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FURTHER STRUCTURAL STUDIES ON THE PETROFORMYNES

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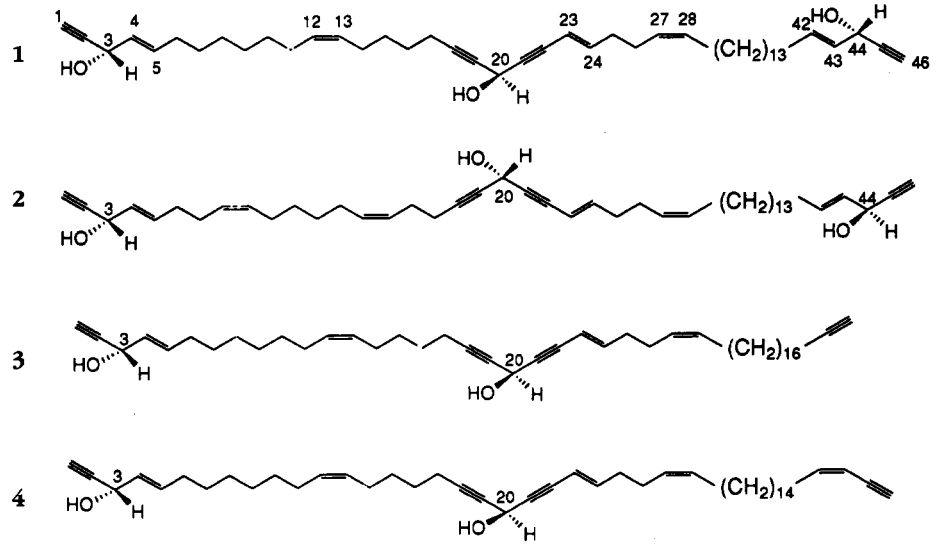
ABSTRACT.—Petroformynes are unusual metabolites contained in the Mediterranean sponge *Petrosia ficiformis*. The structure and absolute stereochemistry of the most abundant of these compounds, which display long unbranched alkyl chains, have only recently been rigorously proven. The structures of twelve minor compounds are now reported, providing a better understanding of the chemistry of *P. ficiformis*. In addition, complete spectroscopic characterization of eleven partial structures (**a–k**) frequently contained in *P. ficiformis* metabolites is provided as an aid to future structure elucidation among compounds of this type.

Polyacetylenes represent a unique class of natural products that possess a variety of biological activities ranging from antimicrobial to cytotoxic and antitumor (1,2). The Mediterranean sponge *Petrosia ficiformis* Poiret (family Petrosiidae, order Petrosida/Haplosclerida) contains unusual polyacetylenes (3–7), the petroformynes, which are characterized by long unbranched alkyl chains, and which also show interesting bioactivity (3). Recently, we have reinvestigated the *Petrosia* polyacetylenes in order to establish their absolute stereochemistry (8). Application of high-field nmr to Mosher's method determined the stereochemistry (*S*) at all chiral centers of petroformynes-1–5, and petroformynes A and B. In addition, the mol wts of petroformynes-1–9 (3), previously deduced from the gc retention time of the parent hydrocarbons, were definitively determined (8) by recording positive fabms spectra with a matrix of *m*-nitrobenzyl alcohol (9). In the course of this study, a series of minor metabolites was also isolated. The present paper deals with isolation and structural elucidation of the novel molecules **5–16**, all closely related to the more abundant petroformynes-1–4 [**1–4**] but exhibiting either different oxidative patterns or isomerization of double bonds. The complete characterization, mainly by nmr spectroscopy, of a series of partial fragments



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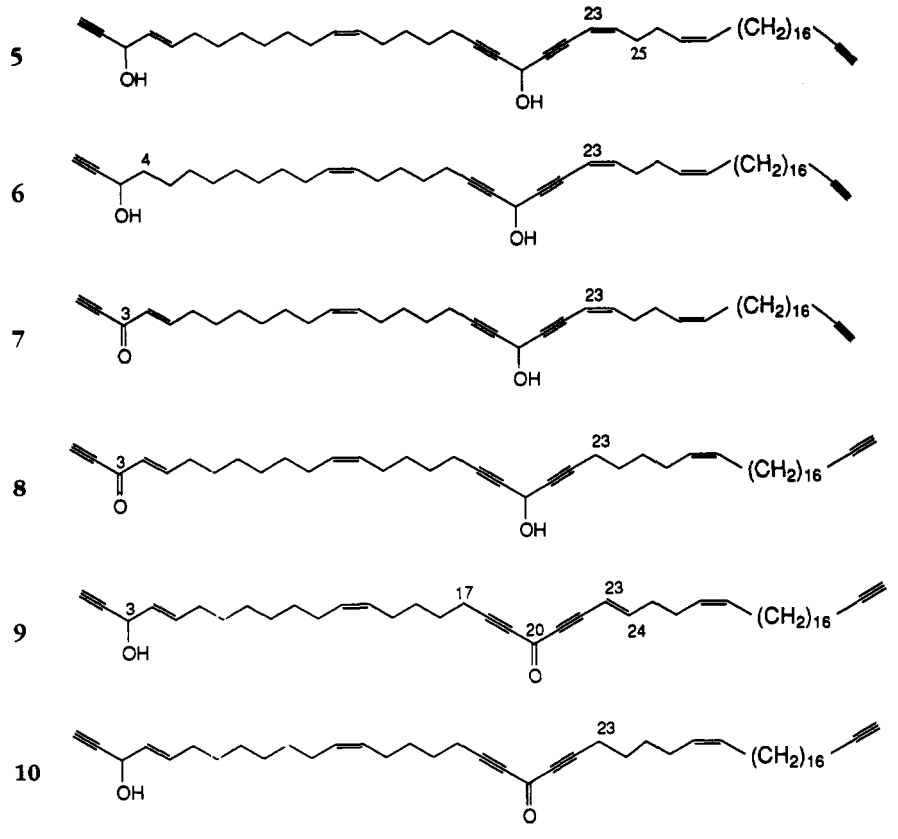
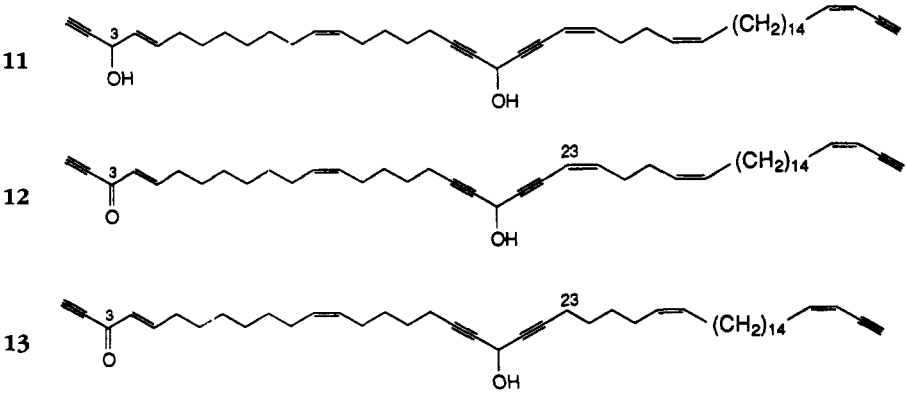


(**a–k**) frequently occurring in *Petrosia* metabolites is also presented as an aid to elucidating the structures of related metabolites.

RESULTS AND DISCUSSION

Specimens of the two varieties of the Mediterranean *P. ficiformis* (red variety from sunlit waters, white variety from dark caves) were collected off Naples. The two varieties of the sponge were separately extracted with Me_2CO. The Et_2O-soluble fraction of the Me_2CO extract was chromatographed on a Si gel column eluting with petroleum ether and increasing amounts of Et_2O according to the previous report (3). The fractions were combined by tlc analysis (SiO_2; petroleum ether-Et_2O, 1:1). This procedure yielded, in



order of increasing polarity, three main fractions, A, B, and C, from the white variety, whereas four acetylenic fractions, A′–D′, were collected from the red variety. Fraction E′ was eluted with $CHCl_3$-MeOH (8:2) from the red variety.

All these fractions, except A and B, which were directly submitted to prep. hplc, were purified by further cc and prep. hplc. Eight novel metabolites [**5–10**, **12**, and **13**] were isolated together with petroformynes-6 and -7 (3) from fractions A and B of the white variety. Compound **11** was isolated from fraction C1 together with petroformynes-3 [**3**] and -4 [**4**]. Fraction B′ of the red variety yielded three new polyacetylenes [**14–16**] together with petroformyne-8 [**17**] (8). The known petroformynes-1–5, -9, and A and B (3) were obtained from other fractions. All of the new compounds demonstrated considerable spectral analogy with the previously reported petroformynes.

Compounds **5–13** showed ir absorptions indicative of the presence of hydroxy functions (3600 cm^{-1}), terminal acetylenes (3300 cm^{-1}), and disubstituted acetylene (2220 cm^{-1}) groups. Their nmr spectra were reminiscent of those of petroformynes-3 [**3**], and -4 [**4**] (3). In fact, compounds **5–10** displayed a terminal acetylene like that of **3** (partial structure **a**, Figure 1), whereas **11–13** exhibited the same terminal structure of **4** (partial structure **b**, Figure 1). The ^{1}H-nmr spectra of **5–13** showed other structural analogies with known petroformynes: an absence of terminal methyls, evidence for long unbranched alkyl chains (strong signals at δ 1.26), and the presence of isolated double bonds (peaks at δ 5.35, 2.02).

Compound **5**, isopetroformyne-3, was the isomer at Δ^{23} of petroformyne-3 [**3**]. Its molecular formula was deduced to be $C_{46}H_{70}O_2$ by hrfabms [*m/z* 677.5278, $(M+Na)^+$,

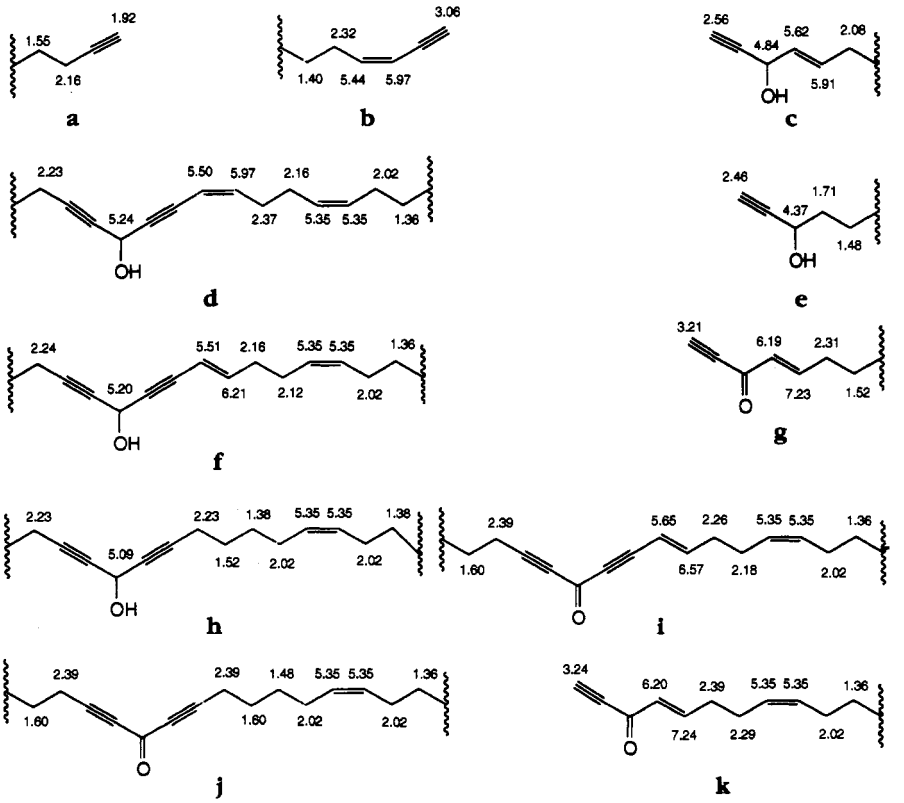


FIGURE 1. Partial structures **a** [5], **b** [11], **c** [5], **d** [5], **e** [6], **f** [15], **g** [7], **h** [8], **i** [9], **j** [10], and **k** [17] with ^{1}H-nmr assignments.

($\Delta -0.7$ ppm)] and ^{13}C-nmr spectroscopy. The analysis of the ^{1}H-nmr spectrum of **5**, in comparison with that of petroformyne-3, identified partial structures **a** and **c**, which were located at the ends of the molecule. In addition, the nmr spectra exhibited signals attributable to the partial structure **d** (Figure 1). The *Z* stereochemistry for the conjugated double bond in unit **d** was inferred from the ^{1}H-nmr coupling constant ($J=10.8$ Hz) of the olefinic protons and from the ^{13}C-nmr resonance (δ 30.4) for the vinyl C-25 signal (10). Conversely, the *E* stereochemistry at Δ^{23} of **3** was previously supported by the chemical shift of C-25 at δ 33.4 (4). Because of the change in the orientation of the Δ^{23} double bond, the signals of H-20 and H-24 in the ^{1}H-nmr spectrum of **5** were shifted downfield and upfield, respectively (H-20 from δ 5.20 to 5.24, H-24 from δ 6.20 to 5.97) (Figure 1). All ^{1}H- and ^{13}C-nmr assignments according to structure **5** were confirmed by ^{1}H-^{1}H COSY, HMQC, and HMBC experiments.

Compound **6**, 4,5-dihydroisopetroformyne-3, showed ^{1}H-nmr data very similar to those of **5**, consistent with the presence of the partial structures **a** and **d** (Figure 1). The ^{13}C-nmr spectrum of **6**, in comparison with that of **5**, exhibited only six olefinic carbons (Table 1), assignable to three *Z* oriented double bonds, while the characteristic ^{13}C-nmr signals (δ 128.6, 134.3) of the $\Delta^{4}E$ oriented double bond were absent. A fabms spectrum of **6** showed a peak at *m/z* 679 $(M+Na)^{+}$, two mass units more than **5**, supporting the Δ^{4} reduction. All homo- and heteronuclear nmr spectra confirmed the presence of the partial unit **e** (Figure 1). In fact, a long-range coupling of 2.1 Hz was observed between the acetylenic proton at δ 2.46 (d, $J=2.1$ Hz) and the carbinol proton at δ 4.37 (dt, $J=2.1$ and 7.0 Hz). This was in turn coupled with a complex multiplet (δ 1.71, H_2-4). The presence of partial structure **e** was further supported by comparison with model compounds (11,12).

Compound **7**, isopetroformyne-6, had a molecular formula of $C_{46}H_{68}O_2$, established by fabms and ^{13}C-nmr data, which was identical to that of petroformyne-6, differing from **3** only by the oxidation to a ketone of the secondary alcohol at C-3. Comparison of the ir, uv, and ^{1}H- and ^{13}C-nmr spectra of **7** with those of petroformyne-6 (3) revealed that the only differences were attributable to the stereochemistry of the Δ^{23} double bond: *Z* for **7** (see partial structure **d**, Figure 1), *E* for petroformyne-6 (partial structure **f**, Figure 1). The presence of the partial structures **a**, **c**, and **d** (Figure 1), along with the close spectral similarities with petroformyne-6, led to structure **7**.

Compound **8**, 23,24-dihydropetroformyne-6, yielded a fabms peak at *m/z* 677 $(M+Na)^{+}$, of two mass units more than that of **7**. The ^{1}H- and ^{13}C-nmr spectra of **8** revealed a close relationship with both **7** and petroformyne-6. The presence of **g** and **a** at the ends of the molecule was very clear. Both ^{1}H-^{1}H COSY and HMQC experiments implied the presence of the partial structure **h** (Figure 1). In particular, the ^{13}C-nmr spectrum of **8** displayed six olefinic carbon signals assignable to Δ^{4} (δ 132.0, 155.5), Δ^{12} (δ 129.6, 130.1) and Δ^{27} (δ 129.3, 130.2) double bonds (Table 1), but without the diagnostic signals for the Δ^{23} (δ 108.4 and 144.8) double bond. The absence of the Δ^{23} conjugated double bond shifted upfield H-20 (from δ 5.20 to 5.09) further supporting structure **8** for this isolate.

The molecular formula of compound **9**, 20-oxo-petroformyne-3, $C_{46}H_{68}O_2$, was identical to that of **7**, as deduced by fabms [*m/z* 675, $(M+Na)^{+}$]. Nevertheless, the spectroscopic properties of **9** were somewhat different from those of **7**. ^{1}H- and ^{13}C-nmr spectra of **9** showed the presence of a carbonyl (δ ^{13}C 198.3), a secondary hydroxy group (δ ^{1}H 4.84, δ ^{13}C 62.9), two terminal acetylenes (δ ^{13}C 74.0, 68.0), and four disubstituted double bonds (Table 1). ^{1}H-^{1}H COSY, HMQC, and HMBC experiments placed the fragments **a** and **c** of **3** at the ends of the molecule but also yielded evidence for the partial structure **i** (Figure 1). Oxidation of the secondary hydroxy group at C-20 of **3** shifted

TABLE 1. Selected ^{13}C-Nmr Chemical Shifts for Compounds **5**–**17**.[a]

Carbon	Compound												
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14a**[b]	**15**	**16**	**17**
1	73.9	72.8	78.9	78.9	74.0	73.9	73.9	78.9	78.9	73.9	78.9[e]	78.9[e]	79.1
2	83.3	80.0	78.4	78.1	83.3	83.7	83.3	78.1	78.1	83.3	78.1	78.4	78.1
3	62.8	62.3	177.8	177.8	62.8	62.7	62.8	177.7	177.6	62.7	177.8	177.5	177.5
4	128.6	37.6	132.0	132.0	128.7	128.6	128.5	132.0	132.0	128.1	132.0[f]	132.2[f]	132.3
5	134.3	24.9	155.5	155.5	134.1	134.1	134.3	155.5	155.5	134.1	155.8[g]	154.5[g]	154.6
6	31.8		32.5	32.5	31.8	31.8	31.8	32.5	32.5	31.6	32.5[h]	32.5[h]	32.5
7	28.5[c]		28.1[c]	28.1[c]	28.6[c]	28.5[c]	28.6[c]	28.4[c]	28.1[c]	28.4[c]	28.4[c]	26.3	26.3
8												129.2	128.1
9												129.1	129.1
10	29.1[c]	29.1[c]	29.1[c]	28.9[c]	29.1[c]	29.1[c]	29.2[c]	29.2[c]	29.2[c]	29.2[c]	29.2[c]	27.2[d]	27.3[d]
11	27.4[d]	27.4[d]	27.4[d]	27.3[d]	27.5[d]	27.4[d]	27.2[d]	27.2[d]	27.2[d]	27.2[d]	27.2[d]	29.1[c]	29.2[c]
12	129.6	129.6	129.6	129.6	129.6	129.6	129.6	129.6	129.6	129.6	131.4	29.3[c]	28.9[c]
13	130.1	130.1	130.1	130.1	130.1	130.1	130.1	130.1	130.1	130.1	130.1	27.1[d]	27.5[d]
14	27.5[d]	27.1[d]	27.0[d]	27.1[d]	27.1[d]	27.1[d]	27.1[d]	27.1[d]	27.1[d]	27.1[d]	27.1[d]	129.6	129.2
15	28.8[c]	28.9[c]	28.8[c]	28.7[c]	28.8[c]	28.9[c]	28.7[c]	28.7[c]	28.7[c]	28.8[c]	28.6[c]	130.2	130.1
16	28.3[c]	28.7[c]	28.6[c]	28.5[c]	28.6[c]	28.8[c]	28.5[c]	28.6[c]	28.9[c]	28.5[c]	28.1[c]	25.6	25.6
17	18.6	18.6	18.7	18.6	18.6	19.0	18.6	18.7	18.6	18.6	18.6	19.0	19.0
18	85.5	85.6	85.3	85.0	95.1	94.5	85.5	84.8	84.8	85.5	85.5	85.3	85.3
19	80.8	80.8	81.1	79.8	82.4	82.4	81.3	81.4	80.6	80.8	80.7	80.6	80.8
20	52.9	52.9	52.9	52.5	198.3	195.1	52.9	52.9	52.5	52.7	52.8	53.2	52.8
21	90.9	90.9	90.8	85.0	90.0	82.4	90.9	90.8	79.8	90.2	90.1	90.3	90.2
22	77.7	77.8	77.8	79.8	88.7	94.5	80.6	80.5	84.9	80.1	80.1	80.1	80.2
23	108.4	108.4	108.4	18.6	107.8	19.0	108.4	108.4	18.6	109.0	108.9	109.0	108.9
24	144.8	144.8	144.3	28.4[c]	153.5	28.6[c]	144.8	144.9	28.6[c]	145.5	145.7	145.8	145.8
25	30.4	30.4	30.4	28.5[c]	33.7	29.3[c]	30.4	30.4	29.3[c]	33.6	33.1	33.2	33.2
26	26.4	26.4	26.4	26.6	25.9	26.6	26.4	26.5	26.7	26.3	26.3	26.3	26.3
27	128.5	128.5	128.5	129.3	127.5	128.9	128.6	128.5	129.3	128.6	128.1	128.3	129.6
28	130.8	130.8	130.8	130.2	131.4	130.5	130.8	130.8	130.2	130.9	130.9	130.9	130.9
29	27.2[d]	27.2[d]	27.2[d]	27.4[d]	27.2[d]	27.2[d]	27.4[d]	27.6[d]	27.6[d]	27.5[d]	27.3[d]	27.8[d]	27.2[d]
30	29.2[c]	28.5[c]	28.5[c]	27.9[c]	28.5[c]	29.4[c]	29.5[c]	29.1[c]	29.2[c]	28.2[c]	27.8[c]	28.5[c]	28.8[c]
40										27.8[c]	27.6[c]	28.2[c]	29.1[c]
41							29.3[c]	29.3[c]	29.3[c]	32.6	32.7[h]	32.7[h]	31.9
42							30.3	30.3	30.3	155.9	155.5[g]	155.9[g]	134.6
43	27.1[d]	27.3[d]	27.1[d]	27.2[d]	27.3[d]	27.2[d]	146.3	146.3	146.3	131.9	131.9[f]	132.0[f]	128.3
44	18.4	18.4	18.4	18.4	18.4	18.4	107.9	107.9	107.9	177.8	177.8	177.5	62.8
45	84.8	84.8	84.8	84.8	84.8	85.0	73.9	73.9	73.9	78.4	78.1	78.1	83.3
46	68.0	68.0	68.0	68.0	68.0	68.0	81.1	81.1	81.1	78.9	78.8[e]	78.6[e]	73.9

[a]WM 500 Bruker Spectrometer; δ values are reported in ppm referenced to $CDCl_3$ (δ 77.0) as internal standard. Assignments deduced from the analysis of mononuclear and heteronuclear spectra and comparison with known petroformynes (4). The methylene values not reported contributed to a large signal at δ 29.8.

[b]δ Values for C-1–C-6 of **14a** are assignable to C-46–C-41 of **14b**, respectively, while δ values for C-41–C-46 of **14a** are attributable to C-1–C-6 of **14b**, respectively.

[c–h]Similar values belonging to the same structure and marked with same superscript are interchangeable.

downfield H-23 (from δ 5.51 to 5.65), H-24 (from δ 6.20 to 6.57), and H_2-17 (from δ 2.23 to 2.39). Comparison with model compounds (7) further confirmed the partial structure **i** and led to structure **9**.

Compound **10**, 23,24-dihydro-20-oxo-petroformyne-3, was the 23,24-dihydro derivative of **9** (fabms peak at *m/z* 677; molecular formula $C_{46}H_{70}O_2$). The ^{1}H-nmr spectrum of **10** showed the presence of **a** and **c** at the ends of the molecule. The ^{13}C-nmr spectrum, analogous to that of **8**, displayed only six sp^2 carbon signals (δ 128.6, 134.1, 129.6, 130.1, 128.9, 130.5) (Table 1) and lacked the diagnostic signals at δ 107.8 (C-23) and 153.5 (C-24), suggesting the presence of fragment **j** (Figure 1). The ^{1}H-nmr signal at δ 2.39, integrating for 4 protons, was in agreement with two methylenes at C-17 and C-23. The carbonyl group at C-20 was supported by the ^{13}C-nmr resonance at δ 195.1. All 2D nmr experiments confirmed structure **10**.

Compound **11**, isopetroformyne-4, with a molecular formula of $C_{46}H_{68}O_2$ [hrfabms *m/z* 675.5108 $(M+Na)^+$ (Δ −1.3 ppm)], was identical to petroformyne-4 [**4**]. Interpretation of ^{1}H-^{1}H COSY, HMQC, and HMBC spectra, in comparison with those of **4**,

revealed that the difference between **11**, containing the partial structures **b**, **c**, and **d**, and **4**, containing **b**, **c**, and **f**, was the orientation of the Δ^{23} double bond (*Z* in **11**; *E* in **4**), whereas the remaining resonances were almost identical.

The fabms spectrum of compound **12**, isopetroformyne-7, showed a peak at *m/z* 673 $(M+Na)^+$, two mass units fewer than **11**. Analysis of the nmr spectra of **12**, compared to those of **11**, identified the partial structures **g**, **b**, and **d**, suggesting the oxidation of OH-3. Isopetroformyne-7 [**12**] is the *Z* Δ^{23} isomer of petroformyne-7.

Compound **13**, 23,24-dihydropetroformyne-7, showed a fabms peak at *m/z* 675 $(M+Na)^+$. The molecular formula was deduced, in conjunction with ^{1}H- and ^{13}C-nmr spectra, as $C_{46}H_{68}O_2$. The ^{1}H- and ^{13}C-nmr spectra of **13** showed, as in **12**, characteristic resonances (Table 1) of partial structures **g** and **b** together with fragment **h**, indicating reduction of the Δ^{23} double bond.

Compounds **14–16** were isolated from the red variety of *P. ficiformis*. Their ir spectra presented an intense band at ν max 1645 cm^{-1} typical of a conjugated carbonyl group. Their nmr spectra were reminiscent of those of petroformyne-1 [**1**] and -2 [**2**], displaying signals easily assignable to the partial structure **f** (Figure 1).

The fabms spectrum of compound **14**, petroformyne-10, showed a peak at *m/z* 689 $(M+Na)^+$, two mass units fewer than the co-occurring petroformyne-1 [**1**]. The nmr spectra of **14** showed other structural analogies with those of **1**: the presence of the terminal 1-yn-3-ol-4-ene moiety (**c**, Figure 1), ten sp^2 ^{13}C-nmr signals, and the absence of methyls. In addition, the nmr spectra exhibited signals attributable to the partial structure **g** (Figure 1), which was easily confirmed by comparison with **7**, **8**, **12**, and **13**. Bearing in mind the close spectral similarities between **14** and **1**, two alternative structures (**14a** or **14b**) were suggested.

The structures **14a/14b** are formally identical to those previously suggested for petroformyne-8 (3). Reanalysis of the ^{1}H- and ^{13}C-nmr data, particularly the number of sp^2 carbons in the ^{13}C-nmr spectra (Table 1), recently led to the revised structure **17** for petroformyne-8 that contains the partial structures **f**, **g**, and **k** (8). The Δ^8 double bond of the latter partial structure induces diagnostic ^{1}H-nmr shifts for H_2-6 and H_2-7. The fabms peak at *m/z* 687 $(M+Na)^+$ for **17**, two mass units fewer than **14a/14b**, supported this revision.

The ^{1}H-nmr spectrum of **15**, 3,44-dioxo-petroformyne-1, showed two sets of signals assignable to the partial structures **f** and **g** while the signals at δ 2.56 (H-1) and 4.84 (H-3), diagnostic for the unit **c**, were absent. The ^{13}C-nmr spectrum of **15** exhibited three pairs of signals at δ 78.9, 78.8, 132.0, 131.9, 155.8, and 155.5 (Table 1), attributable to two partial structures **g** consistent with two terminal 1-yn-3-oxo-4-ene groups. The fabms peak of **15** at *m/z* 687 $(M+Na)^+$, two mass units fewer than **1**, supported structure **15**.

In a similar manner, the ^{1}H- and ^{13}C-nmr spectra of **16**, 3,44-dioxo-petroformyne-2, were very similar to those of **17**, suggesting the presence of the fragments **f** and **k**, but with the partial structure **c** substituted by **g**. Three groups of ^{13}C-nmr coupled signals at δ 78.9, 78.6, 132.2, 132.0, 154.5, and 155.9 (Table 1) and the fabms peak at *m/z* 685 $(M+Na)^+$ further supported structure **16**.

The absolute stereochemistry of **14** was determined by applying the modified Mosher's method (13). The (*S*)- and (*R*)-MTPA esters of **14** were prepared by treatment of **14** with (*R*)- and (*S*)-MTPA chloride. The $\Delta\delta$ ($\delta_{S\text{-ester}}$-$\delta_{R\text{-ester}}$) values are summarized in the structures **14c/14d** (Figure 2). They are identical to those recorded for the MTPA esters of petroformynes-3 [**3**] and -4 [**4**] (8), and indicate an *S* absolute stereochemistry at C-3/C-44 and C-20.

It is interesting to note that all petroformynes studied until now exhibit *S* absolute configuration at all the chiral centers and have positive $[\alpha]_D$ values. In contrast, 3-

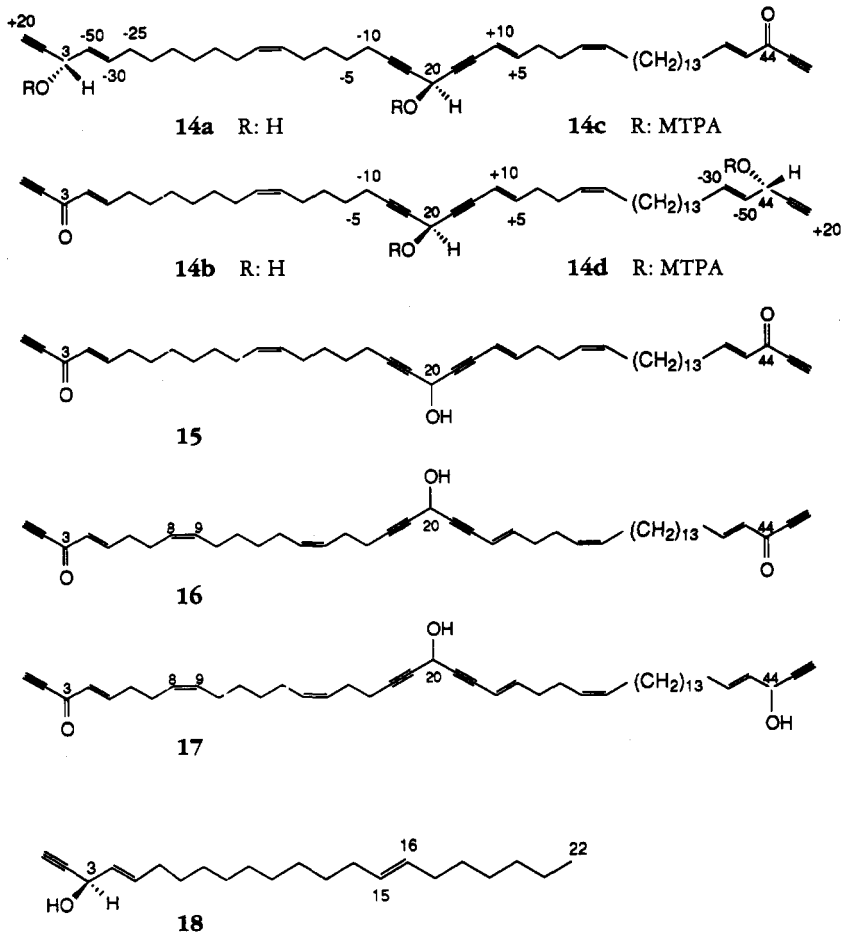


FIGURE 2. Structures of polyacetylenes **14–18** and $\Delta\delta$ (δ_S-δ_R) values (Hz) obtained for the MTPA esters of petroformyne-10 [**14**].

hydroxydocosa-4(*E*),15(*E*)-dien-1-yne [**18**], a metabolite from *Cribrochalina vasculum*, displayed an *R* configuration at C-3 (8,12) and also a negative optical rotation {[α]D $-44°$ (c=0.20, MeOH)}. Strangely enough, the optical rotation of compound **14** is negative although its absolute stereochemistry at all the chiral centers is *S*. This prompted us to attempt to remeasure the [α]D value of **14** on a larger amount of material to avoid any error due to the presence of optically active impurities. Many samples of *P. fciformis* were recollected, but, unfortunately, they showed different metabolite patterns, all exhibiting **14** in very small amounts.

The absolute stereochemistry of compounds **5–13** remains to be determined, because of the scarcity of material, while small amounts of MTPA esters of compounds **15–17** did not allow us to confidently record ^{1}H-nmr spectra. However, the consideration that these compounds are closely related to the co-occurring petroformynes-1–4 [**1–4**], for which the absolute stereochemistry has recently been established as *S* at all the chiral centers (8), and that the minor metabolite petroformyne-10 [**14**] also displayed the same absolute configuration, suggests identical stereochemistry for all secondary alcohol functions of these minor petroformynes, although polyacetylenes with opposite stereochemistry co-occur in a *Petrosia* sp. recently collected from Sukumo Bay, Japan (11).

TABLE 2. Biological Activity of Some Petroformynes.

Compound	5	6	9	10	11	13	14	15	16	17
Brine Shrimp Assay (LD_{50}, ppm)	0.26	6.8	1.0	6.3	0.60	0.50	0.04	0.18	5.0	0.77

Most of these new polyacetylenes were tested for their toxic potential by means of the *Artemia salina* bioassay and showed LD_{50} values ranging from 0.04 to 6.8 ppm (Table 2).

EXPERIMENTAL

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 a 500 MHz Bruker WM 500 spectrometer. Chemical shifts are reported in ppm referenced to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon). ^{1}H- and ^{13}C-nmr assignments were supported by ^{1}H-^{1}H COSY, HMQC, and HMBC experiments. Fabms and hrfabms 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 using a Uvidec-100-III detector. Both Spherisorb-S5 ODS-2 analytical [5 μm, 4.6 mm (i.d.)×25 cm] and semi-prep. [5 μm, 10 mm (i.d.)× 25 cm] columns were employed. Commercial Merck Si gel 60 (70–230 mesh ASTM) was used for cc, and Merck precoated Si gel plates were used for tlc. The chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2N H_2SO_4 and heated at 80° for 5 min to detect the spots.

ANIMAL MATERIAL.—The sponge *P. ficiformis* (red and white varieties) was collected in the Bay of Naples, Italy, by scuba diving at depths of 10–15 m. Voucher specimens are available for inspection at ICMIB [Nos. S-015 (white) and S-106 (red)].

EXTRACTION AND ISOLATION.—The fresh sponge of both varieties (450 g of white and 500 g of red, dry wt after extraction) was immediately extracted with Me_2CO (10 liters). After concentration, the aqueous residues were extracted with Et_2O (3×250 ml). The combined Et_2O extracts were taken to dryness, yielding oily residues [5.2 g (white) and 6.0 g (red)]. The fractionation of polyacetylenes was basically performed as previously reported (3). This yielded, in order of increasing polarity, fractions A (90 mg), B (15 mg), and C (800 mg) from the white sponge variety. In an analogous manner, fractions A′ (80 mg), B′ (510 mg), C′ (1,000 mg), D′ (370 mg), and E′ (1,600 mg) were obtained from the red sponge variety. All these fractions, except A and B of the white sponge variety, which were directly purified by reversed-phase hplc, were again chromatographed on a Si gel column, eluting with light petroleum ether and increasing amounts of Et_2O. This procedure yielded fractions C1 (110 mg), B′1 (63 mg), B′2 (68 mg), C′1 (120 mg), D′1+D′2 (90 mg), and D2 (60 mg) from corresponding fractions. Fraction E′ was first methylated with CH_2N_2, and was then submitted to cc. This yielded fraction E'_M (750 mg).

Prep. hplc isolation of petroformynes [**5–13**] from fractions A, B, and C1 was carried out separately by isocratic elution with MeOH-H_2O (95:5). This yielded **5** (0.6 mg), **6** (1.6 mg), **7** (2.7 mg), **8** (4.2 mg), **9** (3.6 mg), **10** (2.9 mg), **11** (1.0 mg), **12** (1.3 mg), and **13** (2.4 mg), together with petroformynes-3 [**3**] (18.1 mg), -4 [**4**] (20.8 mg), -6 (2.0 mg), and -7 (2.8 mg). Prep. hplc purification of petroformynes **14–16**, and -8 [**17**] from fractions B1 and B2 was conducted by isocratic elution using MeOH-H_2O (96:4). This afforded **14** (10 mg), **15** (8.6 mg), **16** (5.7 mg), and **17** (6.7 mg). The flow rate for all of the separations was 6 ml/min. Hplc isolation of petroformynes-1–4 [**1–4**], -5, -9, and A and B from other fractions was performed as previously reported (3,8).

The $[\alpha]_D$ values recorded for the most abundant petroformynes-1–4 [**1–4**], and A and B, were clearly positive (+13.5°, +10.2°, +11.5°, +13.1°, +5.0°, and +4.5°, respectively) in agreement with those previously reported (3,4), while the $[\alpha]_D$ of petroformyne-5 was +6.8°. Conversely, the $[\alpha]_D$ measurements of some minor petroformynes were negative, but considering the small quantities of the new compounds, some values will require checking at a later date.

ISOPETROFORMYNE-3 [**5**].—Pale yellow oil; $[\alpha]^{21}_D$ +20° (*c*=0.06, $CHCl_3$); ir (liquid film) ν max 3303, 2220, 1495 cm^{-1}; uv (MeOH) λ max 231 nm (ϵ 40,000); fabms *m/z* 677 $(M+Na)^+$; hrfabms *m/z* 677.5278 (Δ −0.7 ppm) for $C_{46}H_{70}O_2$; ^{1}H nmr δ 2.56 (1H, d, *J*=2.0 Hz, H-1), 4.84 (1H, d, *J*=5.9 Hz, H-3), 5.62 (1H, dd, *J*=15.3 and 6.1 Hz, H-4), 5.91 (1H, ddd, *J*=15.3, 6.8, and 7.2 Hz, H-5), 2.08 (2H, ddd, *J*=7.0, 7.0, and 7.2 Hz, H_2-6), 1.40 (4H, m, H_2-7, H_2-42), 1.36 (6H, m, H_2-10, H_2-15, H_2-30), 2.02 (6H, m, H_2-11, H_2-14, H_2-29), 1.55 (4H, m, H_2-16, H_2-43), 2.23 (2H, td, *J*=7.1, 7.1, and 2.0 Hz, H_2-17), 5.24 (1H,

d, J=1.8 Hz, H-20), 5.50 (1H, dd, J=10.8 and 1.5 Hz, H-23), 5.97 (1H, dt, J=10.8, 7.4, and 7.4 Hz, H-24), 2.37 (2H, ddd, J=7.1, 7.2, and 7.3 Hz, H_2-25), 2.16 (4H, m, H_2-26, H_2-44), 5.32–5.42 (4H, m, protons of isolated double bonds), 1.92 (1H, t, J=2.6 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

4,5-Dihydroisopetroformyne-3 [**6**].—Colorless oil; $[\alpha]^{21}D$ +1.8° (c=0.16, $CHCl_3$); ir (liquid film) ν max 3309, 2220, 1456 cm^{-1}; uv (MeOH) λ max 230 nm (ϵ 32,000); fabms m/z 679 $(M+Na)^+$; ^{1}H nmr δ 2.46 (1H, d, J=2.1 Hz, H-1), 4.37 (1H, td, J=6.5, 6.5, and 2.0 Hz, H-3), 1.71 (2H, m, H_2-4), 1.48 (2H, m, H_2-5), 1.36 (4H, m, H_2-10, H_2-30), 2.02 (6H, m, H_2-11, H_2-14, H_2-29), 1.39 (2H, m, H_2-15), 1.53 (4H, m, H_2-16, H_2-43), 2.23 (2H, td, J=7.1, 7.1, and 1.9 Hz, H_2-17), 5.24 (1H, d, J=1.8 Hz, H-20), 5.50 (1H, dd, J=10.8 and 1.5 Hz, H-23), 5.97 (1H, dt, J=10.8, 7.4, and 7.4 Hz, H-24), 2.37 (2H, ddd, J=7.1, 7.4, and 7.5 Hz, H_2-25), 2.16 (4H, m, H_2-26, H_2-44), 5.32–5.42 (4H, m, protons of isolated double bonds), 1.92 (1H, t, J=2.6 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

Isopetroformyne-6 [**7**].—Colorless oil; $[\alpha]^{21}D$ +2.6° (c=0.27, $CHCl_3$); ir (liquid film) ν max 3300, 2204, 2089, 1645, 1457 cm^{-1}; uv (MeOH) λ max 231 nm (ϵ 25,000); fabms m/z 675 $(M+Na)^+$; ^{1}H nmr δ 3.21 (1H, s, H-1), 6.19 (1H, d, J=15.8 Hz, H-4), 7.23 (1H, ddd, J=15.8, 7.1, and 7.1 Hz, H-5), 2.31 (2H, m, H_2-6), 1.52 (6H, m, H_2-7, H_2-16, H_2-43), 1.35 (6H, m, H_2-10, H_2-15, H_2-30), 2.02 (6H, m, H_2-11, H_2-14, H_2-29), 5.32–5.42 (4H, m, protons of isolated double bonds), 2.24 (2H, td, J=6.9, 6.9, and 1.9 Hz, H_2-17), 5.24 (1H, br s, H-20), 5.50 (1H, d, J=9.7 Hz, H-23), 5.98 (1H, dt, J=9.7, 5.0, and 5.0 Hz, H-24), 2.37 (2H, ddd, J=4.8, 7.1, and 7.3 Hz, H_2-25), 2.16 (4H, m, H_2-26, H_2-44), 1.93 (1H, t, J=2.5 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

23,24-Dihydropetroformyne-6 [**8**].—Pale yellow oil; $[\alpha]^{21}D$ −3.8° (c=0.42, $CHCl_3$); ir (liquid film) ν max 3300, 2182, 2087, 1647, 1461 cm^{-1}; uv (MeOH) λ max 232 nm (ϵ 14,700); fabms m/z 677 $(M+Na)^+$; ^{1}H nmr δ 3.21 (1H, s, H-1), 6.19 (1H, d, J=15.8 Hz, H-4), 7.23 (1H, ddd, J=15.8, 7.0, and 7.0 Hz, H-5), 2.31 (2H, ddd, J=6.9, 7.3, and 7.5 Hz, H_2-6), 1.52 (8H, m, H_2-7, H_2-16, H_2-24, H_2-43), 1.38 (8H, m, H_2-10, H_2-15, H_2-25, H_2-30), 2.02 (8H, m, H_2-11, H_2-14, H_2-26, H_2-29), 5.32–5.38 (4H, m, protons of isolated double bonds), 2.23 (4H, m, H_2-17, H_2-23), 5.09 (1H, d, J=1.7 Hz, H-20), 2.16 (2H, m, H_2-44), 1.93 (1H, t, J=2.6 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

20-Oxo-petroformyne-3 [**9**].—Pale yellow oil; $[\alpha]^{21}D$ −1.0° (c=0.35, $CHCl_3$); ir (liquid film) ν max 3297, 2183, 1611, 1456 cm^{-1}; uv (MeOH) λ max 230 nm (ϵ 19,000); fabms m/z 675 $(M+Na)^+$; ^{1}H nmr δ 2.56 (1H, d, J=2.1 Hz, H-1), 4.84 (1H, br s, H-3), 5.61 (1H, dd, J=15.3 and 6.1 Hz, H-4), 5.91 (1H, dt, J=15.3, 7.1, and 7.2 Hz, H-5), 2.08 (2H, m, H_2-6), 1.42 (4H, m, H_2-7, H_2-15), 1.36 (4H, m, H_2-10, H_2-30), 2.02 (6H, m, H_2-11, H_2-14, H_2-29), 5.32–5.43 (4H, m, protons of isolated double bonds), 1.60 (2H, m, H_2-16), 2.39 (2H, t, J=7.1 Hz, H_2-17), 5.65 (1H, d, J=15.9 Hz, H-23), 6.57 (1H, dt, J=15.9, 7.0, and 6.9 Hz, H-24), 2.26 (2H, m, H_2-25), 2.18 (4H, m, H_2-26, H_2-44), 1.53 (2H, m, H_2-43), 1.93 (1H, t, J=2.6 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

23,24-Dihydro-20-oxo-petroformyne-3 [**10**].—Colorless oil; $[\alpha]^{21}D$ +6.8° (c=0.29, $CHCl_3$); ir (liquid film) ν max 3309, 2208, 1618, 1457 cm^{-1}; uv (MeOH) λ max 231 nm (ϵ 15,700); fabms $(M+Na)^+$ m/z 677; ^{1}H nmr δ 2.56 (1H, d, J=2.1 Hz, H-1), 4.83 (1H, br s, H-3), 5.61 (1H, dd, J=15.3 and 6.1 Hz, H-4), 5.91 (1H, dt, J=15.3, 7.2, and 7.2 Hz, H-5), 2.08 (2H, m, H_2-6), 1.42 (2H, m, H_2-7), 1.36 (4H, m, H_2-10, H_2-30), 2.03 (8H, m, H_2-11, H_2-14, H_2-26, H_2-29), 5.34–5.39 (4H, m, protons of isolated double bonds), 1.48 (4H, m, H_2-15, H_2-25), 1.60 (4H, m, H_2-16, H_2-24), 2.39 (4H, t, J=7.0 Hz, H_2-17, H_2-23), 1.53 (2H, m, H_2-43), 2.18 (2H, td, J=7.1, 7.1, and 2.6 Hz, H_2-44), 1.93 (1H, t, J=2.6 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

Isopetroformyne-4 [**11**].—Pale yellow oil; $[\alpha]^{21}D$ +25° (c=0.10, $CHCl_3$); ir (liquid film) ν max 3301, 1459 cm^{-1}; uv (MeOH) λ max 228 nm (ϵ 41,000); fabms m/z 675 $(M+Na)^+$; hrfabms m/z 675.5108 (Δ −1.3 ppm) for $C_{46}H_{68}O_2$; ^{1}H nmr δ 2.56 (1H, d, J=2.2 Hz, H-1), 4.82 (1H, br d, J=5.9 Hz, H-3), 5.62 (1H, dd, J=15.3 and 6.1 Hz, H-4), 5.91 (1H, dt, J=15.3, 7.2, and 7.2 Hz, H-5), 2.08 (2H, m, H_2-6), 1.40 (6H, m, H_2-7, H_2-15, H_2-41), 1.35 (4H, m, H_2-10, H_2-30), 2.02 (6H, m, H_2-14, H_2-29), 5.32–5.42 (4H, m, protons of isolated double bonds), 1.53 (2H, m, H_2-16), 2.23 (2H, td, J=7.0, 7.0, and 2.0 Hz, H_2-17), 5.25 (1H, br s, H-20), 5.50 (1H, dd, J=10.8 and 1.6 Hz, H-23), 5.97 (2H, m, H-24, H-43), 2.37 (2H, ddd, J=7.3, 7.4, and 7.4 Hz, H_2-25), 2.16 (2H, ddd, J=6.7, 7.0, and 7.1 Hz, H_2-26), 2.32 (2H, ddd, J=7.4, 7.5, and 7.9 Hz, H_2-42), 5.44 (1H, dd, J=10.8 and 2.2 Hz, H-44), 3.06 (1H, d, J=2.2 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

Isopetroformyne-7 [**12**].—Colorless oil; $[\alpha]^{21}D$ +2.5° (c=0.13, $CHCl_3$); ir (liquid film) ν max 3304, 2091, 1647, 1455 cm^{-1}; uv (MeOH) λ max 229 nm (ϵ 31,000); fabms m/z 673 $(M+Na)^+$; ^{1}H nmr δ 3.21 (1H, s, H-1), 6.19 (1H, d, J=15.8 Hz, H-4), 7.23 (1H, dt, J=15.8, 7.0, and 7.0 Hz, H-5), 2.32 (2H, ddd, J=7.4, 7.4, and 7.4 Hz, H_2-6), 1.54 (4H, m, H_2-7, H_2-16), 1.35 (6H, m, H_2-10, H_2-15, H_2-30), 2.02 (6H,

m, H_2-11, H_2-14, H_2-29), 5.32–5.41 (4H, m, protons of isolated double bonds), 2.24 (2H, m, H_2-17), 5.25 (1H, br d, J=5.3 Hz, H-20), 5.50 (1H, dd, J=10.0 and 1.4 Hz, H-23), 5.98 (2H, m, H-24, H-43), 2.36 (2H, ddd, J=7.1, 7.1, and 7.2 Hz, H_2-25), 2.16 (2H, m, H_2-26), 1.41 (2H, m, H_2-41), 5.44 (1H, dd, J=10.9 and 1.8 Hz, H-44), 3.06 (1H, d, J=1.8 Hz, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

23,24-Dihydropetroformyne-7 [**13**].—Pale yellow oil; $[\alpha]^{21}D$ −16.1° (c=0.24, $CHCl_3$); ir (liquid film) ν max 3301, 2186, 2093, 1646 cm^{-1}; uv (MeOH) λ max 230 nm (ϵ 22,370); fabms m/z 675 $(M+Na)^+$; ^{1}H nmr δ 3.21 (1H, s, H-1), 6.19 (1H, d, J=15.8 Hz, H-4), 7.23 (1H, ddd, J=15.8, 7.0, and 7.1 Hz, H-5), 2.32 (4H, m, H_2-6, H_2-42), 1.53 (6H, m, H_2-7, H_2-16, H_2-24), 1.35 (8H, m, H_2-10, H_2-15, H_2-25, H_2-30), 2.08 (8H, m, H_2-11, H_2-14, H_2-26, H_2-29), 5.34–5.41 (4H, m, protons of isolated double bonds), 2.23 (4H, td, J=7.0, 7.0, and 1.6 Hz, H_2-17, H_2-23), 5.09 (1H, s, H-20), 1.41 (2H, m, H_2-41), 6.01 (1H, dt, J=11.0, 7.6, and 7.7 Hz, H-43), 5.44 (1H, d, J=11.0 Hz, H-44), 3.06 (1H, br s, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

Petroformyne-10 [**14a/14b**].—Pale yellow oil; $[\alpha]^{21}D$ −3.5° (c=1.0, $CHCl_3$); ir (liquid film) ν max 3292, 2200, 2087, 1642 cm^{-1}; uv (MeOH) λ max 230 nm (ϵ 31,000); fabms m/z 689 $(M+Na)^+$. Alternative structures **14a** and **14b** are proposed; assignments with an asterisk refer to **14b**. ^{1}H nmr δ 2.55 (1H, br s, H-46 or H-1*), 4.84 (1H, br s, H-44 or H-3*), 5.61 (1H, dd, J=15.2 and 5.7 Hz, H-43 or H-4*), 5.90 (1H, dt, J=15.2, 6.5, and 6.5 Hz, H-42 or H-5*), 2.08 (2H, ddd, J=6.8, 6.9, and 6.9 Hz, H_2-41 or H_2-6*), 1.40 (4H, m, H_2-40 or H_2-7*), 3.21 (1H, s, H-1 or H-46*), 6.18 (1H, d, J=15.8 Hz, H-4 or H-43*), 7.24 (1H, ddd, J=15.8, 6.8, and 6.8 Hz, H-5 or H-42*), 2.30 (2H, ddd, J=7.1, 7.2, and 6.7 Hz, H_2-6 or H_2-41*), 1.51 (2H, ddd, J=7.1, 7.2, and 7.3 Hz, H_2-16), 1.35 (6H, m, H_2-10, H_2-15, H_2-30), 2.02 (6H, m, H_2-11, H_2-14, H_2-29), 5.33–5.42 (4H, m, protons of isolated double bonds), 2.24 (2H, t, J=6.0 Hz, H_2-17), 5.20 (1H, br s, H-20), 5.51 (1H, d, J=15.8 Hz, H-23), 6.19 (1H, dt, J=15.8 and 6.5 Hz, H-24), 2.12–2.16 (4H, m, H_2-25, H_2-26), 1.26 (protons of other isolated methylenes); ^{13}C-nmr data, see Table 1.

3,44-Dioxopetroformyne-1 [**15**].—Pale yellow oil; $[\alpha]^{21}D$ −2.5° (c=0.86, $CHCl_3$); ir (liquid film) ν max 3269, 2200, 2092, 1645 cm^{-1}; uv (MeOH) λ max 232 nm (ϵ 38,000); fabms m/z 687 $(M+Na)^+$; ^{1}H nmr δ 3.215 and 3.209 (1H each, s, H-1, H-46), 6.21 (3H, m, H-4, H-24, H-43), 7.24 (2H, ddd, J=15.7, 6.0, and 6.4 Hz, H-5, H-42), 2.31 (4H, m, H_2-6, H_2-41), 1.52 (6H, m, H_2-7, H_2-16, and H_2-40), 1.35 (6H, m, H_2-10, H_2-15, H_2-30), 2.02 (6H, m, H_2-11, H_2-14, H_2-29), 5.34–5.42 (4H, m, protons of isolated double bonds), 2.24 (2H, t, J=6.8 and 6.8 Hz, H_2-17), 5.20 (1H, br s, H-20), 5.51 (1H, d, J=15.9 Hz, H-23), 2.12–2.16 (4H, m, H_2-25, H_2-26), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

3,44-Dioxopetroformyne-2 [**16**].—Colorless oil; $[\alpha]^{21}D$ +2.3° (c=0.57, $CHCl_3$); ir (liquid film) ν max 3260, 2223, 2090, 1646 cm^{-1}; uv (MeOH) λ max 228 nm (ϵ 42,000); fabms m/z 685 $(M+Na)^+$; ^{1}H nmr δ 3.24 (1H, s, H-1), 6.20 (2H, d, J=15.7 Hz, H-4, H-43), 7.23 (2H, ddd, J=15.7, 7.0, and 7.0 Hz, H-5 and H-42), 2.39 (2H, ddd, J=6.7, 6.7, and 6.8 Hz, H_2-6), 2.29 (4H, m, H_2-7, H_2-41, $H_2$16, H_2-17), 5.35–5.38 (6H, m, protons of isolated double bonds), 2.02 (6H, m, H_2-10, H_2-13, H_2-29), 1.36 (6H, H_2-11, H_2-12, H_2-30), 5.20 (1H, br s, H-20), 5.51 (1H, d, J=16.4 Hz, H-23), 6.20 (1H, m, H-24), 2.12–2.16 (4H, m, H_2-25, H_2-26), 1.52 (2H, ddd, J=6.8, 7.2, and 7.4 Hz, H_2-40), 3.21 (1H, s, H-46), 1.26 (methylene protons); ^{13}C-nmr data, see Table 1.

Petroformyne-8 [**17**].—Colorless oil; $[\alpha]^{21}D$ +3.6° (c=0.67, $CHCl_3$); ir (liquid film) ν max 3292, 2200, 2087, 1642 cm^{-1}; uv (MeOH) λ max 231 nm (ϵ 30,000); fabms m/z 687 $(M+Na)^+$; ^{1}H nmr δ 3.24 (1H, s, H-1), 6.20 (1H, d, J=15.8 Hz, H-4), 7.23 (1H, ddd, J=15.8, 6.8, and 6.8 Hz, H-5), 2.39 (2H, ddd, J=7.0, 7.0, and 7.1 Hz, H_2-6), 2.29 (6H, m, H_2-7, H_2-16, H_2-17), 5.32–5.42 (6H, m, protons of isolated double bonds), 2.02 (6H, m, H_2-10, H_2-13, H_2-29), 1.36 (6H, m, H_2-11, H_2-12, H_2-30), 5.20 (1H, br s, H-20), 5.51 (1H, dd, J=15.8 and 1.4 Hz, H-23), 6.20 (1H, dt, J=15.8, 6.5, and 6.5 Hz, H-24), 2.12–2.17 (4H, m, H_2-25, H_2-26), 1.40 (2H, m, H_2-40), 2.07 (2H, ddd, J=7.0, 7.3, and 7.8 Hz, H_2-41), 5.92 (1H, ddd, J=15.3, 7.0, and 7.5 Hz, H-42), 5.61 (1H, dd, J=15.3 and 6.1 Hz, H-43), 4.83 (1H, br d, J=5.8 Hz, H-44), 2.56 (1H, d, J=2.1 Hz, H-46); ^{13}C-nmr data, see Table 1.

PREPARATION OF (*S*)- AND (*R*)-MTPA ESTERS.—(*S*)- and (*R*)-MTPA esters (**14c/14d**) of compound **14** were prepared as previously reported (8). The observed $\Delta\delta$ (δ_S-δ_R) values are reported in Figure 2.

BIOASSAYS.—Brine shrimp (*A. salina*) assays were performed in triplicate in DMSO (1% of final volume), using 10 animals suspended in artificial sea water, as reported by Meyer *et al.* (14). Briefly, for each dose tested survivor shrimps were counted after 24 h and data statistically analyzed by the Finney program (15) which yields LD_{50} values with 95% confidence intervals.

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