Callyspongynic Acid, a Polyacetylenic Acid Which Inhibits α-Glucosidase, from the Marine Sponge *Callyspongia truncata*[1]

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A new polyacetylenic acid, callyspongynic acid (**1**), was isolated as an α-glucosidase inhibitor from the marine sponge *Callyspongia truncata*. Its structure was determined by spectroscopic and chemical methods.

Callyspongia truncata is a pale violet, encrusting sponge that is a rich source of polyacetylenes with a variety of biological activities including antifouling,[2] larval metamorphosis inducing,[2] and inhibition of fertilization.[3] In our search for α-glucosidase inhibitors from Japanese marine invertebrates, the lipophilic extract of *C. truncata* collected off the Kii Peninsula, 450 km west of Tokyo, showed considerable activity. Bioassay-guided isolation afforded a new polyacetylenic acid, callyspongynic acid (**1**). This paper describes the isolation, structure determination, and bioactivity of this compound.

The EtOH extract of the frozen sponge (1 kg) was partitioned between H_2O and $CHCl_3$, and the active $CHCl_3$ layer was fractionated by the modified Kupchan procedure.[4] The $CHCl_3$ fraction was purified by ODS flash and silica gel chromatographies followed by ODS HPLC with aqueous MeCN to yield callyspongynic acid (**1**; 64.3 mg, 0.0064% yield based on wet weight).

The molecular formula of callyspongynic acid (**1**) was established as $C_{32}H_{38}O_3$ on the basis of HRFABMS data. The ^1H and ^{13}C NMR spectra demonstrated the presence of a shielded carboxyl group (δ_C 163.1) and a secondary alcohol (δ_H 4.75, δ_C 63.1), accounting for all three oxygen atoms. In addition to these signals, a disubstituted olefin (δ_H 5.85 and 5.56, δ_C 133.9 and 130.8), an allylic methylene (δ_H 2.08, δ_C 32.8), seven propargylic methylenes (δ_H 2.15, 2.23, 2.24, 2.24, 2.25, 2.28, and 2.36, δ_C 18.5, 18.5, 18.5, 18.8, 19.2, 19.3, and 19.6), eight methylenes, five disubstituted acetylenes (δ_C 66.3, 66.7, 77.1, 78.0, 79.0, 80.0, 80.1, 81.1, 81.3, and 83.3), and a terminal acetylene (δ_H 2.85, δ_C 74.5 and 84.7) were observed.

Interpretation of the COSY spectrum together with HSQC data resulted in four structural units, **a**−**d** (Figure 1). Unit **a** was composed of five contiguous methylenes (C-4 to C-8). The carbon chemical shifts of C-4 and C-8 (δ 19.2 and 19.3, respectively) indicated that they were linked to acetylenes. Units **b** (C-11 to C-13) and **c** (C-16 to C-18) were also linked to acetylenes, as judged from the carbon chemical shift values. Unit **d** (C-23 to C-32) contained a terminal acetylene, which was long-range coupled to the oxygenated methine (H-30), which was in turn correlated with a disubstituted *E*-olefin linked to a 1,5-disubstituted *n*-pentyl group.

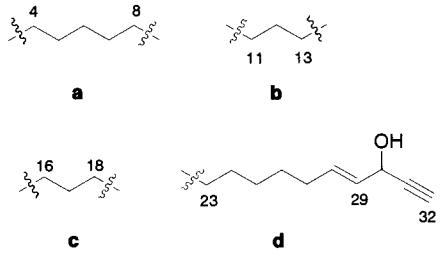

Figure 1. Partial structures of **1**.

Further structural analysis was carried out by interpretation of HMBC data. H-4 was correlated with three nonprotonated carbons (δ 163.1, 83.3, and 79.0), thus demonstrating the connectivities from C-1 to C-8. H-8 was further correlated with two acetylenic carbons (δ 81.3 and 80.1), both of which were in turn coupled to H-11 in unit **b**, thus establishing the connectivities from C-1 to C-13. H-12 and H-17 were correlated with carbons at δ 81.1 (C-14) and 80.0 (C-15), respectively, both of which were in turn correlated with H-13 and H-16, thereby allowing the connection of units **b** and **c** through an acetylene. H-18 exhibited cross-peaks with four acetylenic carbons, all of which were in turn correlated with H-23 of unit **d**. Therefore units **c** and **d** were connected through two acetylenic bonds.

The absolute stereochemistry at C-30 was determined by the modified Mosher method.[5] Callyspongynic acid (**1**) was hydrogenated followed by esterification with (*R*)- or (*S*)-MTPACl in pyridine to afford (30*S*)-MTPA ester and (30*R*)-MTPA ester, respectively.[6] Distribution of $\Delta\delta$ ($\delta_S - \delta_R$) values led to the 30*R*-stereochemistry.

Polyacetylenic acids of marine sponge origin show a wide variety of biological activities such as cytotoxic, antimicrobial, antifungal, and enzyme inhibition.[7−18] Callyspongynic acid (**1**) inhibited α-glucosidase with an IC_{50} value of 0.25 μg/mL, but was inactive against β-glucosidase, β-galactosidase, thrombin, and trypsin at 100 μg/mL. Since corticatic acid A[7] (**2**) and petrosynol[8] (**3**) were also inhibitory against α-glucosidase with IC_{50} values of 0.16 and 4.08 μg/mL, respectively, but the polyacetylene hydrocarbon callytetrayne[2] (**4**) and methyl callyspongynate were inactive even at 100 μg/mL, perhaps the carboxylic acid and the allylic alcohol linked to an acetylene are important for activity. C_{32} polyacetylenes were also reported from marine sponges of the genera *Adocia*,[9] *Pellina*,[10,11] *Petrosia*,[12] and *Xestospongia*,[13] which all belong to the order Haprosclerida.

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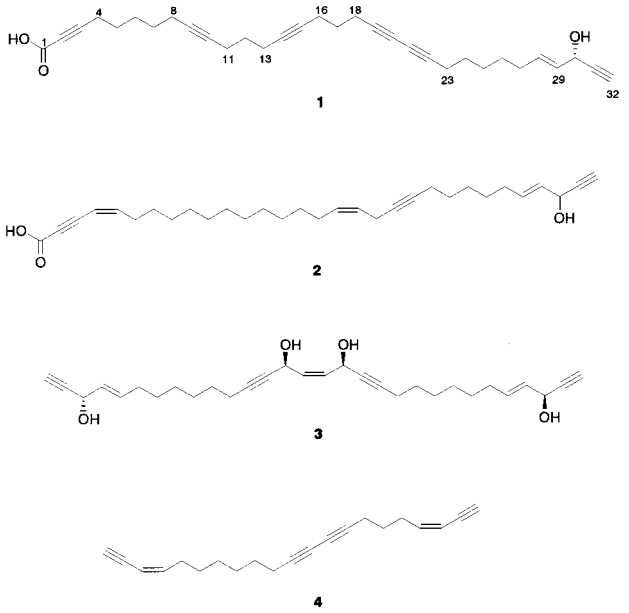
Interestingly, a C_{32} polyacetylene was also isolated from a *Theonella* sponge (order Lithistida).[14]



Experimental Section

General Experimental Procedures. The IR spectrum was measured on a JASCO FT/IR-5300 spectrometer. NMR spectra were recorded on a JEOL A600 NMR spectrometer. NMR chemical shifts were referenced to the residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, and δ_H 7.24 and δ_C 77.0 for $CDCl_3$). Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. FAB mass spectra were measured on a JEOL JMX-SX 102/SX 102 tandem mass spectrometer using PEG 600 sulfate as a marker and TEA as a matrix. UV spectra were recorded on a Shimadzu UV mini 1240 UV−vis spectrophotometer. TLC was visualized by spraying with anisaldehyde solution [EtOH/*p*-anisaldehyde/acetic acid/sulfulic acid (370:9.1:10:12.3)] followed by heating on a hot plate for 1 min. α-Glucosidase (Type V from yeast, G0660) and the substrate, *p*-nitrophenyl α-D-glucopyranoside (N-1377), were purchased from Sigma Chemical Co (St. Louis, MO).

Animal Material. Sponge specimens were collected at a depth of 5 m off the Kii Peninsula (33°53′ N, 135°4′ E), frozen immediately, and kept at −20 °C until processed. The sponge was identified as *Callyspongia truncata*; a voucher specimen was deposited at the Zoological Museum, University of Amsterdam, under code number ZMA POR 16717.

Extraction and Isolation. The frozen sponge (1.0 kg) was homogenized and extracted with EtOH. The extract was concentrated and partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was partitioned between *n*-hexane and MeOH/H_2O (9:1). The MeOH/H_2O (9:1) layer was diluted with H_2O to make it up to MeOH/H_2O (6:4), which was then extracted with $CHCl_3$. The $CHCl_3$ layer was separated by ODS flash chromatography with MeOH/H_2O (1:1 and 7:3), MeCN/H_2O (7:3 and 85:15), MeOH, and $CHCl_3$/MeOH/H_2O (7:3:0.5). The fractions eluted with MeOH/H_2O (1:1 and 7:3) were combined and fractionated on a silica gel open column with $CHCl_3$/MeOH (99:1, 98:2, 95:5, and 9:1) and $CHCl_3$/MeOH/H_2O (8:2:0.1, 7:3:0.5, 6:4:1, and 5:5:2). The active fraction was finally purified by ODS HPLC (MeCN/H_2O, 6:4 to 9:1, gradient elution) to afford callyspongynic acid (**1**) (64.3 mg, 0.0064% yield based on wet weight).

Callyspongynic acid (1): colorless oil; $[\alpha]_D$ +5.4 (*c* 0.5, EtOH); UV (MeOH) 204 nm (ϵ 6890); IR (film) ν_{max} 3290, 2935, 2858, 2235, 1687, 1566, 1429, 1377, 1275, 1089, 1016, 970, 738, 625 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; negative HRFABMS (PEG 600 sulfate/TEA as matrix) *m*/*z* 469.2747 $(M - H)^-$ [$C_{32}H_{37}O_3$; Δ +0.5 mmu].

Table 1. NMR Data of Callyspongynic Acid (**1**) in CD_3OD

no.	^1H [mult., *J* (Hz)]	^{13}C [mult]	HMBC
1		163.1 (s)	
2		79.0 (s)	
3		83.3 (s)	
4	2.28 (t, 6.9)	19.2 (t)	C-1, C-2, C-3, C-5
5	1.55 (m)	28.9 (t)	C-3,[a] C-4[a]
6	1.49 (m)	29.1 (t)	C-4[a]
7	1.48 (m)	29.7 (t)	C-8,[a] C-9[a]
8	2.15 (tt, 6.9, 2.1)	19.3 (t)	C-7, C-9, C-10
9		81.3 (s)	
10		80.1(s)	
11	2.23 (t, 7.0)	18.5 (t)	C-9,[a] C-10[a]
12	1.60 (quint, 7.0)	29.7 (t)	C-10, C-11,[a] C-13,[a] C-14
13	2.24 (t, 7.0)	18.5 (t)	C-11,[a] C-12,[a] C-14,[a] C-15[a]
14		81.1 (s)	
15		80.0 (s)	
16	2.24 (t, 6.9)	18.5 (t)	C-14,[a] C-15,[a] C-17[a]
17	1.65 (tt, 7.0, 6.9)	29.1 (t)	C-15, C-16,[a] C-19
18	2.36 (t, 7.1)	18.8 (t)	C-16, C-17, C-19, C-20, C-21, C-22
19		77.1 (s)	
20		66.7 (s)	
21		66.3 (s)	
22		78.0 (s)	
23	2.25 (t, 6.9)	19.6 (t)	C-19, C-20, C-21, C-22, C-24,[a] C-25[a]
24	1.52 (m)	29.1 (t)	C-22, C-23, C-25,[a] C-26[a]
25	1.42 (m)	29.3 (t)	C-23,[a] C-24,[a] C-27[a]
26	1.42 (m)	29.5 (t)	C-24,[a] C-25,[a] C-27,[a] C-28[a]
27	2.08 (br dt, 6.4, 6.2)	32.8 (t)	C-25,[a] C-26,[a] C-28, C-29
28	5.85 (ddt, 15.2, 1.2, 6.4)	133.9 (d)	C-26, C-27, C-30
29	5.56 (ddt, 15.2, 6.2, 1.7)	130.8 (d)	C-27, C-30, C-31
30	4.75 (br d, 6.2)	63.1 (d)	C-28, C-29, C-31, C-32
31		84.7 (s)	
32	2.85 (d, *J* = 2.3)	74.5 (d)	C-30, C-31

[a] Conclusive assignment was not made due to overlapped signals.

Enzyme Inhibition Assays. Enzyme inhibition assay was performed according to Cannell et al.[19] with some modification. To each well of microtiter plates were added 20 μL of test solution and 90 μL of enzyme solution (0.05 unit/mL, 0.1 M potasium phosphate, 3.2 mM MgCl, pH 6.8). After preincubation at 37 °C for 30 min, 90 μL of substrate solution (0.05 mg/mL, 0.1 M potassium phosphate, 3.2 mM MgCl, pH 6.8) was added to each well. The absorbance at 410 nm was measured after incubation at 37 °C for 10 min, using a TOSOH MPR-A4i II micro plate reader. IC_{50} values were defined as the concentration that inhibits 50% of α-glucosidase activity.

Preparation of MTPA Esters. Callyspongynic acid (**1**; 4 mg) was hydrogenated with 10% palladium carbon in MeOH at room temperature overnight. The reaction mixture was filtered and evaporated. Each half of the hydrogenated compound (2 mg) which revealed a $[M - H]^-$ ion peak at *m*/*z* 495 in FABMS was reacted with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic chloride (MTPACl) in dry pyridine, respectively. The reaction mixture was partitioned between H_2O and hexane. The hexane layer was purified by ODS HPLC with MeOH to give (*S*)- and (*R*)-MTPA esters, respectively.

(*S*)-MTPA Ester: ^1H NMR ($CDCl_3$, 600 MHz) δ 0.89 (3H, t, *J* = 7.7 Hz, H-32), δ 1.52 (2H, m, H-29), δ 1.58 (2H, q, *J* = 6.7 Hz, H-3), δ 1.64 (2H, m, H-31), δ 2.27 (2H, t, *J* = 7.5 Hz, H-2), δ 5.00 (1H, qt, *J* = 5.00, 6.54 Hz, H-30); FABMS (positive, NBA + NaCl) *m*/*z* 727 $(M + H)^+$, 749 $(M + Na)^+$.

(*R*)-MTPA Ester: ^1H NMR ($CDCl_3$, 600 MHz) δ 0.77 (3H, t, *J* = 7.5 Hz, H-32), δ 1.56 (2H, m, H-29), δ 1.58 (2H, m, H-3), δ 1.57 (2H, m, H-31), δ 2.27 (2H, t, *J* = 7.5 Hz, H-2), δ 5.00 (1H, qt, *J* = 5.39, 6.92 Hz, H-30); FABMS (positive, NBA + NaCl) *m*/*z* 727 $(M + H)^+$, 749 $(M + Na)^+$. Δδ $(\delta_S - \delta_R)$ values: H-32, +0.67; H-31, +0.11; H-30, +0.01; H-29, −0.04.

Preparation of Methyl Callyspongynate. Callyspongynic acid (**1**; 2 mg) was treated with diazomethane and evaporated. The reaction mixture was separated by a silica gel open column with $CHCl_3$, $CHCl_3$/MeOH (39:1, 19:1, and 9:1), and $CHCl_3$/MeOH/H_2O (8:2:0.1, 7:3:0.5, and 6:4:1), to give methyl callyspongynate, which showed a $(M + Na)^+$ ion peak at *m*/*z* 507 in positive FABMS (matrix, NBA + NaCl).

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