# Further Petroformynes from Both Atlantic and Mediterranean Populations of the Sponge *Petrosia ficiformis*

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Five novel polyacetylenes (**5**−**9**) were isolated from two different populations of the sponge *Petrosia ficiformis* collected in the Mediterranean Sea and the Atlantic Ocean. Their structures were established by extensive NMR analysis and by comparison with known petroformynes.

Marine sponges of the genera *Petrosia* frequently afford polyacetylene metabolites,[2−12] and most of them exhibit various bioactivities such as antifungal, cytotoxic, or antiviral activities. Petroformynes, characteristic metabolites of the Mediterranean sponge *Petrosia ficiformis* Poiret (family Petrosiidae, order Petrosida/Haplosclerida), are polyacetylenes with high molecular weight and long, unbranched alkyl chains; they also show interesting biological activities.[11] In our previous studies[7−12] more than 20 petroformynes have been isolated and structurally characterized. More recently, the absolute stereochemistry of the most abundant petroformynes, petroformynes 1−4 (**1**−**4**), petroformyne 5, and petroformynes A and B have been established as *S* at all the chiral centers by applying the advanced Mosher's method (Chart 1).[13]

During our continuing studies on bioactive substances from marine organisms, we have investigated the $Et_2O$ soluble fraction from the $Me_2CO$ extract of two varieties (red and white) of the sponge *P. ficiformis*, collected off Tarifa, Spain. This work has resulted in the isolation of two novel polyacetylenes, **5** and **6**, together with the known petroformyne 1 (**1**) and its derivatives petroformyne 5,[9,13] with an additional hydroxy group at C-6 and petroformyne 10,[12] oxidized at one of the ends of **1**. Interestingly, the metabolite pattern of the Tarifa specimens was found to be somewhat different from that of the Naples collection.[13] Petroformynes 3 (**3**) and 4 (**4**), characteristic metabolites of the white variety of Neapolitan *P. ficiformis*, are completely absent in the same variety of the Tarifa collection. Instead, this variety contains the same petroformynes as those found in the red variety of both the Naples and the Tarifa collections. To verify this difference, we have reanalyzed the sponge collected along the coast of Naples. In the course of this study, three further minor petroformynes, **7**−**9** (in addition to those previously reported[11,12]), have been isolated. This paper describes the isolation and structure elucidation of these new compounds.

## Results and Discussion

Specimens of the two varieties of *P. ficiformis* (red from lighted waters, white from dark caves) were collected off Naples, Italy, and off Tarifa, Spain, respectively, and kept frozen prior to extraction. The workup for the extraction and isolation of polyacetylenes was basically performed as previously reported.[12] This common procedure yielded a new high-molecular-weight acetylenic acid (**5**), named petroformynic acid, from the white Tarifa *P. ficiformis*, together with the previously described petroformynes 1 (**1**), 5, and 10. A new acetylenic alcohol, named isopetroformyne 1 (**6**), was isolated from the red Tarifa *P. ficiformis*, together with petroformyne 1(**1**) and petroformyne 10. The minor novel petroformynes **7**−**9** were obtained from the white collection from Naples, together with the compounds previously reported (Chart 2).[11,12] All new compounds showed considerable structural analogies with the known petroformynes, in particular exhibiting the typical petroformyne partial structures **a**−**h** (Figure 1).

Petroformynic acid (**5**) was isolated as a colorless liquid from the polar fraction of the liposoluble extract of white Tarifa *P. ficiformis*. In the IR spectrum, absorptions for both acetylenic (2212 $cm^{-1}$) and carboxylic (1695 and 3400 $cm^{-1}$) acid functionalities were observed.

The presence of the carboxylic acid functional group conjugated to a triple bond was further suggested by comparing the $^{13}$C-NMR signal at $\delta$ 157.0 (s) with $^{13}$C-NMR data of related compounds.[14] To prove this assignment, **5** was treated with $CH_2N_2$ giving the methyl ester derivative **5a**, which showed, in the $^1$H-NMR spectrum, the expected singlet at $\delta$ 3.79 integrating for three protons. The molecular formula of **5**, $C_{31}H_{50}O_2$, was deduced by the HRFABMS of its methyl ester **5a**, $C_{32}H_{52}O_2$. The presence of the substructures **a** and **b** was easily recognized by interpretation of $^1$H−$^1$H COSY, TOCSY, DEPT, HMQC, and HMBC spectra and by comparison with compound **10**.[9] Thus, the remaining part of the molecule (R═$C_{18}H_{34}$) had to contain 16 methylenes and one double bond, with the position of this isolated double bond remaining to be determined, although its stereochemistry was indicated to be *Z* on the basis of the $^{13}$C-NMR resonances of the allyl carbons ($\delta$ 27.4, 27.5).[9] The partial structures **a**, **b**, and R were linearly connected as in **5**.

Petroformynic acid (**5**) showed strong structural similarities with compound **10**, previously isolated from the red *P. ficiformis* from Naples.[9] In fact, the differences between **5** and **10** lay only in the middle part (R) of the molecules where the latter contained three double bonds and 24 methylenes.

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**Chart 1**

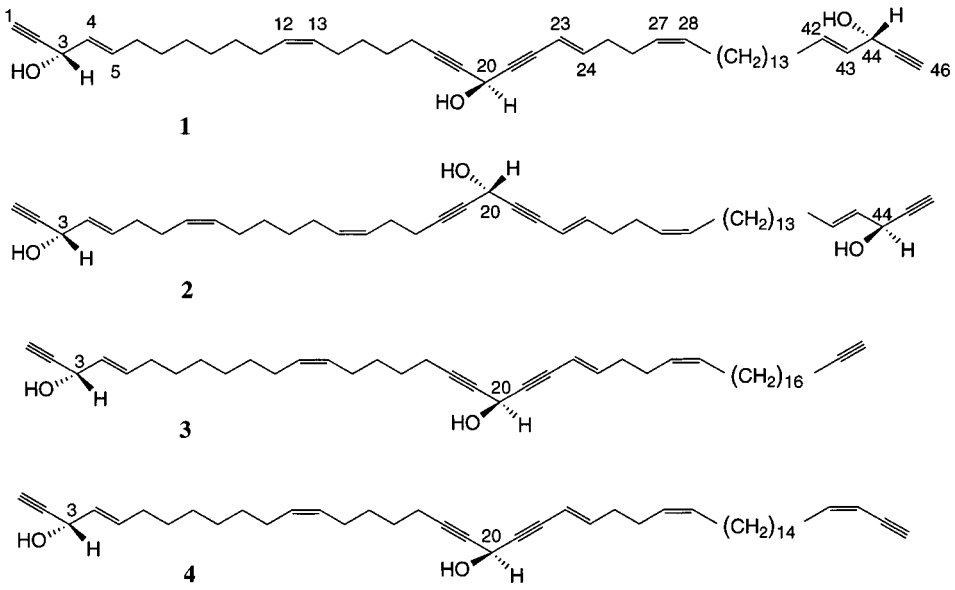
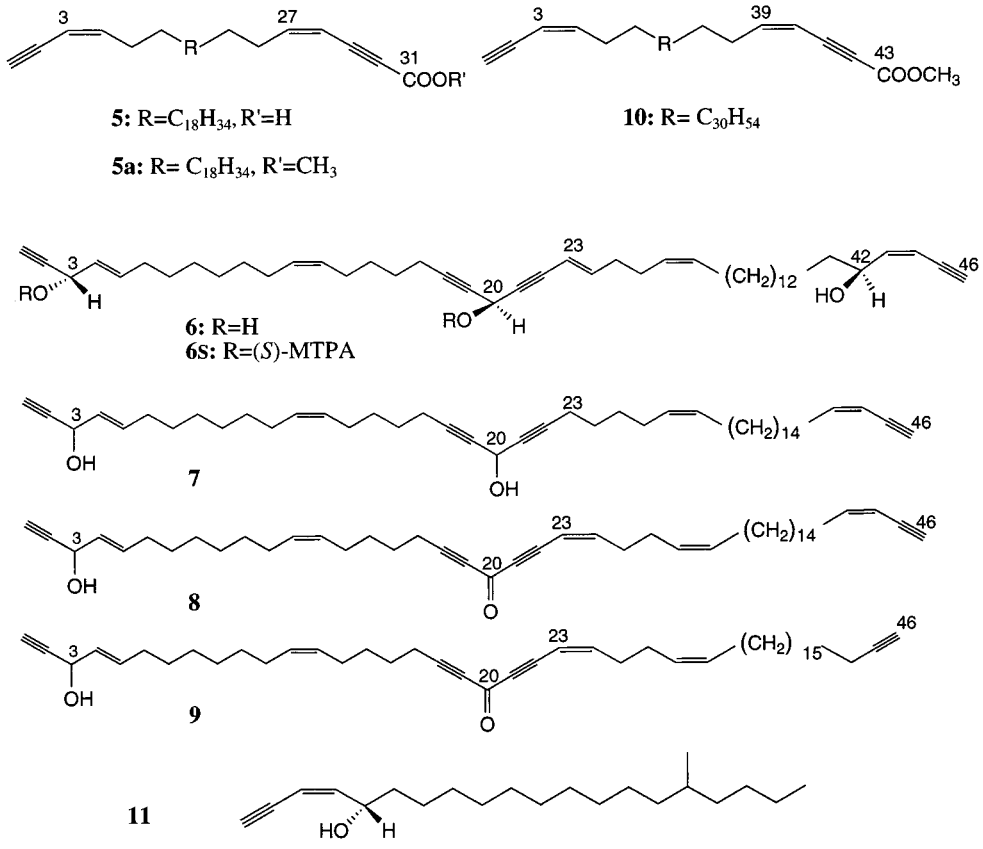



**Chart 2**




Compounds **6**−**9** showed IR absorption indicative of the presence of hydroxy functions (3600 $cm^{-1}$), terminal acetylenes (3300 $cm^{-1}$), and disubstituted triple bonds (2220 $cm^{-1}$). Their NMR spectra were reminiscent of those of petroformynes 3 (**3**) and 4 (**4**). In fact, a common partial structure **c** (Figure 1), which is most frequently encountered in polyacetylene metabolites, was present in all four new compounds. In addition, compounds **7** and **8** displayed another terminal acetylene function as petroformyne 4 (**4**) (partial structure **a**, Figure 1), while compound **9** showed, at the other end of the molecule, the same terminal structure as petroformyne 3 (**3**) (partial structure **d**, Figure 1). The $^1$H-NMR spectra of **6**−**9** showed other structural analogies with known petroformynes: the absence of terminal methyls; the evidence for long, unbranched alkyl chains (strong signals at $\delta$ 1.26); and the presence of isolated double bonds (peaks at $\delta$ 5.35 and 2.02).

Isopetroformyne 1 (**6**) showed spectral data closely related to those of petroformyne 1. Its molecular formula, $C_{46}H_{68}O_3$, was deduced by FABMS [*m*/*z* 691, $(M + Na)^+$] and $^{13}$C-NMR data, indicating the presence

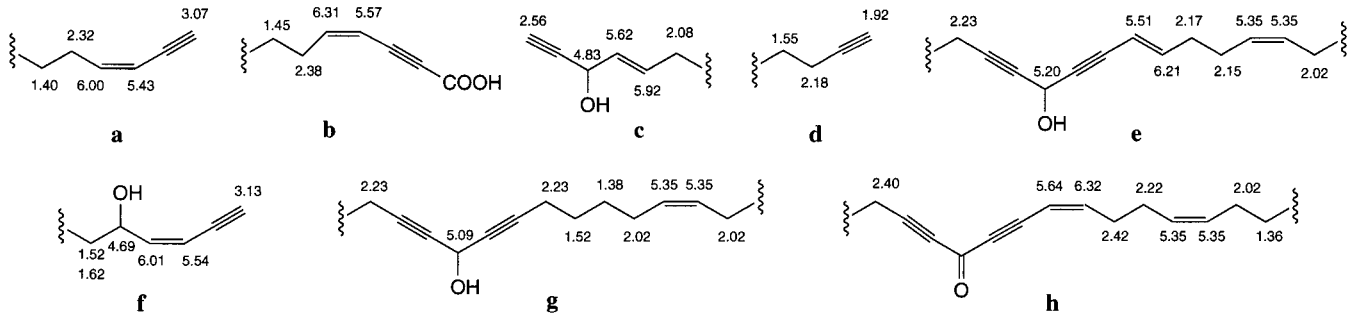



**Figure 1.** Partial structures **a** (**5**), **b** (**5**), **c** (**6**), **d** (**9**), **e** (**6**), **f** (**6**), **g** (**7**), and **h** (**8**) with $^1$H-NMR assignments.

**Table 1.** Selected $^1$H- and $^{13}$C NMR Data[a] and HMBC Correlations of Compound **6**

| position no. | $\delta$, $^1$H[b] (mult. $J$ in Hz) | $\delta$, $^{13}$C[c] (mult) | HMBC (C−H, $J$ = 10 Hz) |
|---|---|---|---|
| 1 | 2.56 (d, 1.6) | 74.0 d | H-1 |
| 2 | | 82.7 s | H-1, H-3 |
| 3 | 4.83 (br s) | 62.8 d | H-1, H-5 |
| 4 | 5.62 (dd, 15.3, 6.1) | 128.5 d | H-3, H-5 |
| 5 | 5.92 (dt, 15.3, 7.0) | 134.3 d | |
| 6 | 2.08 (m) | 31.8 t | |
| 7 | 1.58 (m) | 28.5[g] t | |
| 11 | 2.02 (m) | 27.2[f] t | |
| 12 | 5.35 (m) | 129.6[e] d | H-11 |
| 13 | 5.35 (m) | 130.1[e] d | H-14 |
| 14 | 2.02 (m) | 27.3[f] t | |
| 15 | 1.38 (m) | 29.1 t | |
| 16 | 1.52 (m) | 28.6[g] t | |
| 17 | 2.23 (m) | 18.6 t | $H_2$-16 |
| 18 | | 85.1 s | $H_2$-17, $H_2$-16 |
| 19 | | *d* | |
| 20 | 5.20 (br s) | 52.9 d | |
| 21 | | *d* | |
| 22 | | *d* | |
| 23 | 5.51 (d, 16.4) | 108.9 d | |
| 24 | 6.21 (dt, 16.4, 7.1) | 145.7 d | |
| 25 | 2.17 (m) | *c* | |
| 26 | 2.15 (m) | *c* | |
| 27 | 5.35 (m) | 128.1[e] d | $H_2$-26 |
| 28 | 5.35 (m) | 130.9[e] d | $H_2$-29 |
| 29 | 2.02 (m) | 27.1[f] t | |
| 41a | 1.52 (m) | 36.5 t | |
| 41b | 1.62 (m) | | |
| 42 | 4.69 (dt, 8.2, 6.5) | 70.5 d | H-44 |
| 43 | 6.01 (dd, 10.9, 8.2) | 147.5 d | |
| 44 | 5.54 (dd, 10.9, 2.1) | 108.9 d | H-42, H-46 |
| 45 | | 82.5 s | H-46 |
| 46 | 3.13 (d, 2.1) | 79.5 d | H-43, H-46 |

[a] Bruker AMX 500 MHz; $CDCl_3$; assignments were deduced from the analysis of single- and two-dimensional spectra. [b] Assignments for unreported methylene protons contributed to a large signal at $\delta$ 1.26. [c] The methylene values not reported resonated between $\delta$ 29.8 and 29.0. [d] Not observed. [e−g] Values in same column with the same superscripts may be interchanged.

of 13 degrees of unsaturation, the same as petroformyne 1. Comparison of the NMR spectra of **6** with those of **1** allowed recognition of the same partial structures **c** and **e** and also provided some more structural details, suggesting the presence of a new substructure **f** (Figure 1). In the $^1$H-NMR spectrum of **6** (Table 1), a new signal at $\delta$ 3.13 (H-46) appeared in the place of the terminal acetylene proton signal at $\delta$ 2.56 in the $^1$H-NMR spectrum of petroformyne 1 (**1**) or at $\delta$ 3.06 in the $^1$H-NMR spectrum of petroformyne 4 (**4**). The $^{13}$C-NMR spectrum of **6** (Table 1) showed two sp$^2$ carbon signals at $\delta$ 108.9 (C-44) and 147.5 (C-43), according to partial structure **f** (Figure 1), differing slightly from those previously reported for petroformynes. Detailed analysis of COSY, TOCSY, HMQC, and HMBC experiments confirmed the fragment **f**, where the sp$^2$ carbon signal at $\delta$ 108.9 (C-44) showed HMBC correlations with the proton at $\delta$ 3.13 (H-46) and the oxymethine at $\delta$ 4.69 (H-42, $\delta_C$ 70.5). The geometry of the $\Delta^{43}$ double bond was assigned as *Z* on the basis of the vicinal coupling constant of 10.9 Hz. Comparison of **6** with the known compound **11**, isolated from the sponge *Cribrochalina vasculum*,[15] further secured the assignment of the moiety **f**. Finally, the remaining portion of the molecule ($C_{21}H_{30}$), assignable to two alkyl chains between fragments **c** and **e** and between the fragments **e** and **f**, was suggested to be the same as petroformyne 1, the main metabolite of the sponge, leading to structure **6**.

To determine the absolute stereochemistry of **6**, the modified Mosher's ester method, which has been successfully applied to determine the absolute stereochemistry of petroformynes,[13] was employed. Because **6** was obtained in a limited amount, insufficient to prepare both the (*S*)- and (*R*)-MTPA derivatives, only the (*S*)-MTPA ester (**6S**) was synthesized. The extensive analysis of 2D NMR spectra of **6S** revealed that the chemical shift of the protons at C-1 ($\delta$ 2.62), C-3 ($\delta$ 6.02), C-4 ($\delta$ 5.50), C-5 ($\delta$ 6.00), and C-6 ($\delta$ 2.04) and at C-17 ($\delta$ 2.20), C-20 ($\delta$ 6.33), C-23 ($\delta$ 5.50), and C-24 ($\delta$ 6.23) were almost identical with those of model *S*-MTPA esters of petroformynes 1−4,[12] while the resonances of the protons at C-41 ($\delta$ 1.73, 1.61), C-42 ($\delta$ 5.98), C-43 ($\delta$ 5.96), C-44 ($\delta$ 5.67), and C-46 ($\delta$ 3.26) were identical with those reported for *S*-MTPA ester of compound **11**.[15] On the basis of this observation, it is likely that **6** has the same *S* absolute stereochemistry at C-3 and C-20 as petroformynes **1**−**4** and has the same *S* absolute configuration at C-42 as **11**.

Compounds **7**−**9** were isolated from white *P. ficiformis* from Naples. Because of the scarcity of material, which prevented detailed chemical and spectroscopic studies, their structures were determined mainly by comparison of $^1$H-NMR and MS data with those of the known polyacetylenes, previously reported from a different collection in the same area of the same sponge variety.

Compound **7** (23,24-dihydropetroformyne 4) yielded a FABMS peak at *m*/*z* 677 $(M + Na)^+$, 2 amu more than that of petroformyne 4 (**4**). The $^1$H NMR spectrum of **7** showed the presence of partial terminal moieties **a** and **c**, as in petroformyne-4 (**4**), and also indicated the presence of partial structure **g** in the middle part of the molecule, suggesting that **7** was the 23,24-dihydro derivative of petroformyne 4.

The molecular formula of **8** [FABMS peak at *m*/*z* 673 $(M + Na)^+$, $C_{46}H_{66}O_2$], 20-oxo-isopetroformyne 4, was two mass units less than that of petroformyne 4. The

presence of the same terminal substructures **a** and **c** as petroformyne 4 was immediately recognized. In addition, an IR band at 1622 $cm^{-1}$ and the absence of the proton signal at $\delta$ 5.1−5.2 region implied the oxidation of the hydroxy group at C-20. Comparison with 20-oxoisopetroformyne 3[12] revealed the presence the same partial structure **h**, which should be located in the middle of the molecule. Finally, the coupling constant ($J$ = 10 Hz) between H-23 and H-24 was consistent with a *cis*-orientation of this double bond.

Compound **9**, 20-oxoisopetroformyne 3, with a molecular formula of $C_{46}H_{68}O_2$ [FABMS peak at *m/z* 675 (M + Na)$^+$], was isomeric with 20-oxopetroformyne 3.[12] Comparison of the spectral data with those of both **8** and 20-oxopetroformyne 3 revealed the presence of the same partial structures **c**, **d**, and **h**. In particular, **9** differs from 20-oxopetroformyne 3 only in the orientation of the $\Delta^{23}$ double bond (*Z* in **9**, *E* in 20-oxopetroformyne 3), whereas the remaining resonances were almost identical.

The finding of closely related metabolites in both Atlantic and Mediterranean red and white *P. ficiformis* raises intriguing questions about their biogenetic origin. Even though our preliminary experiments of feeding labeled acetate to *P. ficiformis* were unsuccessful, further studies should be conducted to verify their biosynthesis.

## Experimental Section

**General Experimental Procedures.** The IR spectra were recorded on a Bio-Rad FTS 7 spectrometer, and UV spectra were obtained on a Varian DMS 90 double beam spectrophotometer. $^1$H- and $^{13}$C-NMR spectra were recorded on AMX 500 MHz, AM 400 MHz, and DPX 300 MHz Bruker spectrometers, respectively. Chemical shifts are reported in parts per million referenced to $CHCl_3$ as internal standard ($\delta$ 7.26 for proton and $\delta$ 77.0 for carbon). $^1$H- and $^{13}$C-NMR assignments were supported by $^1$H−$^1$H COSY, TOCSY, HMQC, and HMBC experiments. FABMS and HR-FABMS spectra were recorded on ZAB VG and ZAB T tandem mass spectrometers using *m*-nitrobenzyl alcohol (positive-ion mode) as matrix. Optical rotations were measured on a JASCO DIP-370 digital polarimeter in $CHCl_3$. Reversed-phase HPLC purifications were performed on a Waters liquid chromatograph with a UVIDEC-100-III detector. Both analytical [5 $\mu$m, 4.6 mm (i.d.) × 25 cm] and semipreparative [5 $\mu$m,10 mm (i.d.) × 25 cm] columns were Spherisorb-S5 ODS2. Commercial Merck Si gel 60 (70−230 mesh, ASTM) was used for column chromatography. Merck precoated Si gel plates were used for TLC. The chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2 N $H_2SO_4$ and heated at 80° for 5 min to detect the spots.

**Collection of Animal Material.** Red and white varieties of the sponge *P. ficiformis* were collected in the Bay of Naples, Italy, and off Tarifa, Spain, by scuba at a depth of −10 m. Voucher specimens are available for inspection at the ICMIB; identification no. S-015 (Tarifa white), s-016 (Tarifa red), s-025 (Naples white), and s-043 (Naples red).

**Extraction and Isolation.** The biological material was extracted using the standard workup as previously reported.[11,12] The new polyacetylenes **5**−**9** were isolated by the following procedure.

The Tarifa sponge *P. ficiformis* (white variety, dry wt 50 g) gave a $CHCl_3$−$CH_3OH$ 8:2 fraction (109.4 mg) containing a mixture of petroformyne 5 and compound **5**, together with some minor related compounds. This fraction was directly submitted to reversed-phase HPLC [MeOH−$H_2O$−TFA (95:5:0.05) as eluent] yielding petroformynic acid (**5**) (2.4 mg) and petroformyne 5 (13.7 mg).[9,13]

The Tarifa sponge *P. ficiformis* (red variety, dry wt 74 g), after the usual workup,[13] afforded a fraction (23.5 mg) eluted by light petroleum ether−$Et_2O$ (7:3), which was further purified by reversed-phase HPLC [MeOH−$H_2O$ (95:5) as eluent] yielding isopetroformyne 1 (**6**) (2.1 mg).

HPLC purification of petroformynes **7**−**9** from the light petroleum ether−$Et_2O$ (8:2) fraction (109 mg) of the Naples sponge *P. ficiformis* (white variety, dry wt 320 g) was carried out following the same procedure for the isolation of isopetroformyne 3 and other minor petroformynes as described by Guo et al.[12] This yielded **7** (0.4 mg), **8** (0.8 mg), and **9** (0.6 mg), together with minor petroformynes reported previously.[12]

**Petroformynic acid (5):** colorless oil; UV (MeOH) $\lambda_{max}$ 230 nm ($\epsilon$ 19 200); IR (liquid film) $\nu_{max}$ 3400 (terminal acetylene), 2927 (CH, aliphatic), 2212 (acetylene), 1695 (conjugated carboxylic acid), 1461, 1252 $cm^{-1}$; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 3.07 (1H, br s, H-1), 5.43 (1H, dd, $J$ = 10.8, 1.7 Hz, H-3), 6.00 (1H, dt, $J$ = 10.8, 7.5 Hz, H-4), 2.32 (2H, app q, $J$ = 7.5 Hz, $H_2$-5), 1.40 (2H, m, $H_2$-6), 5.34 (2H, m, protons of the isolated double bond), 2.02 (4H, m, allylic protons), 1.45 (2H, m, $H_2$-25), 2.38 (2H, app q, $J$ = 7.6 Hz, $H_2$-26), 6.31 (1H, dt, $J$ = 11.0, 7.6 Hz, H-27), 5.57 (1H, d, $J$ = 11.0 Hz, H-28); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 81.1 (d, C-1), 80.6 (s, C-2), 107.9 (d, C-3), 146.3 (d, C-4), 30.3 (t, C-5), 28.7 (t, C-6), 129.9 (d, carbons of isolated double bond), 27.4, 27.5 (t, allylic carbons), 28.5 (t, C-25), 31.1 (t, C-26), 152.7 (d, C-27), 106.2 (d, C-28), 85.9 (s, C-29), 83.9 (s, C-30), 157.0 (s, C-31); FABMS *m/z* 453 (M + H)$^+$, 475 (M + Na)$^+$.

**Preparation of Methyl Ester of 5.** A solution of $CH_2N_2$ (0.5 mL) was added to a solution of **5** (2.4 mg) in $CHCl_3$ (1 mL) at room temperature. After 30 min, the reaction was stopped, and the solvent was removed by $N_2$. Purification of the residue by Si gel column chromatography on a Pasteur pipette eluting with light petroleum−$Et_2O$ (97:3) yielded the pure petroformynic acid methyl ester **5a** (1.2 mg).

**Petroformynic acid methyl ester (5a):** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 3.07 (1H, br s, H-1), 5.44 (1H, br d, $J$ = 11 Hz, H-3), 6.00 (1H, dt, $J$ = 11, 7 Hz, H-4), 2.35 (4H, m, $H_2$-5 and $H_2$-26), 5.35 (2H, m, protons of the isolated double bond), 2.02 (4H, m, allylic protons), 6.26 (1H, dt, $J$ = 11, 7 Hz, H-27), 5.54 (1H, d, $J$ = 11 Hz, H-28), 3.79 (3H, s, $COOCH_3$); FABMS *m/z* 467 (M + H)$^+$; HRFABMS *m/z* 467.3885 (M + H)$^+$ (calcd for $C_{32}H_{51}O_2$, 467.3889).

**Isopetroformyne 1 (6):** colorless oil; $[\alpha]^{21}_D$ +2.5° (*c* 0.21, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 228 nm ($\epsilon$ 13 088); IR (liquid film) $\nu_{max}$ 3292 (terminal acetylene), 2200 (acetylene), 2087, 1456, 1252 $cm^{-1}$; FABMS *m/z* 691 (M + Na)$^+$; $^1$H- and $^{13}$C-NMR data, see Table 1.

**Preparation of (*S*)-MTPA Ester.** (*S*)-MTPA ester (**6S**) of compound **6** was prepared in same manner as previously reported.[13]

***S*-MTPA ester of isopetroformyne 1 (6S):** colorless oil; $[\alpha]^{21}_D$ +4.5° (*c* 0.1, $CHCl_3$); $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 2.62 (1H, br s, H-1), 6.02 (1H, m, H-3), 5.50 (1H, dd, $J$ = 15, 6 Hz, H-4), 6.00 (1H, m, H-5), 2.04 (2H, m, $H_2$-6), 1.32 (2H, m, $H_2$-7), 1.47 (2H, m, $H_2$-16), 2.20 (2H, m, $H_2$-17), 6.33 (1H, s, H-20), 5.50 (1H, d, $J$ = 16 Hz, H-23), 6.23 (1H, dt, $J$ = 16, 7 Hz, H-24), 2.15 (4H, m, $H_2$-25 and $H_2$-26), 5.32 (1H, m, H-27), 1.73 and 1.61 (2H, m, $H_2$-41), 5.98 (1H, m, H-42), 5.96 (1H, m, H-43), 5.67 (1H, m, H-44), 3.26 (1H, br s, H-46), 5.35 (4H, m, protons of isolated double bonds), 1.26 (methylene protons).

**23,24-Dihydropetroformyne 4 (7):** colorless oil; $[\alpha]^{21}_D$ +5° (*c* 0.05, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 228 nm ($\epsilon$ 27 000); IR (liquid film) $\nu_{max}$ 3303 (terminal acetylene), 2220 (acetylene), 1459 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 2.56 (1H, d, $J$ = 2 Hz, H-1), 4.84 (1H, m, H-3), 5.62 (1H, dd, $J$ = 15, 6 Hz, H-4), 5.91 (1H, dt, $J$ = 15, 7 Hz, H-5), 2.08 (2H, m, $H_2$-6), 2.02 (8H, m, $H_2$-11, $H_2$-14, $H_2$-26, $H_2$-29), 2.23 (4H, m, $H_2$-17 and $H_2$-23), 5.09 (1H, br s, H-20), 1.52 (2H, m, $H_2$-24), 1.38 (2H, m, $H_2$-25), 2.32 (2H, app q, $J$ = 7 Hz, $H_2$-42), 6.01 (1H, dt, $J$ = 11, 7 Hz, H-43), 5.44 (1H, d, $J$ = 11 Hz, H-44), 3.06 (1H, d, $J$ = 2 Hz, H-46), 5.35 (4H, m, protons of isolated double bonds), 1.26 (methylene protons); FABMS *m*/*z* 677 (M + Na)$^+$.

**20-Oxoisopetroformyne 4 (8):** Colorless oil; $[\alpha]^{21}_D$ +0.25° (*c* 0.10, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 228 nm ($\epsilon$ 19 000); IR (liquid film) $\nu_{max}$ 3297 (terminal acetylene), 2183 (acetylene), 1622 (C=O, ketone), 1459 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 2.56 (1H, d, $J$ = 2 Hz, H-1), 4.84 (1H, m, H-3), 5.62 (1H, br dd, $J$ = 15, 6 Hz, H-4), 5.91 (1H, dt, $J$ = 15, 7, Hz, H-5), 2.08 (2H, m, $H_2$-6), 2.02 (6H, m, $H_2$-11, $H_2$-14, $H_2$-29), 2.40 (2H, t, $J$ = 7 Hz, $H_2$-17), 5.64 (1H, d, $J$ = 10 Hz, H-23), 6.32 (1H, dt, $J$ = 10, 7 Hz, H-24), 2.42 (2H, m, $H_2$-25), 2.22 (2H, m, $H_2$-26), 2.32 (2H, app q, $J$ = 7 Hz, $H_2$-42), 6.01 (1H, dt, $J$ = 11, 7 Hz, H-43), 5.44 (1H, br d, $J$ = 11 Hz, H-44), 3.06 (1H, d, $J$ = 2 Hz, H-46), 5.35 (4H, m, protons of isolated double bonds), 1.26 (methylene protons); FABMS *m*/*z* 673 (M + Na)$^+$.

**20-Oxoisopetroformyne 3 (9):** colorless oil; $[\alpha]^{21}_D$ +2.7° (*c* 0.13, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 228 nm ($\epsilon$ 21 000); IR (liquid film) $\nu_{max}$ 3309 (terminal acetylene), 2189 (acetylene), 1626 (C=O, ketone), 1461, 1252 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 2.56 (1H, d, $J$ = 2 Hz, H-1), 4.84 (1H, m, H-3), 5.62 (1H, br dd, $J$ = 15, 6 Hz, H-4), 5.91 (1H, dt, $J$ = 15, 7 Hz, H-5), 2.08 (2H, m, $H_2$-6), 2.02 (6H, m, $H_2$-11, $H_2$-14, $H_2$-29), 2.40 (2H, t, $J$ = 7 Hz, $H_2$-17), 5.64 (1H, d, $J$ = 10 Hz, H-23), 6.32 (1H, dt, $J$ = 10, 7 Hz, H-24), 2.42 (2H, m, $H_2$-25), 2.22 (2H, m, $H_2$-26), 1.55 (2H, m, $H_2$-43), 2.18 (2H, m, $H_2$-44), 1.92 (1H, t, $J$ = 2 Hz, H-46), 5.35 (4H, m, protons of isolated double bonds), 1.26 (methylene protons); FABMS *m*/*z* 675 (M + Na)$^+$.

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**Supporting Information Available:** Copies of $^1$H-NMR spectra of compounds **5**, **5a**, **6**, **6s**, and **7**−**9** (8 pages). Ordering information is given on any current masthead page.

## References and Notes

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