Tetrahedron 65 (2009) 5204–5208

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Cytotoxic polyacetylenes related to petroformyne-1 from the marine sponge *Petrosia* sp.

Reiko Ueoka [a], Yuji Ise [b], Shigeki Matsunaga [a,*]

[a] *Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*
[b] *Misaki Marine Biological Station, The University of Tokyo, Miura, Kanagawa 238-0225, Japan*

ARTICLE INFO

Article history:
Received 11 April 2009
Received in revised form 25 April 2009
Accepted 29 April 2009
Available online 7 May 2009

Keywords:
Petroformyne
Sponge
Isolation
NMR

ABSTRACT

Four polyacetylenes related to petroformyne-1 were isolated from the marine sponge *Petrosia* sp. Their structures were determined on the basis of spectroscopic data and the modified Mosher analysis. They exhibit cytotoxic activity against P388 murine leukemia cells.

1. Introduction

Long-chain polyacetylenes are characteristic metabolites in the marine sponge of the genus *Petrosia*.[1] They exhibit potent cytotoxic activity in general. Petroformynes, which represent this class of metabolites, were isolated from the Mediterranean *Petrosia ficiformis*.[2] Recently, closely related polyacetylenes with the same lengths but different arrangements of the same sets of functional groups were isolated from the Korean *Petrosia* sp.[3]

In the course of our search for cytotoxic constituents from marine invertebrates, we isolated four new polyacetylenes from the marine sponge *Petrosia* sp. collected at Kurose Hole, 30 km north of Hachijo Island. Their structures were elucidated on the basis of NMR and MS/MS data. This paper describes the isolation, structure elucidation, and biological activities of these compounds, and suggests the necessity to reexamine the structure of petroformyne-1.

2. Results and discussion

The organic layer of the extract of the sponge was dried and subjected to the modified Kupchan procedure[4] to yield 60% MeOH, $CHCl_3$, and *n*-hexane layers. The $CHCl_3$ layer was separated by ODS flash chromatography, silica gel open column chromatography, and reversed-phase HPLC to give neopetroformynes A–D (**1**–**4**).

Neopetroformyne A (**1**) had a molecular formula of $C_{46}H_{68}O_3$, which was suggested by HRFABMS [*m*/*z* 691.5070, $(M+Na)^+$, Δ +0.3 mmu]. Analysis of the ^1H NMR data in conjunction with the HSQC spectrum revealed the presence of two acetylenic protons, ten sp^2 methines, three oxygenated methines, and a number of methylenes. The ^{13}C NMR spectrum further showed the presence of six acetylenic carbons without hydrogen. Partial structures **a**–**c** with unit **a** duplicating were deduced from the COSY data, and confirmed by the HMBC data (Fig. 1). The locations of units **b** and **c** in the alkyl chain were determined by an analysis of the FABMS/MS data for **1** (Fig. 2a). An intense product ion at *m*/*z* 306 was ascribed to the C-1 to C-19 fragment with a proton shift.[5] The $[M+Na]^+$ ion was selected as the precursor ion. Analysis of the product ions indicated that unit **a** and unit **b** were connected via one methylene, unit **b** and unit **c** were connected via one methylene, and unit **c** and unit **a** were connected via ten methylenes. Connection between unit **a** and unit **b** was supported by the TOCSY correlations between H-6 and H-10 and between H-6 and H-11.

The geometries of the Δ^4-, Δ^{17}- and Δ^{42}-olefines were determined as *E* on the basis of a coupling constant of 15.4 Hz, 15.7 Hz, and 15.4 Hz between the olefinic protons, respectively. The *Z*-geometries of the Δ^{21}- and Δ^{27}-olefines were assigned on the basis of the chemical shifts of allylic carbons.[6]

The absolute stereochemistry of **1** was determined by the modified Mosher method[7] applied to the three hydroxyl groups (Fig. 3a). Treatment of **1** with *R*-(−)- or *S*-(+)-MTPACl yielded (*S*)- or (*R*)-MTPA esters **5** and **6**, respectively. The $\Delta\delta$ values indicated the 3*S*,14*S*,44*S* configuration.

* Corresponding author. Tel.: +81 3 5841 5297; fax: +81 3 5841 8166.
E-mail address: assmats@mail.ecc.u-tokyo.ac.jp (S. Matsunaga).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.04.091

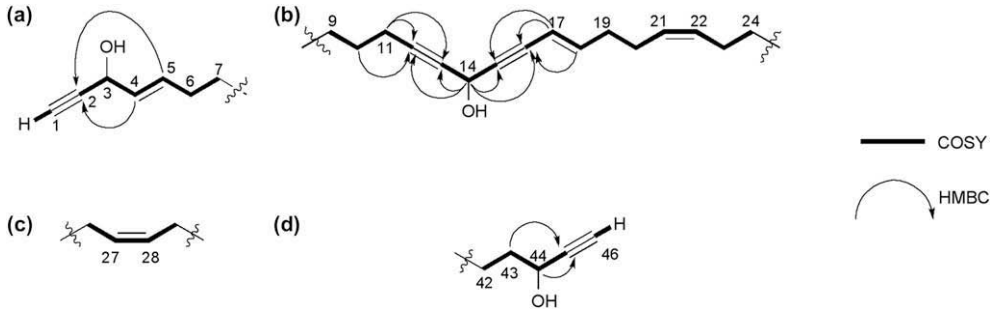

Figure 1. Partial structures **a**–**c** for **1** and partial structure **d** for **3**.

Neopetroformyne B (**2**) had a molecular formula of $C_{45}H_{66}O_3$, which was established by HRFABMS [*m*/*z* 677.4914, $(M+Na)^+$, Δ +0.4 mmu]. The ^1H and ^{13}C NMR spectra were almost identical with those of **1**, indicating that **2** is a lower homologue of **1**: the same partial structures (**a**×2, **b**, and **c**) were deduced from the 2D NMR data. The planar structure of **2** was determined by FABMS/MS analysis (Fig. 2b). The alkyl chain between unit **c** and unit **a** was shorter than that of **1** by one methylene unit. The geometries of olefins were determined by analysis of ^1H–^1H coupling constants and ^{13}C chemical shifts of allylic carbons as described above. The absolute stereochemistry was assigned as 3*S*,14*S*,43*S*, because the ^1H NMR spectrum of the (*S*)-MTPA ester **7** of **2** was almost superimposable on that of **5**.

Neopetroformyne C (**3**) had a molecular formula of $C_{46}H_{70}O_3$, which was established by HRFABMS [*m*/*z* 693.5228, $(M+Na)^+$, Δ +0.5 mmu], suggesting one less unsaturation than **1**. The ^1H NMR data suggested the replacement of one of units **a** in **1** by unit **d** (Fig. 1). The planar structure of **3** was assigned by the FABMS/MS data (Fig. 2c). The intense product ion at *m*/*z* 306 suggested that the C-1 to C-19 portion was conserved in **3**. The tandem MS data demonstrated that the Δ^{42}-olefin in **1** was saturated in **3**. The geometries of the olefins were determined as described for **1**. The absolute stereochemistry was assigned as 3*S*,14*S*,44*S* by applying the modified Mosher method to the (*S*)- and (*R*)-MTPA esters (**8** and **9**, respectively) (Fig. 3b).

Neopetroformyne D (**4**) had a molecular formula of $C_{45}H_{66}O_4$, which was established by HRFABMS [*m*/*z* 693.4862, $(M+Na)^+$, Δ +0.3 mmu]. The ^1H NMR spectrum displayed one additional oxygenated methine signal instead of a propargylic methylene in **2**. Partial structures **a** (×2) and **c** were deduced from the 2D NMR data. C-11 in partial structure **b** was oxidized to a secondary alcohol. Tandem FABMS data afforded an intense ion at *m*/*z* 322, in agreement with oxidation at C-11 (Fig. 2d). All the other product ions were larger than the corresponding product ions of **2** by 16 u, indicating that **4** differed from **2** only in the oxidation state of C-11. The geometries of the olefins were determined as described for **1**. The absolute stereochemistry was assigned as 3*S*,43*S* by comparison of the ^1H NMR spectrum of the (*S*)-MTPA ester **10** with that of **5**. However, the absolute stereochemistry of C-11 and C-14 was not assigned due to the paucity of the material.

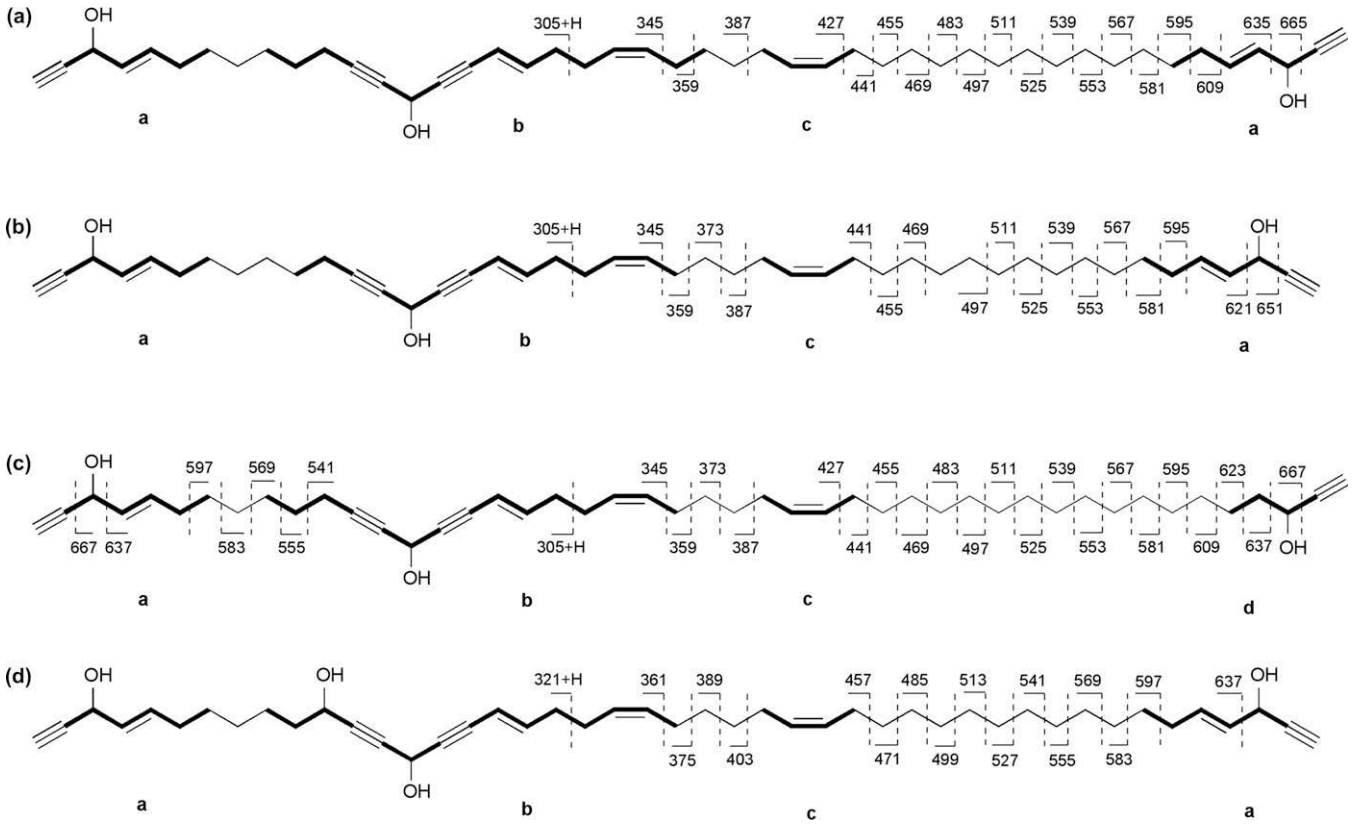

Figure 2. a)–d) The structures and FABMS/MS data of **1**–**4** from the $[M+Na]^+$ ion, respectively.

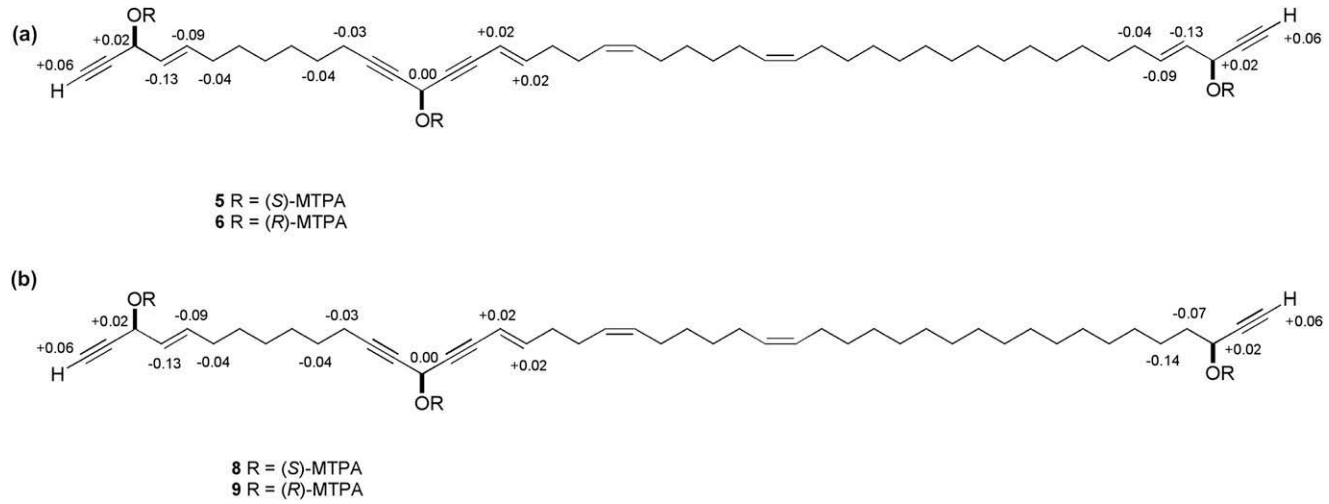

Figure 3. $\Delta\delta_{S-R}$ values for the MTPA esters of **1** and **3**.

Neopetroformyne A–D (**1**–**4**) exhibit cytotoxic activity against P388 murine leukemia cells with IC_{50} values of 0.089 μg/mL, 0.2 μg/mL, 0.45 μg/mL, and 0.45 μg/mL, respectively.

3. Conclusion

Neopetroformyne A has the same molecular formula as petroformyne-1 as well as the four sets of partial structures (Fig. 4). The structure of petroformyne-1 was assigned by analysis of ozonolysis products and EIMS data of the tri-TMS ether. The NMR data of neopetroformyne A were indistinguishable from those of petroformyne-1 and the same products are expected to be formed by ozonolysis of the two compounds. We consider it necessary to procure the FABMS/MS data of petroformyne-1 in order to confirm the proposed structure.[8]

4. Experimental section

4.1. General procedures

Optical rotations were measured on a JASCO DIP-1000 digital polarimeter in MeOH. NMR spectra were recorded on a JEOL delta 600 NMR spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks at δ_H 3.31 and δ_C 49.15 for CD_3OD, and at δ_H 7.27 and δ_C 77.23 for $CDCl_3$. FAB mass spectra were measured on a JMS-700 T mass spectrometer.

4.2. Animal material

The sponge *Petrosia* sp. was collected by dredging at a depth of 150 m at Kurose Hole, 30 km north of Hachijo island (33°21′N; 139°40′E), in 2007, immediately frozen, and kept at −20 °C until used. The voucher specimen was deposited at the Misaki Marine Biological Station, The University of Tokyo.

4.3. Extraction and isolation

The sample (600 g) was extracted with MeOH (2×3 L) and EtOH (1×3 L), and the extracts were combined and concentrated in vacuo. The residue was suspended in H_2O (500 mL) and extracted with $CHCl_3$ (3×500 mL) and *n*-BuOH (2×500 mL). The $CHCl_3$ extract was partitioned between 90% MeOH and *n*-hexane. The 90% MeOH layer was diluted with H_2O to yield a 60% MeOH solution and then extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated and separated by ODS flash chromatography to give six fractions (A–F). The fraction E (100% MeOH fraction) was separated by silica gel open column chromatography to give 14 fractions (A′–N′). The active fraction C′ (*n*-hexane/EtOAc (7:3) fraction) was further separated by reversed-phase HPLC (COSMOSIL $5C_{18}$-AR-II, 20×250 mm) with 60% 1-PrOH to give 20.1 mg of neopetroformyne A (**1**), 1.2 mg of neopetroformyne B (**2**), and the active fraction A″. The active fraction A″ was purified by reversed-phase HPLC (Phenomenex 5-Phenylhexyl, 10×250 mm) with 60% 1-PrOH to give 0.3 mg of neopetroformyne C (**3**). The active fraction E′ (*n*-hexane/EtOAc (6:4) fraction) was separated by reversed-phase HPLC (COSMOSIL $5C_{18}$-AR-II, 10 x 250 mm) with 60% 1-PrOH to give 0.2 mg of neopetroformyne D (**4**).

*4.3.1. Neopetroformyne A (**1**)*

Yellowish oil; $[\alpha]_D^{20.5}$ +19 (*c* 0.45, MeOH); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRFABMS *m*/*z* 691.5070 (calcd for $C_{46}H_{68}O_3Na$, 691.5067).

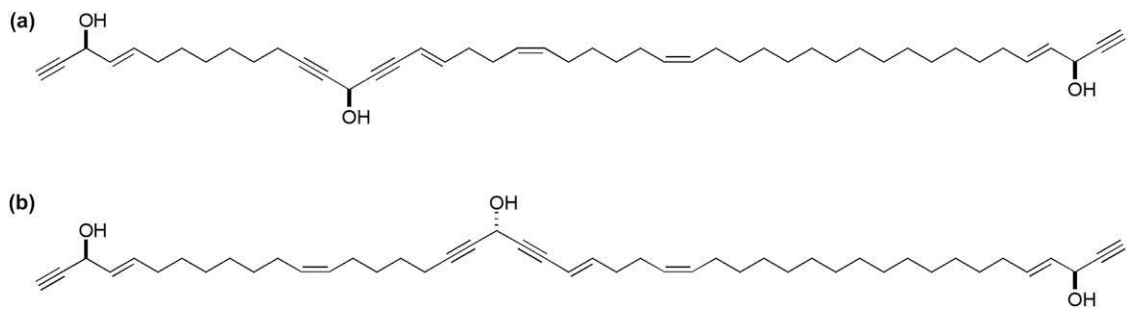

Figure 4. The structures of a) **1** and b) petroformyne-1.

Table 1
^{1}H and ^{13}C NMR data for Neopetroformyne A–D (**1**–**4**) in CD_3OD

No.	**1**		**2**		**3**		**4**	
	δ_H, mult.	δ_c	δ_H, mult.	δ_c	δ_H, mult.	δ_c	δ_H, mult.	δ_c
1	2.86[b] s	74.7	2.87 br	74.7	2.87 s	74.7	2.86 br	74.5
2		84.9		84.9		84.8		84.5
3	4.75 (d, 6.1)	63.3	4.75 (d, 6.0)	63.3	4.75 (d, 5.9)	63.3	4.75 (d, 5.5)	63.0
4	5.55 m	130.7[c]	5.55 m	130.8[b]	5.56 m	130.9[b]	5.56 m	130.5
5	5.85 (dt, 7.2, 15.4)	134.2[d]	5.85 (dt, 7.2, 15.6)	134.2[c]	5.85 (dt, 7.2, 15.6)	134.2	5.85 (dt, 7.2, 15.6)	134.2
6	2.07 m	33.1[e]	2.07 m	33.1[d]	2.08 m	33.1	2.08 m	32.7
7	1.43 m	29.8	1.43 m	29.8	1.43 m	29.8	1.43 m	29.8
8	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]
9	1.44 m	29.0–32.0[a]	1.43 m	29.0–32.0[a]	1.44 m	29.0–32.0[a]	1.48 m	26.2
10	1.52 (quint, 7.2)	29.7	1.52 (quint, 7.3)	29.7	1.52 (quint, 7.1)	29.7	1.67 m	38.7
11	2.23 br t	19.5	2.23 br t	19.4	2.23 br t	19.4	4.34 br t	62.7
12		85.1		85.1		85.1		85.7
13		79.5		79.5		79.5		82.6
14	5.14 br s	53.0	5.14 br s	53.0	5.14 br s	53.0	5.21 br s	52.7
15		87.2		87.2		87.2		86.7
16		82.7		82.7		82.7		82.8
17	5.53 m	110.6	5.53 m	110.6	5.54 m	110.6	5.54 m	110.2
18	6.14 (dt, 6.7, 15.7)	146.1	6.14 (dt, 6.7, 16.0)	146.1	6.14 (dt, 6.4, 15.5)	146.1	6.15 (dt, 6.4, 16.0)	146.1
19	2.17 m	34.3	2.17 m	34.3	2.17 m	34.3	2.17 m	34.2
20	2.16 m	27.7	2.16 m	27.7	2.16 m	27.7	2.16 m	27.5
21	5.35 m	129.6	5.35 m	129.6	5.34 m	129.6	5.34 m	129.2
22	5.40 m	131.9	5.40 m	131.9	5.40 m	131.9	5.40 m	131.7
23	2.05 m	28.2[f]	2.04 m	28.1[e]	2.05 m	28.2[c]	2.04 m	28.2
24–25	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]
26	2.05 m	28.2[f]	2.04 m	28.2[e]	2.05 m	28.2[c]	2.04 m	28.2
27	5.35 m	130.9[c]	5.35 m	130.9[b]	5.36 m	130.9[b]	5.35 m	130.8
28	5.35 m	131.1[c]	5.35 m	131.1[b]	5.36 m	131.1[b]	5.35 m	130.8
29	2.05 m	28.3[f]	2.04 m	28.3[e]	2.05 m	28.3[c]	2.04 m	28.2
30–38	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]	1.30–1.39[a] m	29.0–32.0[a]
39	1.30–1.39[a] m	29.0–32.0[a]	1.43 m	29.8	1.30–1.39[a] m	29.0–32.0[a]	1.43 m	29.8
40	1.43 m	29.8	2.07 m	33.2[d]	1.30–1.39[a] m	29.0–32.0[a]	2.08 m	32.7
41	2.07 m	33.2[e]	5.85 (dt, 7.2, 15.6)	134.3[c]	1.30–1.39[a] m	29.0–32.0[a]	5.85 (dt, 7.2, 15.6)	134.2
42	5.85 (dt, 7.2, 15.4)	134.3[d]	5.55 m	130.9[b]	1.46 m	26.5	5.56 m	130.5
43	5.55 m	130.8[c]	4.75 (d, 6.0)	63.3	1.65 m	39.1	4.75 (d, 5.5)	63.0
44	4.75 (d, 6.1)	63.3		84.9	4.27 (t, 6.6)	62.8		84.5
45		84.9	2.87 br	74.7		86.3	2.86 br	74.5
46	2.87[b] s	74.7			2.76 s	73.5		

[a] ^{1}H and ^{13}C chemical shifts were overlapped.
[b] Assignments may be interchanged.
[c] Assignments may be interchanged.
[d] Assignments may be interchanged.
[e] Assignments may be interchanged.
[f] Assignments may be interchanged.

*4.3.2. Neopetroformyne B (**2**)*

Yellowish oil; $[\alpha]_D^{20.2}$ +21 (*c* 0.06, MeOH); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRFABMS *m*/*z* 677.4914 (calcd for $C_{45}H_{66}O_3Na$, 677.4910).

*4.3.3. Neopetroformyne C (**3**)*

Colorless oil; $[\alpha]_D^{22.0}$ −15 (*c* 0.015, MeOH); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRFABMS *m*/*z* 693.5228 (calcd for $C_{46}H_{70}O_3Na$, 693.5223).

*4.3.4. Neopetroformyne D (**4**)*

Colorless oil; $[\alpha]_D^{21.4}$ +20 (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data, see Table 1; HRFABMS *m*/*z* 693.4862 (calcd for $C_{45}H_{66}O_4Na$, 693.4859).

4.4. Assay for the cytotoxicity against P388 cells

Cytotoxicity was determined as described.[9]

4.5. Preparation of MTPA esters

To a solution of the compound (**1**: 0.5 mg, **2**: 0.2 mg, **3**: 0.1 mg, **4**: 70 μg) in CH_2Cl_2 (100 μL) was added (*R*)-MTPACl (5 μL) and DMAP (1 mg) and the mixture was left at rt for 5 min. The mixture was partitioned between 0.1 M $NaHCO_3$ and $CHCl_3$, and the $CHCl_3$ layer was successively washed with 0.1 N HCl and H_2O. The organic layer was concentrated and separated by preparative TLC to afford the (*S*)-MTPA ester. A (*R*)-MTPA ester was prepared in the same way.

Acknowledgements

We thank Y. Ohfune and R. Miyake, Osaka City University, for the measurement of tandem FABMS. We are grateful to the officers and crew of R/V Tansei-maru of JAMSTEC for collection of the sponge specimen.

Supplementary data

NMR spectra for compounds **1**–**10** are available free of charge. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.04.091.

References and notes

1. Minto, R. E.; Blacklock, B. J. *Prog. Lipid Res.* **2008**, *47*, 233–306.
2. (a) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *Tetrahedron Lett.* **1989**, *30*, 3563–3566; (b) Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *Tetrahedron* **1994**, *50*, 13261–13268; (c) Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *J. Nat. Prod.* **1995**, *58*, 712–722; (d) Guo, Y.; Gavagnin, M.; Salierno, C.; Cimino, G. *J. Nat. Prod.* **1998**, *61*, 333–337.

3. (a) Seo, Y.; Cho, K. W.; Rho, J.-R.; Shin, J. *Tetrahedron* **1998**, *54*, 447–462; (b) Kim, J. S.; Im, K. S.; Jung, J. H.; Kim, Y.-L.; Kim, J.; Shim, C. J.; Lee, C.-O. *Tetrahedron* **1998**, *54*, 3151–3158; (c) Shin, J.; Seo, Y.; Cho, K. W. *J. Nat. Prod.* **1998**, *61*, 1268–1273; (d) Kim, J. S.; Lim, Y. J.; Im, K. S.; Jung, J. H.; Shim, C. J.; Lee, C. O.; Hong, J.; Lee, H. *J. Nat. Prod.* **1999**, *62*, 554–559; (e) Lim, Y. J.; Kim, J. S.; Im, K. S.; Jung, J. H.; Lee, C.-O.; Hong, J.; Kim, D.-K. *J. Nat. Prod.* **1999**, *62*, 1215–1217; (f) Lim, Y. J.; Park, H. S.; Im, K. S.; Lee, C.-O.; Hong, J.; Lee, M.-Y.; Kim, D.-K.; Jung, J. H. *J. Nat. Prod.* **2001**, *64*, 46–53; (g) Lim, Y. J.; Lee, C.-O.; Hong, J.; Kim, D.-K.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2001**, *64*, 1565–1567.
4. Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178–179.
5. Okamoto, C.; Nakao, Y.; Fujita, T.; Iwashita, T.; van Soest, R. W. M.; Fusetani, N.; Matsunaga, S. *J. Nat. Prod.* **2007**, *70*, 1816–1819.
6. Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *J. Nat. Prod.* **1990**, *53*, 345–353.
7. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
8. We were not able to compare the tandem FABMS data, because petroformyne-1 was no more available at Istituto per la Chimica di Molecole d'Interesse Biologico del CNR.
9. Ueoka, R.; Nakao, Y.; Fujii, S.; van Soest, R. W. M.; Matsunaga, S. *J. Nat. Prod.* **2008**, *71*, 1089–1091.