201. Sponge-Derived Polyunsaturated C_{16} Di- and Tribromocarboxylic Acids

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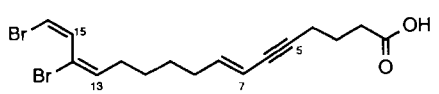
One previously described and two new bromo-substituted polyunsaturated C_{16} fatty acids were isolated from an Indonesian sponge, *Oceanapia* sp. Their common structural feature is an (13*E*,15*Z*)-14,16-dibromodiene terminus. They differ in their C(5) to C(10) portions in unsaturation and halogenation. All three compounds are unstable oils. The mixture exhibits mild cytotoxicity against KB cells.

Introduction. – Sponges of the genera *Xestospongia* and *Petrosia* (order Petrosida, family Petrosiidae) yielded a number of brominated polyunsaturated fatty acids [1–7]. A majority of them (21) are C_{18} acids, but six C_{16} and one C_9 acid are also represented. The unsaturation, except for the C_9 compound, includes olefinic and acetylenic functions. The first of these metabolites, (7*E*,13*E*,15*Z*)-14,16-dibromohexadeca-7,13,15-trien-5-ynoic acid (**1**), was reported by *Schmitz* and *Gopichand* [1] as a CNS-active constituent of the sponge *Xestospongia muta*. Its unique structural feature is its (13*E*,15*Z*)-14,16-dibromodiene terminus.

We now report the isolation of **1** and of two new compounds, (5*Z*,7*E*,9*E*,13*E*,15*Z*)-6,14,16-tribromohexadeca-5,7,9,13,15-pentaenoic acid (**2**) and (7*E*,9*E*,13*E*,15*Z*)-14,16-dibromohexadeca-7,9,13,15-tetraen-5-ynoic acid (**3**) from a sponge, *Oceanapia* sp. (order Petrosida, family Oceanapiidae), collected in October, 1992, off Manado, Sulawesi, Indonesia. All three acids have a common dibromodiene terminus.



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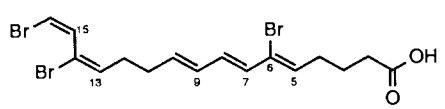

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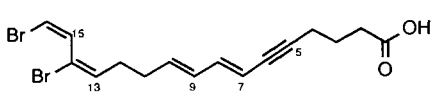

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[1]) *Harbor Branch Oceanographic Institution* Contribution No. 1003.

Results and Discussion. – The freeze-dried sponge was extracted with EtOH. The residue after solvent removal was partitioned between CH_2Cl_2 and H_2O and yielded 2.64 g of lipid extract. A portion of this (420 mg) was subjected to high-speed countercurrent chromatography, followed by reversed-phase HPLC, which resulted in the isolation of three unstable yellow oils, **1** (7.9 mg), **2** (7.5 mg), and **3** (26.9 mg). The structures of these compounds were established using ^{1}H- and ^{13}C-NMR and mass spectroscopy and, in the case of **1**, by comparison with known data [1].

The ^{13}C-NMR spectrum of **1** has 16 signals. The presence of an acid (δ 178.7), an acetylenic (δ 87.1 and 80.1), and 3 olefinic moieties (δ 143.2, 134.5, 131.0, 113.6, 112.4, 109.9) is readily apparent. The ^{1}H-NMR spectrum of **1** shows the presence of 1 disubstituted (*E*)-olefin at δ 5.44 (*d*, J = 14.4 Hz) and 6.03 (*dt*, J = 15.3, 7.8 Hz), 1 disubstituted (*Z*)-olefin at δ 6.68 (*dd*, J = 7.7 Hz) and 6.38 (*d*, J = 7.8 Hz), and a trisubstituted olefin at δ 6.06 (*t*, J = 7.7 Hz; see *Table*). These NMR data indicate that compound **1** is a C_{16} polyunsaturated acetylenic acid. A comparison with published ^{1}H-NMR data establishes the identity of **1** and (7*E*, 13*E*,15*Z*)-14,16-dibromohexadeca-7,13,15-trien-5-yonic acid [1].

Table. *^{1}H-NMR Data of Compounds* **1**, **2**, *and* **3**. δ in ppm and J in Hz.

	1	**2**	**3**
CH_2(2)	2.50 (*t*, J = 7.5)	2.40 (*t*, J = 7.4)	2.50 (*t*, J = 7.4)
CH_2(3)	1.85 (*quint.*, J = 7.1)	1.79 (*quint.*, J = 7.4)	1.86 (*quint.*, J = 7.1)
CH_2(4)	2.38 (*dt*, J = 1.6, 6.8)	2.38 (*q*, J = 7.3), 5.90 (*t*, J = 7.2)	2.42 (*dt*, J = 1.4, 6.6)
H–C(5)			
H–C(7)	5.44 (*d*, J = 14.4)	6.11 (*d*, J = 14.4)	5.49 (br. *d*, J = 15.6)
H–C(8)	6.03 (*dt*, J = 15.3, 7.8)	6.53 (*dd*, J = 10.5, 14.4)	6.47 (*dd*, J = 10.7, 15.5)
CH_2(9) or H–C(9)	2.01 (*q*, J = 6.6)	6.14 (*dd*, J = 11.6, 14.6)	6.07 (*dd*, J = 11.7, 14.5)
CH_2(10) or H–C(10)	1.40 (*m*)	5.79 (*dt*, J = 15.3, 7.1)	5.70 (*dt*, J = 6.6, 15.3)
CH_2(11)	1.40 (*m*)	2.25 (*q*, J = 7.1)	2.22 (*q*, J = 6.6)
CH_2(12)	2.07 (*q*, J = 6.6)	2.13 (*q*, J = 7.3)	2.12 (*q*, J = 7.1)
H–C(13)	6.06 (*t*, J = 7.7)	6.07 (*dt*, J = 1.2, 7.5)	6.05 (*t*, J = 6.3)
H–C(15)	6.68 (*dd*, J = 1.1, 7.7)	6.68 (*dd*, J = 1.1, 7.7)	6.68 (*d*, J = 7.5)
H–C(16)	6.38 (*d*, J = 7.8)	6.39 (*d*, J = 7.8)	6.39 (*d*, J = 7.5)

Compound **2** has the molecular formula $C_{16}H_{21}Br_3O_2$, as shown by the HR-EI-MS data. Some signals in the ^{1}H-NMR spectrum of **2** are identical with those of **1** (*Table*). The lack of an acetylene moiety is indicated by the IR and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum shows 8 olefinic-proton and 5 aliphatic-proton signals. Decoupling experiments connect CH_2(2) to CH_2(3) to CH_2(4) and the latter to H–C(5) (δ 5.90 (*t*, J = 7.2 Hz)). Additional decoupling experiments reveal the H–C(7) to H–C(13) sequence which is readily extended to H–C(16) by comparison with the ^{1}H-NMR data of **1**. Since the COOH group is unconjugated (IR: 1700 cm^{-1}; ^{13}C-NMR: 178.5 ppm), it necessarily must be attached to C(2) and the third Br-atom to C(6). Coupling constants between H–C(7) and H–C(8), H–C(9) and H–C(10), H–C(15) and H–C(16) allow the assignment of the (7*E*,9*E*,15*Z*)-configuration. NOE's from H–C(5) to H–C(7) and from CH_2(12) to H–C(15) establish the (5*Z*,13*E*)-configuration[2]). *Schmitz* and *Gopichand* [1] did not assign the C(13)=C(14) geometry of compound **1**. But since the ^{1}H-NMR chemical shift for H–C(13) of **2** is almost the same as for **1**, the latter could also have (13*Z*)-configuration.

HR-EI-MS of compound **3** leads to a molecular formula $C_{16}H_{18}Br_2O_2$, differing from **1** by H_2. ^{1}H-NMR spectral comparison (*Table*) shows that **3** differs from **1** by an additional olefin double bond at C(9)=C(10).

Since compounds **1–3** are unstable when pure, cytotoxicity was tested on the mixture, which showed only weak cytotoxicity (2+ at 10 μg/ml) against KB cells. Compound **3** showed mild antimicrobial activity against Gram-positive bacteria.

[2]) An unusually long relaxation time of 10 s was required to observe an NOE between CH_2(12) and H–C(15).

Experimental Part

General. Anal. TLC: pre-coated HP-TLC plates (silica gel *60 F254* and *RP-18 F254s*). Countercurrent chromatography: *Ito-Multi-Layer-Coil* separator-extractor. Reversed-phase HPLC: *YMC-Pack AQ-ODS*. UV: λ_{max} (ε) in nm. IR: NaCl plates; in cm^{-1}. NMR: δ in ppm rel. to Me_4Si (= 0 ppm), J in Hz.

Isolation. A sponge was collected from Manado Bay, Sulawesi, Indonesia, on October 1, 1992, from a depth of 10–40 m. The sponge is a semi-spherical to thickly encrusting mass, with broad papillar oscular projections, firm in life and when preserved. The sponge is yellowish-orange to white, with a reddish tinge on light-exposed surfaces, and is beige in EtOH preservative. It is most closely comparable to *Oceanapia papula* DESQUEYROUX-FAUNDEZ 1987 (Porifera, Demospongiae, Petrosida, Oceanapiidae). A voucher specimen was deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No. 033: 890). A freeze-dried sponge (dry weight 104 g) was extracted with EtOH (4 × 900 ml). The solvent was evaporated and the resulting residue partitioned between CH_2Cl_2 and H_2O to yield 2.64 g of nonpolar extract. A portion of the nonpolar extract (420 mg) was applied to a high-speed countercurrent chromatography (HS-CCC) coil with heptane/MeCN/CH_2Cl_2 10:7:3 (lower mobile phase). The second fraction from HS-CCC separation was purified by reversed-phase HPLC (MeOH/H_2O 85:15): **1** (7.9 mg), **2** (7.5 mg), and **3** (26.9 mg) as yellow oils.

(7E,13E,15Z)-14,16-Dibromohexadeca-7,13,15-trien-5-ynoic Acid (**1**). ^{1}H-NMR (300 MHz, $CDCl_3$): *Table*. ^{13}C-NMR (75 MHz, $CDCl_3$): 178.7 (*s*), 143.2 (*d*); 136.5 (*d*); 131.0 (*d*); 113.6 (*s*); 112.4 (*d*); 109.9 (*d*); 87.1 (*s*); 80.1 (*s*); 32.6 (2*t*); 30.9 (*t*); 28.2 (*t*); 28.0 (*t*); 23.6 (*t*); 18.7 (*t*). EI-MS: 402, 323, 321, 279, 263, 243, 183, 117, 91.

(5Z,7E,9E,13E,15Z)-6,14,16-Tribromohexadeca-5,7,9,13,15-pentaenoic Acid (**2**). UV (MeOH): 208 (4600), 252 (sh, 7300), 264 (11700), 272 (14600), 284 (11900). IR (neat): 3500–2500 (br.), 1700, 1590, 1430, 975. ^{1}H-NMR (300 MHz, $CDCl_3$): *Table*. ^{13}C-NMR (75 MHz, $CDCl_3$): 178.5 (*s*); 135.5 (*d*); 135.3 (*d*); 133.1 (*d*); 132.2 (*d*); 131.0 (*d*); 130.2 (*d*); 129.5 (*d*); 126.1 (*d*); 113.9 (*s*); 112.5 (*d*); 33.1 (*t*); 31.7 (*t*); 30.9 (*t*); 30.8 (*t*); 23.5 (*t*). EI-MS: 486, 484, 482, 177, 117, 80. HR-EI-MS: 481.911118 ($C_{16}H_{21}Br_3O_2$, calc. 481.90943).

(7E,9E,13E,15Z)-14,16-Dibromohexadeca-7,9,13,15-tetraen-5-ynoic Acid (**3**). UV (MeOH): 204 (15000), 256 (sh, 29000), 268 (37100), 278 (28700). IR (neat): 3500–2500 (br.), 3010, 2930, 2200, 1700, 1590, 980. ^{1}H-NMR (300 MHz, $CDCl_3$): *Table*. ^{13}C-NMR (75 MHz, $CDCl_3$): 179.5 (*s*); 140.8 (*d*); 135.4 (*d*); 135.0 (*d*); 130.9 (*d*); 130.7 (*d*); 114.0 (*s*); 112.5 (*d*); 109.9 (*d*); 90.8 (*s*); 80.8 (*s*); 32.8 (*t*); 31.6 (*t*); 30.7 (*t*); 23.7 (*t*); 19.0 (*t*). EI-MS: 404, 402, 400, 323, 321, 241, 177, 117, 91. HR-EI-MS: 399.967407 ($C_{16}H_{18}Br_2O_2$, 399.96754).

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