Three Novel C_{21} Polyacetylenes from the Marine Sponge *Callyspongia* sp.

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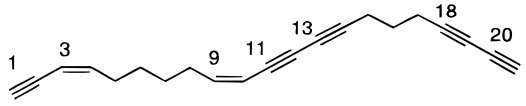
Three new C_{21} linear polyacetylenes, callypentayne (**1**), callyberyne B (**2**), and callytetrayne (**3**), along with the known compound siphonodiol (**4**), have been isolated from the Japanese marine sponge *Callyspongia* sp. The structures of **1**−**3** were proposed on the basis of extensive NMR experiments.

Several examples of polyacetylenic compounds from marine organisms have been reported in the last 20 years.[1] Our chemical interest in marine organisms led to isolation of three new polyacetylenes, callyberynes A−C (**1**−**3**), along with siphonodiol (**4**)[2] from the Japanese marine sponge *Callyspongia* n.sp. (Callyspongiidae). We now report the isolation and structure elucidation of **1**−**3**.



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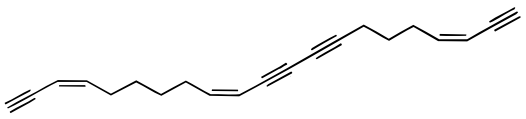

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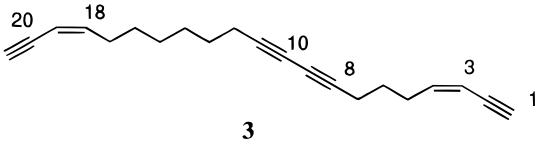

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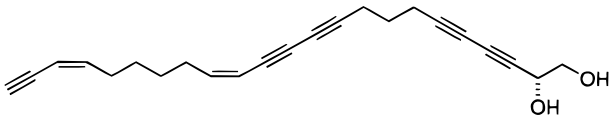

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A MeOH/CH_2Cl_2 (3:1) extract of the sponge was divided into EtOAc- and H_2O-soluble portions. The EtOAc-soluble portions were chromatographed on silica gel columns. Final purification by reversed-phase HPLC afforded compounds **1**−**3**.

Callypentayne (formerly called callyberyne A)[3] (**1**) was obtained as a colorless oil, and its molecular formula, $C_{21}H_{20}$, was determined by HREIMS. An IR absorption (3290, 2225 cm^{-1}) was attributable to terminal and disubstituted acetylene functions. The ^{13}C NMR spectrum indicated the presence of 10 acetylene carbons [δ 82.9 (s) × 2, 81.3 (d), 80.4 (s), 77.9 (s), 76.8 (s), 72.5 (s), 68.2 (s), 66.2 (d), 65.5 (s)] and four sp^2 carbons [δ 147.7 (d), 145.7 (d), 108.3 (d), 108.2 (d)], suggesting two disubstituted double bonds. The ^1H NMR spectrum contained two terminal acetylene protons [δ 3.09 (enyne), δ 1.99 (diyne)], four olefinic protons [(δ 6.05, 6.00, 5.48, 5.46)], and seven methylenes (δ 2.48 (2H), 2.42 (2H), 2.36 (4H), 1.79 (2H), and 1.46 (4H)].

A terminal acetylenic proton at δ 3.09 (enyne) and δ 1.99 (diyne) suggested the partial structures **a** (one terminal yne) and **e** (one terminal diyne), respectively. The $^1H-^1H$ COSY (Figure 1) and $^{13}C-^1H$ COSY experiments implied the partial structures **b** [$-CH{=}CH(CH_2)_4CH{=}CH-$, from C-3 to C-10] and **d** [$-(CH_2)_3-$, from C-15 to C-17]. The partial structure **c** (one diyne) was suggested by the remaining four acetylene carbons and by HREIMS. The HMBC experiment revealed long-range couplings from H-1 to C-3 and -4, from H-3 to C-1, and from H-4 to C-2. This suggested a linkage between partial structures **a** and **b**. Additional HMBC correlations between H-9 and C-11, H-10 and C-12, and H-10 and C-13 established the connectivity between partial structures **b** and **c**. Furthermore, the HMBC spectrum showed couplings between H-15 and C-11, -12, -13, and -14, H-16 and C-14, -18, and H-17 and C-18, -19, -20, and -21. Thus, the structure of **1** was connected. The Z configurations of the two double bonds were determined by their coupling constants of 10.7 Hz. Callypentayne (**1**) can thus be designated as (3Z,9Z)-henicosa-3,9-diene-1,11,13,18,20-pentyne.

Callyberyne B (**2**) was isolated as a colorless oil and was determined to have a molecular formula of $C_{21}H_{22}$ by HREIMS of the molecular ion at *m*/*z* 274.1728, differing from the molecular formula of **1** by H_2. Comparison of the physicochemical data of **2** with those of **1** revealed that the only difference was that **2** had a double bond instead of a triple bond at the C18 position. This conclusion was confirmed by the observation of six carbon signals in the double bond region in the ^{13}C NMR spectrum and by the presence of two terminal acetylene protons at δ 3.10 and 3.08 (enyne) in the ^1H NMR spectrum. The connectivity of the COSY and HMBC experiments (see Experimental Section) supported the proposed structure of **2**. The Z configurations of the three double bonds were determined by their coupling constants of 10.7 Hz. Callyberyne B (**2**) can thus be designated as (3Z,9Z,18Z)-henicosa-3,9,18-triene-1,11,-13,20-tetryne.

Callytetrayne (formerly callyberyne C)[3] (**3**) was isolated as a colorless oil, and its molecular formula was determined as $C_{21}H_{24}$ by HREIMS, differing from the

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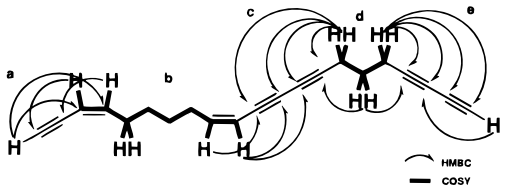

Figure 1. $^1H-^1H$ COSY (bold lines) and HMBC (arrows) correlations for callyberyne A (**1**).

molecular formula of **2** by the addition of H_2. The physicochemical data of **3** resembled those of **2**. Examination of the ^{13}C NMR and ^1H NMR data obtained for **3** revealed that the C12 double bond in **2** was saturated. This was supported by the presence of four sp^2 carbons [δ 146.0 (d), 144.4 (d), 109.2 (d), 108.1 (d)] and two terminal acetylene protons at δ 3.09 and δ 3.08. A combination of COSY and HMBC experiments (see Experimental Section) enabled us to construct the structure of **3**. The Z configurations of the two double bonds were determined by their coupling constants of 10.7 Hz. Thus, callytetrayne (**3**) is represented by (3Z,-18Z)-henicosa-3,18-diene-1,8,10,20-tetryne.

Biogenetically, **1**−**3** are considered most likely to be produced by decarboxylation of a C-22 fatty acid precursor in the sponges. Reports of polyacetylenes with no oxygen functional groups from marine organisms are relatively rare.[4]

Experimental Section

General Experimental Procedures. The following instruments were used: a JASCO FT/IR-5300 (IR), a JEOL JMS-HX-100 mass spectrometer (HRMS), and a Varian UNITY 600 NMR spectrometer (^1H and ^{13}C NMR).

Sponge Material. The marine sponge *Callyspongia* n.sp. (180 g, wet weight) was collected off the coast of Tokushima prefecture, Japan, and was kept frozen (−20 °C) until used. The marine sponge was identified by Professor P. R. Bergquist of Auckland University. The voucher sample (TS032) of the organism under consideration is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan. It is a mass of thin, erect branches arising from a single base of attachment 3.0 cm in diameter. The overall dimensions are 30 cm wide and 25 cm high. Individual branches are of irregular shape ranging from 0.5−1.5 cm in diameter and are oval in cross section. The spicules are small strongyles of very uniform size 50−70 μm × 2.5 μm. As is typical for the genus, the fibrous skeleton is a reticulum with irregularly spaced tracts in the endosome and a more organized almost rectangular arrangement in the immediate subsurface region. Endosomal fibres contain from one to eight rows of spicules. The tangential, dermal skeleton is a regular reticulation of fine fibers that contain one to two rows of spicules. The sponge surface is smooth with no spines or protruding spicule brushes. The species is distinct within the genus in combining ramose habit, strongylote spicules, and a smooth dermal skeletal arrangement. *Callyspongia* is a large genus, and description of any new species, this one included, requires more material than is available and also careful review of type specimens. This cannot be undertaken at present.

Extraction and Isolation of Metabolites. The frozen sample (180 g) was lyophilized and exhaustively extracted with MeOH/CH_2Cl_2 (3:1) (2 L × 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 mL × 3). The EtOAc-soluble portion (7.0 g) was repeatedly subjected to silica gel flash column chromatography (using increasing concentrations of MeOH in CH_2Cl_2 as eluent), followed by reversed-phase HPLC (70−80% MeOH) to give **1** (33.0 mg, 0.018% wet weight), **2** (3.6 mg, 0.002%), **3** (3.9 mg, 0.002%), and the known compound, siphonodiol (**4**) (123.1 mg, 0.068%).

Callypentayne (1): colorless oil; FT-IR (film) 3290, 2225 cm^{-1}; UV ($CHCl_3$) λ_{max} 241 (ϵ 26 000), 225 (ϵ 30 000), 269 (ϵ 40 000), 285 (ϵ 34 000) nm; ^1H NMR ($CDCl_3$) δ 6.05 (dt, 1H, J = 10.7, 7.4 Hz, H-9), 6.00 (ddt, 1H, J = 10.7, 0.8, 7.4 Hz, H-4), 5.48 (dt, 1H, J = 10.7, 1.1 Hz, H-10), 5.46 (ddt, 1H, J = 10.7, 2.2, 1.4 Hz, H-3), 3.09 (dd, 1H, J = 2.2, 0.8 Hz, H-1), 2.48 (dt, 2H, J = 0.8, 6.9 Hz, H-15), 2.42 (dt, 2H, J = 1.1, 6.9 Hz, H-17), 2.36 (m, 4H, H-5, 8), 1.99 (t, 1H, J = 1.1 Hz, H-21), 1.79 (quint, 2H, J = 6.9 Hz, H-16), 1.46 (m, 4H, H-6,7); ^{13}C NMR ($CDCl_3$) δ 147.7 (d, C-9), 145.7 (d, C-4), 108.3 (d, C-3), 108.2 (d, C-10), 82.9 × 2 (s, C-14,19), 81.3 (d, C-1), 80.4 (s, C-2), 77.9 (s, C-12), 76.8 (s, C-18), 72.5 (s, C-11), 68.2 (s, C-20), 66.2 (d, C-21), 65.5 (s, C-13), 30.5 (t, C-8), 30.0 (t, C-5), 28.2 (t, C-7), 28.1 (t, C-6), 26.7 (t, C-16), 18.7 (t, C-15), 18.2 (t, C-17); HREIMS m/z 272.1557, calcd for $C_{21}H_{20}$ m/z 272.1565; COSY (H/H) 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 9/10, 15/16, 16/17; HMBC (H/C) 1/3, 1/4, 3/1, 4/2, 9/11, 10/12, 10/13, 15/11, 15/12, 15/13, 15/14, 16/14, 16/18, 17/18, 17/19, 17/20, 17/21, 21/19.

Callyberyne B (2): colorless oil; FT-IR (film) 3290, 2230 cm^{-1}; UV ($CHCl_3$) λ_{max} 240 (ϵ 41 000), 225 (ϵ 39 000), 269 (ϵ 59 000), 286 (ϵ 47 000) nm; ^1H NMR ($CDCl_3$) δ 6.03 (dt, 1H, J = 10.7, 7.4 Hz, H-9), 5.988 (ddt, 1H, J = 10.7, 0.8, 7.4 Hz, H-18), 5.994 (ddt, 1H, J = 10.7, 0.8, 7.4 Hz, H-4), 5.50 (dt, 1H, J = 10.7, 1.2 Hz, H-10), 5.48 (ddt, 1H, J = 10.7, 2.2, 0.8 Hz, H-3), 5.46 (ddt, 1H, J = 10.7, 2.2, 0.8 Hz, H-19), 3.10 (dd, 1H, J = 2.2, 0.8 Hz, H-21), 3.08 (dd, 1H, J = 2.2, 0.8 Hz, H-1), 2.45 (ddt, 1H, J = 7.4, 1.1, 7.4 Hz, H-17), 2.37 (dt, 2H, J = 0.8, 7.4 Hz, H-15), 2.35 (m, 2H, H-5, 8), 1.69 (quint, 2H, J = 7.4 Hz, H-16), 1.46 (m, 4H, H-6, 7); ^{13}C NMR ($CDCl_3$) δ 147.5 (d, C-9), 145.8 (d, C-4), 144.3 (d, C-18), 109.3 (d, C-19), 108.33 (d, C-3), 108.28 (d, C-10), 84.2 (s, C-14), 81.8 (d, C-21), 81.3 (d, C-1), 80.5 (s, C-2), 80.2 (s, C-20), 78.2 (s, C-12), 72.2 (s, C-11), 65.6 (s, C-13), 30.5 (t, C-8), 30.0 (t, C-5), 28.4 (t, C-17), 28.22 (t, C-7), 28.15 (t, C-6), 27.5 (t, C-16), 19.2 (t, C-15); HREIMS m/z 274.1728, calcd for $C_{21}H_{22}$ m/z 274.1722; COSY (H/H) 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 9/10, 15/16, 16/17, 17/18, 18/19; HMBC (H/C) 1/3, 1/4, 4/2, 9/11, 10/12, 10/13, 15/11, 15/12, 15/13, 15/14, 16/14, 17/19, 17/20, 17/21, 18/20, 21/18, 21/19.

Callytetrayne (3): colorless oil; FT-IR (film) 3290, 2225 cm^{-1}; ($CHCl_3$) λ_{max} 239 (ϵ 13 000), 269 (ϵ 1000), 286 (ϵ 800) nm; ^1H NMR ($CDCl_3$) δ 5.99 (ddt, 1H, J = 10.7, 1.0, 7.4 Hz, H-18), 5.98 (ddt, 1H, J = 10.7, 0.8, 7.4 Hz, H-4), 5.49 (ddt, 1H, J = 10.7, 2.2, 1.4 Hz, H-3), 5.45 (ddt, 1H, J = 10.7, 2.2, 1.4 Hz, H-19), 3.09 (dd, 1H, J = 2.2, 0.8 Hz, H-1), 3.08 (dd, 1H, J = 2.7, 1.0 Hz, H-21), 2.43 (dt, 2H, J = 1.1, 7.4 Hz, H-5), 2.33 (dt, 2H, J = 1.1, 7.4 Hz, H-17), 2.29 (dt, 2H, J = 1.1, 7.4 Hz, H-7), 2.25 (dt, 2H, J = 1.1, 7.4 Hz, H-12), 1.66 (quint, 2H, J = 7.4 Hz, H-6), 1.52 (quint, 2H, J = 7.4 Hz, H-13), 1.42 (m, 2H, H-16), 1.41 (m, 2H, H-14), 1.33 (m, 2H, H-15);

^{13}C NMR ($CDCl_3$) δ 146.0 (d, C-18), 144.4 (d, C-4), 109.2 (d, C-3), 108.1 (d, C-19), 81.7 (d, C-1), 81.2 (d, C-21), 80.5 (s, C-20), 80.2 (s, C-2), 77.7 (s, C-11), 77.0 (s, C-8), 65.7 (s, C-9), 65.3 (s, C-10), 30.1 (t, C-17), 29.4 (t, C-5), 28.6 (t, C-15), 28.53 (t, C-14), 28.49 (t, C-16), 28.2 (t, C-13), 27.5 (t, C-6), 19.2 (t, C-12), 18.8 (t, C-7); HREIMS *m*/*z* 276.1876, calcd for $C_{21}H_{24}$, *m*/*z* 276.1878; COSY (H/H) 3/4, 4/5, 5/6, 6/7, 12/13, 13/14, 14/15, 15/16, 16/17, 17/18, 18/19; HMBC (H/C) 1/3, 1/4, 3/1, 5/2, 5/3, 6/8, 7/8, 7/9, 7/10, 7/11, 12/8, 12/9, 12/10, 12/11, 17/19, 17/20, 17/21, 18/20, 19/21, 21/18, 21/19.

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References and Notes

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(2) Tada, H.; Yasuda, F. *Chem. Lett.* **1984**, 779−780.
(3) The structures of callypentayne and callytetrayne were independently presented by us at the 43rd Annual Meeting of the Japanese Society of Pharmacognosy on Sep 4, 1996, in Japan (where they were called callyberynes A and C) and by Professor Fusetani and his collaborators at the 38th Symposium on the Chemistry of Natural Products on October 14, 1996, in Japan. A manuscript by Professor Fusetani describing the structures of callypentayne and callytetrayne was submitted to this journal shortly before this manuscript (*J. Nat. Prod.* **1997**, *60*, 126−130), and we have thus adopted his name for these two compounds to avoid confusion in the literature.
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