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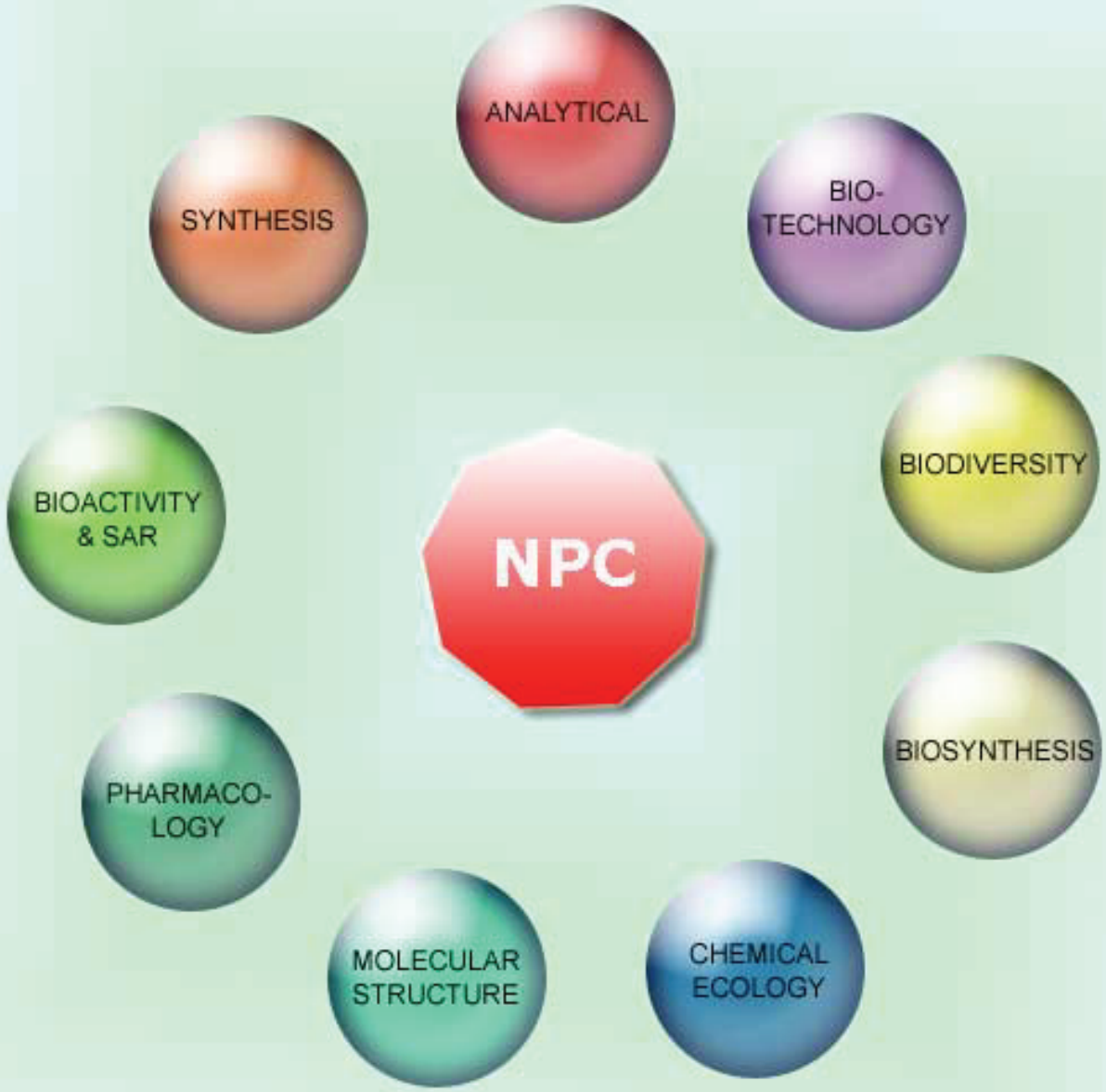

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A New Cytotoxic Polyacetylenic Alcohol from a Sponge *Callyspongia* sp.

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A new cytotoxic polyacetylenic alcohol **1** was isolated from an Indonesian sponge *Callyspongia* sp. The structure of compound **1** was elucidated by spectral analyses and by applying modified Mosher's method. Compound **1** killed the cultured NBT-II rat bladder carcinoma cells at 5 and 10 μg/mL.

Keywords: *Callyspongia*, Sponge, Polyacetylene, Cytotoxicity.

Polyacetylenes with linear carbon skeletons have been reported from both terrestrial and marine organisms. Among the marine-derived polyacetylenes, compounds vary in the level of unsaturation, carbon chain lengths, oxygenation patterns and terminal such as in polyacetylenic alcohols [1]. Particularly, marine sponges of the genera *Callyspongia*, *Theonella, Haliclona* and *Petrosia* produce polyacetylenic alcohols incorporating a 1-yn-3-ol terminal, with antiviral, antimicrobial, antitumor, cytotoxic, neurotogenic and α-glucosidase inhibiting activities [1-7]. Two reports have described this pharmacophore as playing key roles in antitumor and neurotogenic activities [8-9]. However, despite featuring the 1-yn-3-ol terminal and having been published for nearly two decades, very little is known about biological activity and structural variation of callyspongyne-type polyacetylenes [10].

In our collaborative project for finding new bioactive molecules from Indonesian marine organisms [11-12], we became interested in the extract of a *Callyspongia* sponge collected off Flores Island. The ^{1}H NMR of the extract showed characteristics of the structurally uncommon callyspongyne-type polyacetylenes [10]. The data prompted us to further examine the structure and bioactivity of the major fraction of the extract, leading to the discovery of a new and cytotoxic polyacetylenic alcohol **1** [13]. This note describes the structure elucidation and cytotoxicity of compound **1** (Figure 1).

Since ESIMS of compound **1** showed a sodiated ion at *m/z* 459.3605, the molecular formula was deduced as $C_{31}H_{48}O$ (Δ +0.4 ppm) with eight degrees of unsaturation. Spectral data indicated the presence of the following functional groups: two terminal and one internal acetylenes at δ_H 2.57, 3.07; δ_C 73.9, 79.5, 80.3, 80.8, 83.3, 81.4; 3280, 2115 cm^{-1}, two double bonds at δ_H 5.48, 5.61, 5.92, 6.01; δ_C 108.6, 128.3, 134.8, 145.2, one secondary alcohol at δ_H 4.84; δ_C 62.8; 3359 cm^{-1}, eight methylenes at δ_H 1.35~2.42, and a methylene chain at δ_H 1.27 brs; δ_C 29.4-29.7. As three acetylenes and two double bonds satisfied all the unsaturation degrees, the compound was suggested to be a new member of linear callyspongyne-type polyacetylenes.

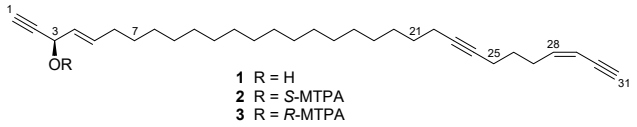

Figure 1: Structures for compounds **1-3**.

Table 1: ^{1}H and ^{13}C NMR Data for Compound **1** in $CDCl_3$.

		1		
Position	^{13}C	^{1}H	COSY	HMBC
1	73.9 CH	2.57 d, *J* = 2.4 Hz		C-3
2	83.3 C	-		
3	62.8 CH	4.84 brs	H-4	C-2,4,5
OH		1.83 brd, *J* = 6.0 Hz		C-2,3,4
4	128.3 CH	5.61 ddt, *J* = 15.2, 6.1, 1.4 Hz	H-3,5	C-2,3,6
5	134.8 CH	5.92 brdt, *J* = 15.2, 6.7 Hz	H-4,6	C-3,6,7
6	31.9 CH_2	2.06 brq, *J* = 7.0 Hz	H-5,7	C-4,5,7
7	28.8 CH_2[a]	1.38 m	H-6,8	C-5,6,8
8	29.1 CH_2[b]	1.27 brs		
9~19	29.4~7 CH_2	1.27 brs		
20	28.9 CH_2[a]	1.35 m		C-21
21	29.2 CH_2[b]	1.48 quint, *J* = 7.3 Hz	H-20,22	C-20,22,23
22	18.7 CH_2	2.13 tt, *J* = 7.3, 2.1 Hz	H-21,25	C-23,24
23	80.8 C	-		
24	79.5 C	-		
25	18.4 CH_2	2.18 tt, *J* = 7.3, 2.1 Hz	H-22,26	C-23,24,26,27
26	28.3 CH_2	1.61 quint, *J* = 7.3 Hz	H-25,27	C-24,25,27,28
27	29.7 CH_2	2.42 brq, *J* = 7.3 Hz	H-26,28	C-25,26,28,29,30
28	145.2 CH	6.01 dt, *J* = 11.0, 7.3 Hz	H-27,29	C-30
29	108.6 CH	5.48 brd, *J* = 11.0 Hz	H-28,31	C-27
30	80.3 C	-		
31	81.4 CH	3.07 d, *J* = 2.1 Hz	H-29	C-30

[a] and [b] indicate that the signals are exchangeable.

One of acetylenic protons at δ_H 2.57 showed an HMBC cross peak with the oxymethine at δ_C 62.8 (C-3), which proton at δ_H 4.84 showed correlations to C-2, 4 and 5 indicating that the alcohol is flanked by a terminal acetylene and a *trans* double bond (*J* = 15.2 Hz). The double bond is connected to methylenes at δ_C 31.9 and 28.8 (C-6 and 7). This moiety has been found in polyacetylenes from marine origins such as petrosynol [3], and the data was consistent with those reported for analogs [10].

Another terminal acetylenic signal at δ_H 3.07 (H-31) showed a COSY cross peak to one of *cis* olefinic protons at δ_H 5.48 (*J* = 11.0 Hz, H-29). The other *cis* vinyl proton at δ_H 6.01 showed a

correlation with a methylene at δ_H 2.42 (H-27), which in turn coupled to a characteristic methylene at δ_H 1.61 appearing as a quintet. This methylene showed COSY cross peaks to the neighbor methylenes at C-25 and 27, and also HMBC cross peaks to one of internal acetylenic carbons at δ_C 79.5 (C-24) in addition to methylenes at C-25 and 27, and an olefin at C-28. The proton signal at H-25 showed two coupling constants: one with J = 7.3 Hz for vicinal coupling to H-26 and the other J = 2.1 Hz for long range coupling with H-22. The signal at H-22 appeared similar to that of H-25, and COSY and HMBC supported the presence of H-20 and 21. The data for C21-C31 are consistent with those reported for analogous moieties [14,15].

Both the methylene groups at H-7 and H-20 showed HMBC correlations to overlapped methylenes at δ_C 28-29 suggesting that the remaining methylenes connect the above two terminal units.

For the sole chiral center at C-3, we applied modified Mosher's method [16] to form esters **2** and **3**. As results, $\Delta\delta_{2\text{-}3}$ values were +0.11, +0.05 and +0.04 for H-4, 5 and 6, while -0.04 for H-1 as shown (Figure 2). Therefore, C-3 was concluded to be *R* configuration and the whole structure of **1** and the MTPA esters (**2**, **3**) can be depicted as in Figure 1.

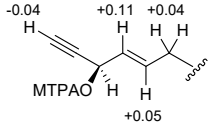

Figure 2: $\Delta\delta_{2\text{-}3}$ values of MTPA esters.

Compound **1** killed the cultured NBT-II rat bladder carcinoma cells at 5 and 10 μg/mL (Supplementary material).

Experimental

General: Reagent-grade solvents were used. HPLC separation was carried out on a unit of Hitachi HPLC instruments with a Cosmosil 5SL-II column. NMR spectra were measured on a Jeol Alpha 500 NMR spectrometer. ESI-MS were measured on a Micromass LCT instrument. FTIR spectra were obtained on a Jasco FTIR-300 spectrophotometer, respectively. Optical rotation was measured on a Jasco DIP-1000 polarimeter.

Sponge: A specimen of the purple soft sponge *Callyspongia* sp. was collected by hand using SCUBA at a depth of 43 m off Labuhan Bajo, Flores, Indonesia in August, 2001. The specimen (01Z25) was examined by one of us (NJdV).

Extraction and Isolation: The specimen (0.95 g after drying) was frozen at the collection site. After brought back to laboratory, it was extracted with acetone (150 mL) three times. The lipophilic portion (0.23 g) was subjected to a silica gel column to give seven fractions. The first fraction (38.6 mg) was purified on a silica HPLC column (Cosmosil 5SL-II, 6 x 250 mm) eluted with *n*-hexane-EtOAc (3-1) to give five subfractions. The third subfraction (22.8 mg) was further purified by silica HPLC (Cosmosil 5SL-II, 6 x 250 mm) with *n*-hexane-EtOAc (6-1, 4.0 mL/min) to give compound **1** (7.3 mg, eluted at 7.02 min).

Compound 1
White powder.
$[\alpha]_D^{25}$: -7.9 (*c* 0.046, $CHCl_3$).
IR (KBr): 3359, 3035, 2921, 2354, 1668 cm^{-1}.
^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$): Table 1.
ESIMS: *m/z* 675, 490, 413, 360, 304, 241, 185.
HRESIMS: *m/z* 459.3605 $[M+Na]^+$; calcd for $C_{31}H_{48}ONa$: 459.3603.

MTPA esters 2 and 3 from compound 1: A solution of compound **1** (0.6 mg, 0.0013 mmol) in 50 μL of dry CH_2Cl_2 was added to a stirring solution of (–)-α-methoxy-α-(tryfluoromethyl)phenyl acetic acid (*S*-MTPA) (1.0 mg, 0.0042 mmol), dicyclohexylcarbodiimide (DCC) (0.5 mg, 0.0024 mmol) and 4-dimethylaminopyridine (DMAP) (0.1 mg, 0.0008 mmol) in CH_2Cl_2. The mixture was allowed to stand for 12 h at room temperature. The reaction mixture was filtered off and the filtrate was purified by HPLC (*n*-hexane-EtOAc, 3-1) to give *S*-MTPA ester **2** (0.7 mg, 0.0010 mmol, 78%). Using (+)-α-methoxy-α-(tryfluoromethyl)phenyl acetic acid (*R*-MTPA) in place of *S*-MTPA, *R*-MTPA ester was also similarly prepared from 0.6 mg of compound **1**, resulting in **3** (1.0 mg, 0.0015 mmol, 89%).

***S*-MTPA ester 2**
White powder.
$[\alpha]_D^{25}$: -196 (*c* 0.007, $CHCl_3$).
^1H NMR ($CDCl_3$): δ 7.26-7.45 (5H, m), 6.07 (1H, m, H-5), 6.02 (1H, m, H-3), 6.01 (1H, m, H-28), 5.61 (1H, dd, J = 16.5, 6.5 Hz, H-4), 5.48 (1H, m, H-29), 3.07 (1H, d, J = 2.5 Hz, H-31), 2.59 (1H, d, J = 2.0 Hz, H-1), 2.42 (2H, brq, J = 15.0, 8.0 Hz, H-27), 2.18 (2H, m, H-25), 2.13 (2H, m, H-22), 2.08 (2H, q, J = 7.0 Hz, H-6), 1.61 (2H, quint, J = 7.0 Hz, H-21), 1.48 (2H, m, H-21), 1.38 (2H, m, H-7), 1.26 (26H, m, H-8~H-20).
HRESIMS: *m/z* 675.4028 $[M+Na]^+$; calcd for $C_{41}H_{55}F_3O_3Na$: 675.4001.

***R*-MTPA ester 3**
White powder.
$[\alpha]_D^{25}$: -116 (*c* 0.010, $CHCl_3$).
^1H NMR ($CDCl_3$): δ 7.26-7.45 (5H, m), 6.03 (1H, m, H-3), 6.02 (1H, m, H-5), 6.01 (1H, m, H-28), 5.50 (1H, m, H-4), 5.48 (1H, m, H-29), 3.07 (1H, d, J = 2.5 Hz, H-31), 2.63 (1H, d, J = 2.0 Hz, H-1), 2.42 (2H, brq, J = 13.5, 7.5 Hz, H-27), 2.18 (2H, m, H-25), 2.13 (2H, m, H-22), 2.04 (2H, q, J = 7.0 Hz, H-6), 1.61 (2H, quint, J = 7.0 Hz, H-26), 1.48 (2H, m, H-21), 1.38 (2H, m, H-7), 1.26 (26H, m, H-8~H-20).
HRESIMS: *m/z* 675.4025 $[M+Na]^+$; calcd for $C_{41}H_{55}F_3O_3Na$: 675.4001.

Preliminary cytotoxicity test: Suspensions of NBT-II cells in 1 mL DMEM (Dulbecco's Modified Eagle Medium) were dispensed into 24 wells. After preincubation for 24 h, DMSO (Dimethylsulfoxide) solution of compound **1** was added to each well to adjust the final concentration at 5 and 10 μg/mL. After 48 h incubation, each well was observed under a microscope.

Supplementary data: ^1H, ^{13}C and 2D NMR spectra of compound **1** and cell assay images are available.

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