

Cytotoxic C₄₇-Polyacetylene Carboxylic Acids from a Marine Sponge *Petrosia* sp.

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Two new polyacetylene carboxylic acids, petroformynic acids B (**3**) and C (**4**), were isolated from a marine sponge *Petrosia* 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 C₄₆-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 C₄₇ 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 C₄₇H₆₈O₄ as established by HRFABMS. The ¹H 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 ¹³C NMR spectrum showed the presence of one carboxylic acid (δ 161.9), 10 sp² 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 propargylic 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 ¹H NMR signals.

We turned our attention to determine the planar structure by tandem FAB mass spectrometric analysis. Three types of ions,

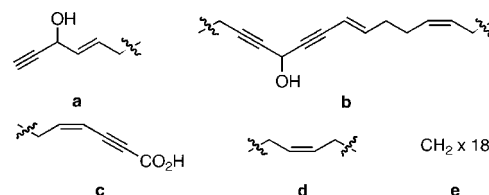


Figure 1. Partial structures assigned for **3**.

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 C₄-saturated chain, units b and d were linked via an ethylene unit, and units d and c were joined through a C₁₂-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.

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 C₄₇H₇₀O₄, suggesting one less unsaturation than **3**. The ¹H and ¹³C NMR spectra of **4** were almost superimposable on those of **3** except for the absence of the Δ⁴³-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 IC₅₀ value of 0.4 μg/mL.

In the structure elucidation of petroformynic acids we have noticed that petroformyne 4 (**2**) and petrotetraandiol 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

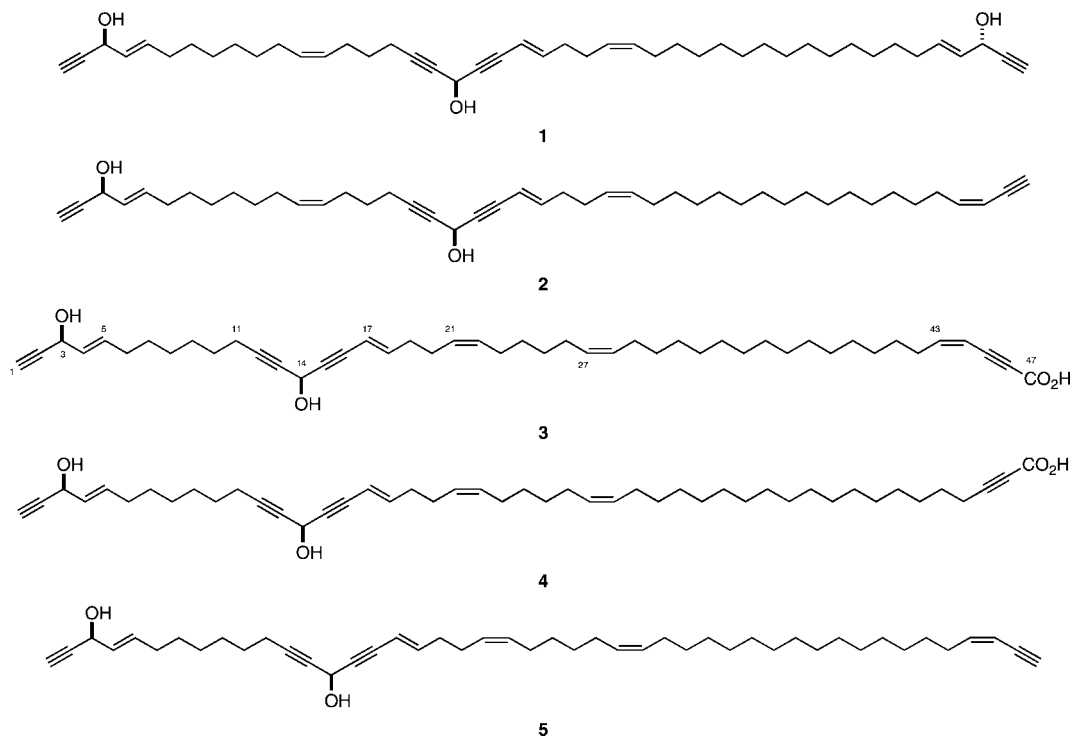
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**Table 1.** NMR Data (600 MHz, CD₃OD) for Petroformynic Acids C (3) and D (4)

position	1			2		
	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**

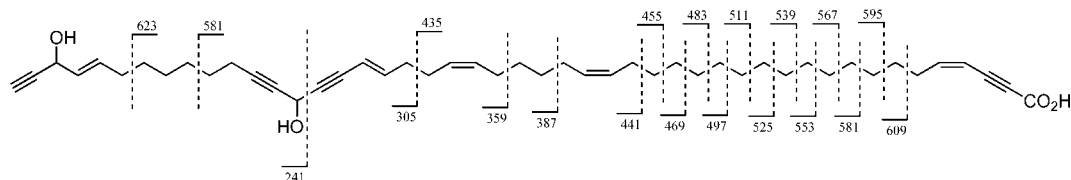
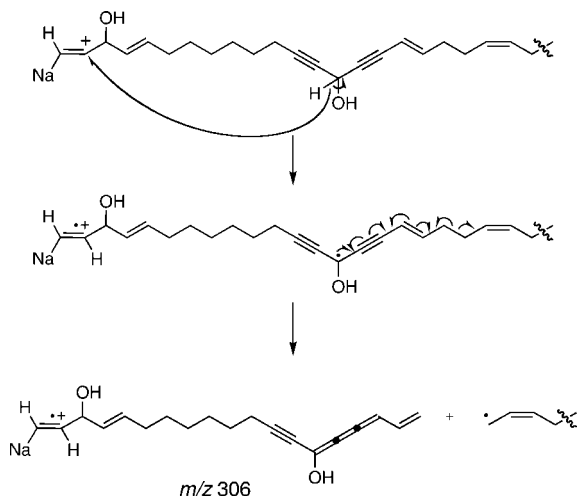
(**5**) and is the first example to support the aforementioned biosynthetic proposal.

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. ¹H NMR and ¹³C NMR spectra were recorded on a JEOL delta 600 NMR spectrometer in CD₃OD at 300 K. Chemical shifts were referenced to solvent peaks: δ_H 3.30 for CD₂HOD and δ_C 49.0 for CD₃OD. 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),

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)

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 CHCl₃. The combined extracts were partitioned between CHCl₃ and H₂O, and the CHCl₃ 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 CHCl₃ and MeOH system to afford an active fraction eluting with CHCl₃/MeOH (4:1). This fraction was purified by ODS HPLC (Nacalai, Cosmosil 5C₁₈ AR-II with 50% *n*-PrOH containing 100 mM NaClO₄) to give 1 (25 mg) and 2 (30 mg).

Petroforminic acid B (3): yellowish, amorphous solids; [α]_D²⁶ +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 C₄₇H₆₈O₄Na, 719.5015); ¹H NMR and ¹³C NMR data in CD₃OD, see Table 1.

Petroforminic acid C (4): yellowish, amorphous solids; [α]_D²⁶ +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 C₄₇H₇₀O₄Na, 721.5172); ¹H NMR and ¹³C NMR data in CD₃OD, 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% NaHCO₃ solution and extracted with EtOAc. The organic layer, which contained the

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

Bis[(*S*)-(-)-MTPA] ester of 3: ¹H NMR (CDCl₃) 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: ¹H NMR (CDCl₃) δ 2.60 (H1), 6.01 (H3), 5.60 (H4), 6.07 (H5), 6.33 (H14), 5.45 (H17), 6.23 (H18).

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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>.

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