^{-1}; ^1H NMR see Table I.

Methyl 18-Bromo (9*Z*, 15*Z*, 17*E*)-octadeca-9,15,17-diene-5,7-diynoate (15):[8] colorless oil; UV (MeOH) λ max 284 (5600), 267 (7100), 253 (9500), 240 (10700), 214 nm (ε 22900); IR (film) 2950, 2850, 2230, 1740 cm^{-1}; ^1H NMR (300 MHz, in $CDCl_3$) δ 2.42 (2H, t, *J*=7.6 Hz, H-2), 1.83 (2H, tt, *J*=7.6, 7.3 Hz, H-3), 2.39(2H, t, *J*=7.3 Hz, H-4), 5.45 (1H, d, *J*=10.9 Hz, H-9), 5.96 (1H, dt, *J*=10.9, 7.2 Hz, H-10), 2.29 (2H, m, H-11), 1.38(4H, m, H-12,13), 2.11 (2H, m, H-14), 5.45 (1H, dt, *J*=10.8, 7.3 Hz, H-15), 5.85 (H, dd, *J*=12.4, 10.8 Hz, H-16), 6.94 (1H, dd, *J*=13.8, 12.1 Hz, H-17), 6.22 (1H, d, *J*=13.8, H-18), 3.62 (3H, s, COO*Me*)

Methyl 18-Bromo-(9*E*, 15*Z*, 17*E*)-octadeca-9,15,17-diene-5,7-diynoate (16):[8] colorless oil; UV (MeOH) λ max 282 (3000), 267 (3800), 252 sh (4500), 240 (5000), 213 nm (ε 9100); IR (film) 3450-2250, 2990, 2885, 2240, 1735, 1435, 1340, 11905, 800, 725 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables I and II.

Methyl 18-Bromo-(15*Z*, 17*E*)-octadeca-15,17-diene-5,7-diynoate (17):[8] colorless oil; UV (MeOH) λ max 245 (sh) nm (ε 8700); IR (film) 2950, 2850, 2230, 1740, 1455 cm^{-1}; ^1H NMR see Table I.

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5. Due to unstability of this class of compounds, the isolated amounts of the free acids do not reflect their quantity in the sponge.
6. The spectral data of xestospongic acid were not reported in reference 4a.
7. This compound was quite labile, particularly when exposed to air, thus had to be characterized immediately after isolation. When kept for several weeks in a refrigerator, the compound became a colorless solid, then a blue solid. Upon addition of MeOH, the blue solid immediately turned red. At present, we have no idea of what happened to the compound.
8. Neither FAB or EI mass spectrum was successfully measured due to extreme instability of the compound.