ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/5860606

Cytotoxic C 47 -Polyacetylene Carboxylic Acids from a Marine Sponge Pertrosia sp.

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · DECEMBER 2007

Impact Factor: 3.8 · DOI: 10.1021/np0702943 · Source: PubMed

CITATIONS
13

READS
46

7 AUTHORS, INCLUDING:

Yoichi Nakao
Waseda University
93 PUBLICATIONS **1,594** CITATIONS
SEE PROFILE

Tsuyoshi Fujita
National Institute for Environmental Studies
155 PUBLICATIONS **1,339** CITATIONS
SEE PROFILE

Rob van Soest
Naturalis Biodiversity Center
536 PUBLICATIONS **7,815** CITATIONS
SEE PROFILE

Nobuhiro Fusetani
Fisheries and Oceans Hakodate
412 PUBLICATIONS **12,290** CITATIONS
SEE PROFILE

Available from: Nobuhiro Fusetani
Retrieved on: 24 January 2016

Cytotoxic C_{47}-Polyacetylene Carboxylic Acids from a Marine Sponge *Pertrosia* sp.

Chikane Okamoto,[†] Yoichi Nakao,[†] Tsuyoshi Fujita,[‡] Takashi Iwashita,[‡] Rob W. M. van Soest,[§] Nobuhiro Fusetani,[†,⊥] and Shigeki Matsunaga*[,†]

Laboratory of Aquatic Natural Products Chemistry, Garaduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyyo-ku, Tokyo 113-8657, Japan, Suntory Institute for Bioorganic Research, 1-1 Wakayamadai, Shimamoto-cho, Osaka 618-0024, Japan, and Zoological Museum of the University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

Received June 20, 2007

Two new polyacetylene carboxylic acids, petroformynic acids B (**3**) and C (**4**), were isolated from a marine sponge *Pertrosia* sp. as cytotoxic constituents. Their structures were determined by interpretation of 2D NMR data and tandem FABMS data. Absolute stereochemistry of **3** was assigned by application of the modified Mosher analysis. Petroformynic acids exhibit moderate cytotoxic activity against P388 cells.

One of the most intriguing classes of sponge metabolites is the C_{46}-linear polyacetylenes named petroformynes, which were isolated from the Mediterranean *Petrosia ficiformis*.[1] Representative members of this class are petroformyne 1 (**1**), a triol with four triple bonds and five double bonds, and petroformyne 4 (**2**), a diol with four triple bonds and four double bonds: other members are either their oxidation products, geometrical isomers, or hydrogenated products at the olefinic bond(s).[2–4] Recently, closely related metabolites differing in the arrangement of functional groups were isolated from a Korean *Petrosia* sp.[5–8]

In the course of our screening for cytotoxic activity from the extracts of Japanese marine invertebrates, a *Petrosia* sp. collected off Katsuo-jima Island, Wakayama Prefecture, showed significant activity. Bioassay-guided fractionation of the extract afforded petroformynic acids A (**3**) and B (**4**), linear C_{47} polyacetylenes terminating in a carboxylic acid group.

The combined organic extracts of the sponge were subjected to a solvent partitioning scheme to afford the cytotoxic 90% MeOH fraction. This material was separated by ODS flash column chromatography followed by silica gel column chromatography and ODS HPLC to furnish petroformynic acid B (**3**, 25 mg, 5.0×10^{-2}% yield based on wet weight) and petroformynic acid C (**4**, 30 mg, 6.0×10^{-2}% yield) as the predominant cytotoxic constituents.[9]

Petroformynic acid B (**3**) has a molecular formula of $C_{47}H_{68}O_4$ as established by HRFABMS. The ^{1}H NMR spectrum contained signals for two oxygenated methines (δ 4.74 and 5.13), one acetylenic proton (δ 2.86), and 10 olefinic protons [δ 5.34, 5.37 (2H), 5.39, 5.53 (2H), 5.56, 5.85, 6.05, and 6.14]. The ^{13}C NMR spectrum showed the presence of one carboxylic acid (δ 161.9), 10 sp^2 carbons [δ 109.0, 112.4, 129.4, 130.7, 130.9 (2C), 131.7, 134.1, 145.9, and 147.7], two shielded oxygenated methines (δ 52.9 and 63.2), and eight acetylenic carbons (δ 74.5, 77.4, 79.4, 82.6, 84.8, 85.0, 87.1, and 91.6) together with the methylene envelope. The chemical shift of 52.9 ppm is characteristic of a doubly propargyric oxymethine carbon.[1] Interpretation of 2D NMR data allowed us to assign five structural units (Figure 1). Further structure elucidation by interpretation of NMR data was hampered by a severe overlap of the ^{1}H NMR signals.

We turned our attention to determine the planar structure by tandem FAB mass spectrometic analysis. Three types of ions, differing in the composition of metal adduct, $[M + Li (Na, K)]^+$, $[M + 2Li (Na, K) - H]^+$, and $[M - H]^-$, were chosen as the precursor, and fragment ions in the high-energy CID spectra were analyzed (Table 2). All of these data were consistent with structure **3**. Units a and b were connected through a C_4-saturated chain, units b and d were linked via an ethylene unit, and units d and c were joined through a C_{12}-saturated chain (Figure 2). A series of fragment ions differing by 14 mass units between *m/z* 441 and 609 demonstrated the length of the alkyl chain connecting units c and d. An intense even-numbered ion at *m/z* 306 could be accounted for by a sequence of reactions shown in Figure 3.

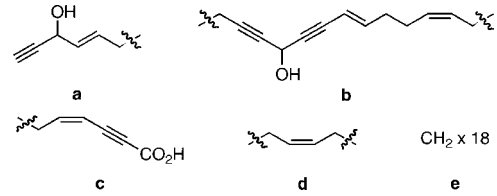


Figure 1. Partial structures assigned for **3**.

The 4*E*,17*E*,43*Z* geometry was assigned on the basis of $^3J_{H,H}$ values ($J_{4,5} = J_{17,18} = 16.2$ Hz and $J_{43,44} = 12.0$ Hz). The 21*Z*,27*Z* geometry was assigned because the corresponding allylic carbons were shielded (Table 1).[4] The 3*S*,14*S* stereochemistry was determined by the modified Mosher's method applied to the methyl ester of **3**.[10]

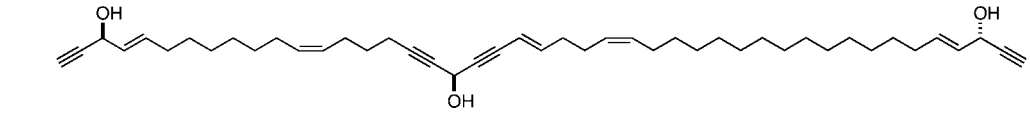
Petroformynic acid C (**4**) has a molecular formula of $C_{47}H_{70}O_4$, suggesting one less unsaturation than **3**. The ^{1}H and ^{13}C NMR spectra of **4** were almost superimposable on those of **3** except for the absence of the Δ^{43}-olefinic signals. The same partial structures a, b, and d were assigned by interpretation of 2D NMR data; the olefin in partial structure c was saturated. The arrangement of these units was again established by interpretation of tandem FABMS data (Table 3). The geometries of olefins in **4** were assigned as described above. Due to the instability of **4**, we were not able to carry out the modified Mosher analysis for **4**. From a biosynthetic point of view it is likely that **4** shares the same stereochemical feature as those of **3**.

Petroformynic acids C (**3**) and D (**4**) inhibited the growth of P388 cells each with an IC_{50} value of 0.4 μg/mL.

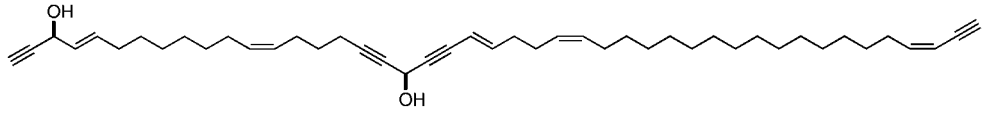
In the structure elucidation of petroformynic acids we have noticed that petroformyne 4 (**2**) and petrotetrayndiol A (**5**) are isomeric and that it is not possible to distinguish the two compounds by the NMR data. We also noticed that the reported mass fragmentation patterns of the TMS derivatives of petroformynes 3 and 4, on which their structure elucidation relied, are very different in spite of their structural similarity.[11] It may be necessary to compare the tandem FABMS data of the two

* To whom correspondence should be addressed. Tel: 81-3-5841-5297. Fax: 81-3-5841-8166. E-mail: assmats@mail.ecc.u-tokyo.ac.jp.
[†] University of Tokyo.
[‡] Suntory Institute.
[§] University of Amsterdam.
[⊥] Present address: Graduate School of Fisheries Sciences, Hokkaido University, Minato-cho, Hakodate 041-8611, Japan.

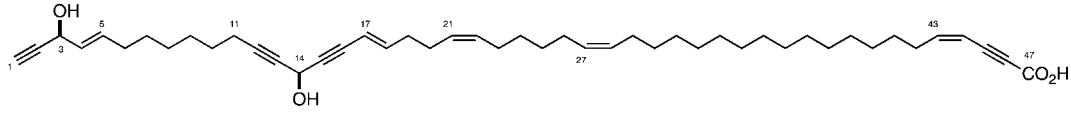
10.1021/np0702943 CCC: $37.00 © 2007 American Chemical Society and American Society of Pharmacognosy
Published on Web 11/07/2007



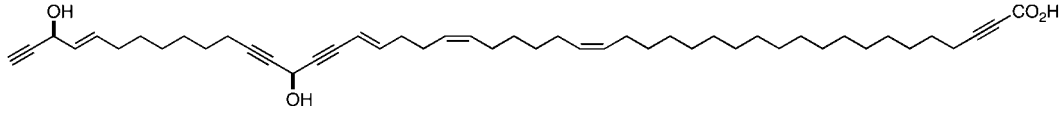
1



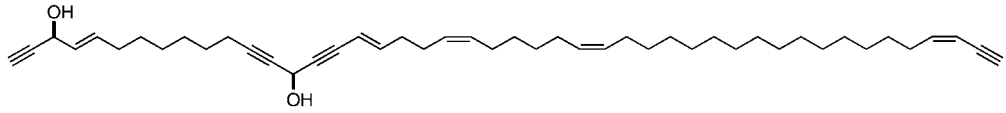
2



3



4



5

Table 1. NMR Data (600 MHz, CD_3OD) for Petroformynic Acids C (**3**) and D (**4**)

	1			**2**		
position	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1	74.5	2.86 (s)	C-2,3	74.5	2.86 (s)	C-3
2	84.8			84.7		
3	63.2	4.74 (brd)	C-2,5,6	63.2	4.73 (brd)	C-1,2,4,5
4	130.7	5.56	C-2,3,5	130.7	5.56	C-2,3,6
5	134.1	5.85 (dt, 16.2, 7.8)	C-3,6	134.0	5.84 (dt, 16.2, 7.8)	C-3,6
6	32.9	2.08	C-4,5	32.8	2.07	C-4,5,7
7	29.7	1.43		29.7	1.42	C-4
8		1.34			1.36	
9	29.5	1.4		29.4	1.43	
10	29.7	1.53	C-12	29.5	1.52	
11	19.3	2.23 (td, 7.2, 3.0)	C-12,13	19.2	2.22 (td, 7.2, 3.0)	C-9,10,12,13
12	85.0			82.6		
13	79.4			79.3		
14	52.9	5.13 (s)	C-13,15,16	52.7	5.12 (s)	C-13,15
15	87.1			87.0		
16	82.6			82.6		
17	112.4	5.53	C-15,16,18	110.4	5.53	C-15
18	145.9	6.14 (dt, 16.2, 6.6)	C-16,19,20	146.0	6.14 (dt, 16.2, 6.6)	C-16,19
19	33.9	2.17	C-17,18,21	34.1	2.17	C-17,20,21
20	27.6	2.16	C-22	27.4	2.15	C-19,22
21	129.4	5.34		129.4	5.33	C-23
22	131.7	5.39		131.7	5.39	C-21
23	27.2	2.05		28.1	2.04	
24–25		1.35–1.37			1.36–1.37	
26	28.0	2.04		28.0	2.04	
27	130.9	5.37		130.8	5.35	
28	130.9	5.37		130.8	5.35	
29	28.0	2.04		28.0	2.04	
30–41		1.27–1.36		30.2–30.8	1.27–1.36	
42	31.5	2.37 (dd, 6.6, 6.6)	C-43	30.4	1.43	C-43,44
43	147.7	6.05 (dt, 12.0, 6.6)	C-42,44,46	29.2	1.54	C-42,44,45
44	109.0	5.53	C-42,45,46	19.2	2.29 (t, 7.2)	C-42,43,45,46,47
45	91.6			85.5		
46	77.4			77.5		
47	161.9			160.0		

compounds in order to distinguish **5** from petroformyne 4 (**2**). Although the petroformyne class of metabolites are postulated to be biosynthesized by decarboxylation,[2] no direct proof of this concept has been obtained, because the precursor and the product polyacetylenes have not been isolated together. Petroformynic acid B can be considered as a precursor of petrotetrayndiol A (**5**) and is the first example to support the aforementioned biosynthetic proposal.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-1000 digital polarimeter in methanol. UV

Table 2. Tandem FAB Mass Spectrum of Compound **3**

precursor ion *m/z*	product ions *m/z* (A %)
703 $(M + Li)^+$	685 (80.5), 668 (35.4), 641 (26.9), 607 (8.3), 593 (2.9), 565 (9.5), 551 (9.9), 549 (7.7), 535 (20.8), 523 (8.8), 513 (19.6), 509 (8.1), 481 (5.9),467 (5.7), 453 (6.5), 439 (13.2), 425 (6.5), 419 (18), 371 (19.5), 357 (5.9), 351 (17.1), 343 (9.6), 329 (8.6), 303 (8.8), 290 (100), 289 (34.5),277 (10), 241 (21)
719 $(M + Na)^+$	701 (72.0), 684 (21.0), 657 (6.2), 623 (7.3), 609 (19.6), 595 (5.4), 581 (11.6), 567 (10.2), 565 (6.4), 553 (4.4), 539 (9.5), 529 (11.4), 525 (12.3), 511 (4.4), 497 (5.7), 483 (6.1), 469 (2.3), 455 (14.0), 441 (8.1), 403 (3.1), 387 (21.0), 373 (6.3), 359 (6.1), 345 (7.7), 319 (4.3), 306 (100.0), 305 (15.2), 293 (4.0)
709 $(M + 2Li - H)^+$	665 (10.7), 615 (2.7), 447 (46.7), 445 (4.6), 381 (14.4), 327 (10.5), 313 (11.7), 299 (10.0), 245 (14.9), 231 (16.3), 217 (12.8), 203 (31.7), 199 (52.7), 189 (16.8), 177 (34.5), 175 (14.9), 161 (11.1), 147 (13.5), 91 (48.9), 78 (100.0)
741 $(M + 2Na - H)^+$	697 (7.5), 649 (2.1), 479 (80.9), 477 (5.3), 413 (7.0), 359 (6.8), 345 (5.3), 331 (3.7), 277 (8.4), 263 (33.2), 249 (7.6), 235 (16.6), 221 (8.8), 209 (49.9), 207 (10.8), 193 (10.4), 179 (10.3), 122 (35.3), 109 (84.5), 70 (100)
773 $(M + 2K - H)^+$	729 (7.3), 511 (95), 509 (4.1), 445 (8.0), 391 (3.8), 377 (2.4), 364 (5.6), 309 (6.7), 295 (27.2), 281 (4.8), 267 (9.6), 253 (7.0), 241 (38.8), 239 (8.1), 225 (6.0), 211 (7.9), 198 (10.4), 155 (21.8), 142 (88.5), 103 (93.5)
695 $(M - H)^-$	651 (62.7), 625 (26.8), 461 (38.8), 433 (42.7), 217 (42.1), 191 (100.0), 163 (29.5)

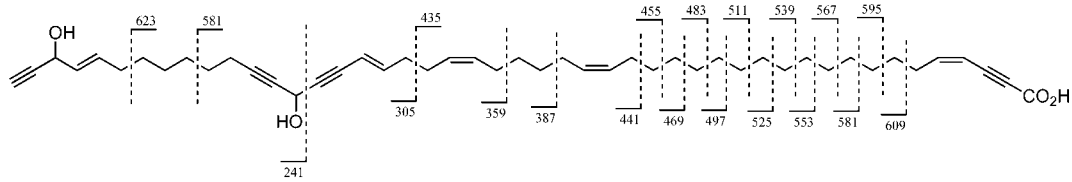


Figure 2. Fragmentation pathway of **3** from the $[M + Na]^+$ ion.

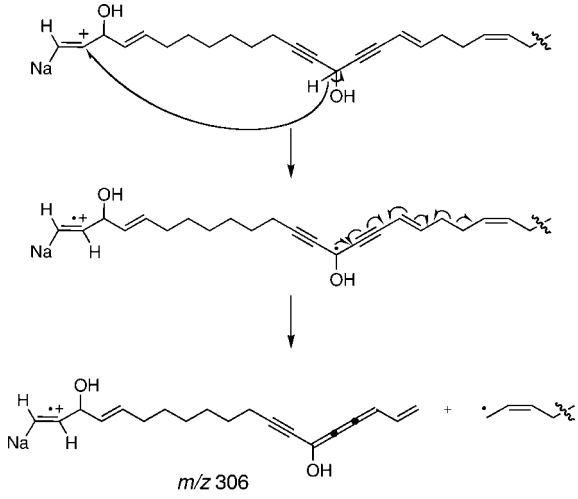


Figure 3. Generation of the fragment ion at *m/z* 306 in the tandem FABMS of **3**.

spectra were recorded on a Shimadzu BioSpec-1600 spectrophotometer in MeOH. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL delta 600 NMR spectrometer in CD_3OD at 300 K. Chemical shifts were referenced to solvent peaks: δ_H 3.30 for $CD_2\underline{H}OD$ and δ_C 49.0 for $\underline{C}D_3OD$. HRFABMS and tandem FAB mass spectra were measured on a JMS HX-110/HX110-A tandem mass spectrometer using 2,2′-dithiodiethanol (positive and negative ion mode) as matrix.

Animal Material. *Petrosia* sp. (ZMAPOR19093) were collected by hand using scuba off Katsuo-jima Island (33°28′ N, 135°51′ E), Wakayama Prefecture, Japan, and frozen until needed. A voucher specimen was deposited in the collections of Zoological Museum of the University of Amsterdam (registration no. ZMAPOR 19093).

Extraction and Isolation. The sponge (500 g, wet weight) was extracted with MeOH and $CHCl_3$. The combined extracts were partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ layer was partitioned between *n*-hexane and 90% MeOH. The 90% MeOH layer was evaporated and subjected to ODS flash chromatography with an aqueous MeOH system to afford two active fractions eluted with 90 and 100% MeOH. These fractions were combined and purified by silica gel column chromatography with a $CHCl_3$ and MeOH system to afford an active fraction eluting with $CHCl_3$/MeOH (4:1). This fraction was purified by ODS HPLC (Nacalai, Cosmosil $5C_{18}$ AR-II with 50% *n*-PrOH containing 100 mM $NaClO_4$) to give **1** (25 mg) and **2** (30 mg).

Petroformynic acid B (3): yellowish, amorphous solids; $[\alpha]^{26}_D$ +6.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 258, 242, 211, 206, 203, 201 nm; FABMS (positive) *m/z* 719 $(M + Na)^+$ and 735 $(M + K)^+$; HRFABMS (positive) *m/z* 719.5018 (calcd for $C_{47}H_{68}O_4Na$, 719.5015); ^{1}H NMR and ^{13}C NMR data in CD_3OD, see Table 1.

Petroformynic acid C (4): yellowish, amorphous solids; $[\alpha]^{26}_D$ +23.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 252, 231, 211, 206, 203, 201 nm; FABMS (positive) *m/z* 721 $(M + Na)^+$ and 737 $(M + K)^+$; HRFABMS (positive) *m/z* 721.5163 (calcd for $C_{47}H_{70}O_4Na$, 721.5172); ^{1}H NMR and ^{13}C NMR data in CD_3OD, see Table 1.

MTPA Esters. To a solution of **3** (2.0 mg) in MeOH (2 mL) was added diazomethane in diethyl ether. The mixture was left for 30 min at room temperature and evaporated to dryness. To a half-portion of the residue dissolved in pyridine (1 drop) was added (*R*)-(–)-MTPACl (5 mg in 50 μL of toluene), and the mixture was left at room temperature for 20 min. The reaction mixture was diluted with 5% $NaHCO_3$ solution and extracted with EtOAc. The organic layer, which contained the

Table 3. Tandem FAB Mass Spectrum of Compound **4**

precursor ion	product ions *m/z* (A %)
705 $(M + Li)^+$	687 (100.0), 670 (48.0), 643 (20.0), 565 (7.9), 551 (9.6), 537 (12.0), 523 (8.1), 515 (23.0), 509 (9.0), 481 (6.0), 467 (5.6), 453 (5.6), 439 (12.4), 421 (20.7), 371 (17.2), 357 (5.2), 343 (9.6), 329 (7.9), 303 (7.9), 290 (81.6), 289 (37.5), 277 (12.9), 243 (24.8)
721 $(M + Na)^+$	703 (84.9), 686 (39.2), 659 (5.6), 609 (3.7), 595 (4.1), 581 (6.9), 567 (7.4), 553 (3.8), 539 (6.9), 531 (5.7), 525 (8.7), 511 (3.1), 497 (4.4), 483 (4.8), 469 (4.3), 455 (10.5), 441 (4.5), 387 (14), 373 (4.5), 359 (3.6), 345 (4.1), 319 (2.9), 306 (100.0), 305 (9.2), 293 (3.9), 241 (4.3)
711 $(M + 2Li - H)^+$	667 (29.4), 617 (8.2), 449 (100), 447 (9.6), 383 (26.8), 329 (19.7), 315 (25.5), 301 (15.2), 247 (31.7), 233 (15.1), 231 (23.8), 219 (18.0), 205 (28.5), 203 (28.5), 199 (83.4), 191 (21.8), 177 (62.7), 163 (19.6), 149 (20.8)
743 $(M + 2Na - H)^+$	699 (15.9), 651 (4.3), 481 (96.5), 479 (9.5), 415 (9.2), 361 (11.8), 347 (9.2), 279 (18.4), 265 (10), 263 (37.8), 251 (10.7), 237 (11.9), 235 (11.2), 223 (15.8), 209 (75.1), 195 (14.4), 181 (14.9), 128 (31.9), 97 (72.9), 84 (87.3), 71 (100.0)
775 $(M + 2K - H)^+$	731 (3.9), 513 (95.5), 511 (5.2), 447 (7.0), 393 (3.8), 379 (2.7), 365 (2.0), 311 (7.4), 297 (3.7), 295 (15.3), 283 (4.6), 269 (6.9), 255 (6.4), 241 (30.7), 227 (5.2), 213 (6.8), 186 (10.1), 172 (11.9), 160 (14.2), 129 (34.7), 116 (50.8), 103 (93.5)
697 $(M - H)^-$	653 (41.1), 627 (24.6), 463 (51.7), 435 (56.7), 217 (31.8), 191 (100.0), 163 (34.3)

bis[(*S*)-MTPA]ester was evaporated and submitted to the ^{1}H NMR analysis. The product with the (*S*)-(+)-MTPACl was prepared in the same manner.

Bis[(*S*)-(–)-MTPA] ester of 3: ^{1}H NMR ($CDCl_3$) δ 2.62 (H1), 6.02 (H3), 5.52 (H4), 6.00 (H5), 6.33 (H14), 5.50 (H17), 6.25 (H18).

Bis[(*R*)-(+)-MTPA] ester of 3: ^{1}H NMR ($CDCl_3$) δ 2.60 (H1), 6.01 (H3), 5.60 (H4), 6.07 (H5), 6.33 (H14), 5.45 (H17), 6.23 (H18).

Acknowledgment. We thank the crew of *R/V Toyoshiomaru*, Hiroshima University, for assistance in collecting the sponge and K. Furihata of the University of Tokyo for his help in the measurements of NMR spectra. This work was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas 16073207 from MEXT.

Supporting Information Available: NMR spectra and tandem FABMS of compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Cimino, G.; Giulio, A. D.; Rosa, S. D.; Marzo, V. D. *Tetrahedron Lett.* **1989**, *30*, 3563–3566.

(2) van Soest, R. W. M.; Fusetani, N.; Andersen, R. J. In *Sponge Science*; Watanabe Y., Fusetani, N., Eds.; Springer: Tokyo, 1998; pp 3–30, and references therein.

(3) Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *Tetrahedron* **1994**, *50*, 13261–13268.

(4) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *J. Nat. Prod.* **1990**, *53*, 345–353.

(5) Seo, Y.; Cho, K. W.; Rho, J.-R.; Shin, J. *Tetrahedron* **1998**, *54*, 447–462.

(6) Shin, J.; Seo, Y.; Cho, K. W. *J. Nat. Prod.* **1998**, *61*, 1268–1273.

(7) 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.

(8) Lim, Y. J.; Lee, C.-O.; Hong, J.; Kim, D.-K.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2001**, *64*, 46–53.

(9) Three polyacetylenes terminating in a carboxylic acid group termed petroformyne A,petroformyne B, and petroformynic acid have so far been reported from *P. ficiformis*.[3,4] The structures of these compounds were assigned except for the position of the olefinic bond(s). Even though our sponge was identified only to the genus level, we named our compounds as petroformynic acids by following the naming of ref 4 in order to avoid confusion by giving different names to related compounds.

(10) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

(11) Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *Tetrahedron Lett.* **1989**, *30*, 3563–3566.

NP0702943