Journal of Natural Products
Vol. 53, No. 2, pp. 345-353, Mar-Apr 1990

MINOR BIOACTIVE POLYACETYLENES FROM *PETROSIA FICIFORMIS*

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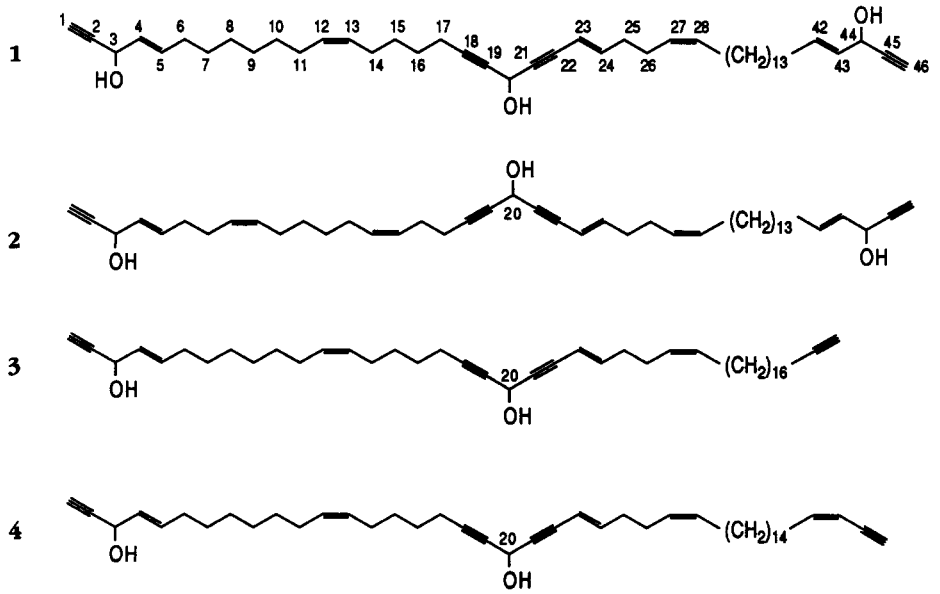
ABSTRACT.—Six novel minor polyacetylenic metabolites were isolated from the marine sponge *Petrosia ficiformis* by means of bioassay-directed purification. Spectral analysis led to partial structure determination, and the brine shrimp lethality assay on the pure compounds allowed a structure/activity relationship study. The potato disc antitumor assay and tests for the inhibition of fertilized sea urchin eggs were also conducted on the most abundant compounds.

Early studies (1,2) on the relationship between the Mediterranean red sponge *Petrosia ficiformis* and its predator, the nudibranch *Peltodoris atromaculata*, led to the isolation and partial structure elucidation of some high-molecular-weight polyacetylenes. Analogous results (3) were also obtained in the same sponge found in dark caves and lacking the symbiotic alga *Aphanocapsa feldmanni*, which is responsible for the red color of the sponge. Several other polyacetylenes, related to the *Petrosia* metabolites but smaller in size, have been isolated from *Xestospongia* and *Cribrochalina* species (4–7), and some interesting biological properties, ranging from antimicrobial to cytotoxic and antimitotic activities, have been described for these compounds (8). We have very recently undertaken a new study of the *Petrosia* polyacetylenes and have obtained preliminary results for the cytotoxic and antimitotic activity of four of the most abundant polyacetylenes (9). Moreover, except for the configuration of chiral centers, we also completed the structure elucidation of these major metabolites **1–4** (9) which were named petroformynes 1–4, respectively.

We now report a bioassay-directed fractionation of the liposoluble extract from *P. ficiformis*, which has led to six minor and novel bioactive polyacetylenes in addition to those previously described (1–3,9). The brine shrimp lethality assay was used to screen throughout the purification procedure and also to test the purified compounds in order to gain a preliminary understanding of the structural prerequisities necessary for this type of activity.



RESULTS AND DISCUSSION

On the day of the collection the sponge (red variety) was thoroughly extracted with Me_2CO. The Et_2O-solution fraction from the MeCO extract was first assayed by means of the *Artemia salina* test (11) and then chromatographed on Si gel columns (1–3,9) (Table 1). All the fractions were assayed for brine shrimp lethality. The active ones were analyzed by tlc [SiO_2, petroleum ether-Et_2O (1:1)] and named A (R_f 0.8), B (R_f 0.65), C (R_f 0.45), D (R_f 0.35), and E (R_f 0.25). A further fraction, named F, was eluted with $CHCl_3$-MeOH (8:2) and also exhibited high bioactivity. The LC_{50} values relative to the activity in the brine shrimp test for these fractions are shown in Table 1. Apart from fraction F, which was first methylated with CH_2N_2, the active fractions were purified by means of reversed-phase hplc. This led to the isolation of the active pure compounds named A1, A2, B1, B2, C5, D3, D4, E2, and E3. Methylated fraction F yielded, after hplc, several active substances, the most abundant of which were named F2 and F5 (Table 1). All compounds showed considerable spectral analogies with the previously reported polyacetylenes (1–3,9). In particular, after hplc and spectral analysis, B1, B2, D3, and D4 were not distinguishable from petroformynes 4, 3, 2, and 1, respectively (1–3,9).

Spectral and chromatographic characteristics of compound E3 were also identical to the ones previously reported (2) for an only partially characterized tetrahydroxypolyacetylene, now named petroformyne 5 [**5a** or **5b**]. The comparison of the nmr spectra of E3 (see Experimental) with that of **1** (9) allowed the addition of further details. The partial structures **a** and **b** were easily recognized:

$$HC\equiv C\text{-}CH(OH)\text{-}CH=CH\text{-}CH_2\text{-}\ [\mathbf{a}] \text{ and}$$
$$\text{-}CH_2\text{-}C\equiv C\text{-}CH(OH)\text{-}C\equiv C\text{-}CH=CH\text{-}CH_2\text{-}CH_2\text{-}CH=CH\text{-}CH_2\text{-}\ [\mathbf{b}]$$

TABLE 1. Brine Shrimp Assay-directed Isolation of Petroformynes.[a]

1st Step	Fraction and Activity	2nd Step	Fraction and Activity
Original Et_2O extract (7 g)	LC_{50} = 4.5		
Petroleum ether	$LC_{50} \gg 100$		
Petroleum ether-Et_2O (9:1)	$LC_{50} \gg 100$		
			5–10 (A, 15 mg) LC_{50} = 0.07
Petroleum ether-Et_2O (6:4)	A + B + C + sterols (1.2 g) LC_{50} = 3.6	Petroleum ether-Et_2O (8:2)	11–15 (B, 30 mg) LC_{50} = 0.06
			26–29 (C, 5 mg) LC_{50} = 0.015
			14–18 (C, 3 mg) LC_{50} = 0.05
Petroleum ether-Et_2O (3:7)	C + D + sterols (1.5 g) LC_{50} = 3.4	Petroleum ether-Et_2O (6:4)	19–21 (C + D, 20 mg) LC_{50} = 0.004
			22–29 (D, 40 mg) LC_{50} = 0.002
Et_2O	E + nonacetylenic lipids (0.6 g) LC_{50} = 9.6	Petroleum ether-Et_2O (4:6)	E (50 mg) LC_{50} = 0.02
$CHCl_3$-MeOH (8:2)	F (0.5 g) LC_{50} = 0.97	hplc	F1–F7 LC_{50} = 0.002–0.004

[a]LC_{50} values are expressed in μg/ml (ppm). Fractions after the second Si gel cc were further purified by means of hplc and the fractions eluted were again assayed (see Table 4).

Furthermore, the ^{1}H-nmr region between δ 2.0 and δ 2.3, characterized by four signals at δ 2.24 (2H, CH_2-C≡C), δ 2.16 (4H,-CH = CH-CH_2-CH_2-CH = CH-), δ 2.07 (2H, CH_2-CH = CH-CH(OH)-C≡CH), and δ 2.02 (6H, CH_2-CH = CH-), differed from that recorded for **2** (9) for the intensity of the signal at δ 2.07 (2 protons instead of 4) according to the presence of only a methylene linked to the terminal allylic carbinols. The coupling pattern (dt, J = 6.4, 6.4 Hz) of the proton signal at δ 4.16 supported the partial structure CH_2-CH(OH)-CH = CH-CH(OH)-C≡CH [**c**]. Suggesting the same carbon skeleton as petroformyne 1 [**1**], and on the basis of the collected evidence, two alternative structures **5a** and **5b** can be proposed for petroformyne 5.

Data acquisition for the minor metabolites A1, A2, C5, E2, F2, and F5 was sometimes limited by their small levels in the sponge.

A1, A2, C5, and E2 presented an intense ir band at ν max 1645 cm^{-1} typical of a conjugated carbonyl group, probably arising from the oxidation of one of the alcohol functions present in the polyacetylenes mentioned above. The nmr analysis supported the presence of the partial structure **b** in all these compounds and showed only few differences with respect to the spectra obtained for structures **1–5**. The discussion will be therefore limited to these differences.

A2, named petroformyne 6 [**6**], showed in the ^{1}H-nmr spectrum (Table 2) some characteristic resonances at δ 3.18 (s), δ 6.20 (d, J = 16.2 Hz), and δ 7.25 (ddd, J = 16.2, 7.2, 7.2 Hz) assigned, bearing in mind the presence of the carbonyl group, to the protons of the partial structure HC≡C-CO-CH = CH-CH_2- [**d**]. The remaining resonances were almost identical to those reported for petroformyne 3 [**3**], leading to structure **6** which, formally, derives from **3** by oxidation of the hydroxy group at C-3. The presence of the carbonyl group was further confirmed by the presence of a small peak in the ^{13}C-nmr spectrum at δ 177.7, only visible using a relatively long relaxation delay during the acquisition. Moreover the ^{13}C resonance of the acetylene C-1 was observed at δ 78.9, while those of the olefinic carbons were at δ 132.2 (C-4) and 155.1 (C-5), in agreement with the conjugation with a carbonyl group. These assignments were further supported by comparison with model compounds (7). 2D-COSY spectrum of A2 (Table 3) confirmed these assignments and also allowed the resolution of the signals between δ 2.32 and 2.0, thus leading to the assignment of all triple- and double-bond-bound methylenes. The presence in petroformyne 6 of the partial structures **b** and **d**, and also of the terminal unconjugated acetylene groups (δ ^{1}H = 1.93, δ ^{13}C = 68.0), along with the close spectral analogies with petroformyne 3 [**3**], led to the structure **6**. The scarcity of material did not allow the performance of chemical reactions such as degradative ozonolysis. However, reduction of A2 with $NaBH_4$ led to a 1:1 mixture of alcohols, one of which had the same retention time, in an hplc isocratic separation, as petroformyne 3 [**3**]. Finally, structure **6** was also supported by the fragment ions obtained in the eims analysis of the TMSi-derivative of this compound.

The analysis of the ^{1}H-nmr spectra of A1, in comparison with those of **4** and 6

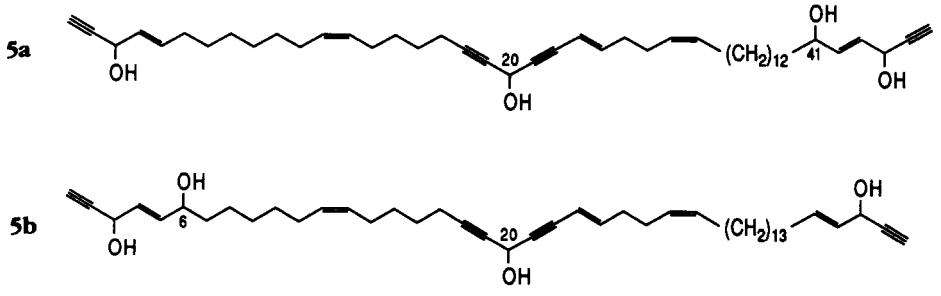


TABLE 2. Selected ^1H and ^{13}C Chemical Shifts for Compounds **6**, **7**, **10** and **11**.[a]

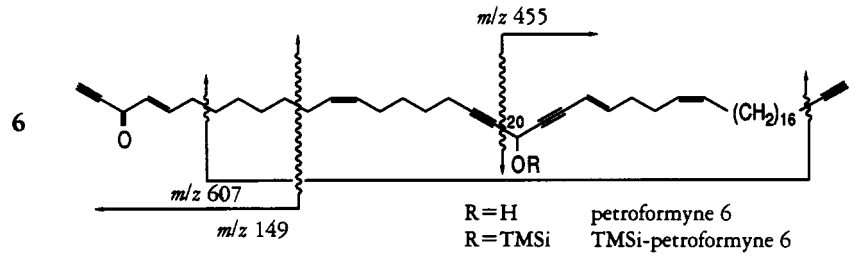
Carbon	Compound					
	6 (A2)		**7** (A1)	**10** (F2)		**11** (F5)
	^1H[b] (*J*, Hz)	^{13}C[c]	^1H[b] (*J*, Hz)	^1H[b] (*J*, Hz)	^{13}C[c]	^1H[a] (*J*, Hz)
1	3.18 s	78.9	3.21 s	2.56 d, 2.0	73.9	2.57 d, 2.0
3		177.7		4.83 bd, 6.0	62.8	4.84 bd, 6.0
4	6.20 d, 16.2	132.1	6.19 d, 15.7	5.60 dd, 15.0 6.0	128.4	5.61 dd, 15.0 6.0
5	7.25 ddd, 16.2 7.2, 7.2	155.1	7.24 ddd, 15.7 7.0, 7.0	5.90 dt, 15.0 7.4	134.7	5.91 dt, 15.0 7.2, 7.2
6	2.32 dt, 7.2 7.0	37.5	2.32[d] bdt, 7.3	2.08	31.9	2.08
7	1.52[d]	28.1[d]	1.53[e]	1.39	28.5	1.36
10	1.35[e]	29.3[e]	1.35[f]			
11	2.02[f]	27.2[f]	2.02[g]			
12	5.35[g]	129.6[g]	5.35[h]			
13	5.38[g]	130.1[g]	5.38[h]			
14	2.00[f]	27.1[f]	2.01[g]			
15	1.38[e]	29.1[e]	1.35[f]			
16	1.53[d]	28.5[d]	1.53[e]			
17	2.23 dt, 7.0 2.5	18.7	2.23 dt, 7.0 2.0			
20	5.20 bs	52.9	5.20 bs			
23	5.52 bd, 16.0	108.9	5.51 bd, 16.0			
24	6.20 dt, 16.0 6.9	145.6	6.20 dt, 16.0 7.0			
25	2.16	33.2	2.16			
26	2.14	26.4	2.14			
27	5.32[g]	128.1[g]	5.32[h]			
28	5.34[g]	130.9[g]	5.34[h]			
29	2.02[f]	27.3[f]	2.02[g]			
30	1.35[e]	29.4[e]	1.35[f]			
37				1.42	28.7	
38				2.38 dt, 14.5 7.2	30.9	1.32
39				6.25 dt, 10.8 7.2	151.7	1.42
40				5.54 d, 10.8	106.4	2.38 dt, 14.4 7.0
41			1.43			6.25 dt, 10.8 7.0
42	1.38[e]	28.5	2.32[d]			5.54 d, 10.8
43	1.53[d]	28.1[d]	6.01 dt, 10.9 7.3			
44	2.15 dt, 7.0 2.6	18.4	5.44 dd, 10.9 1.8			
46	1.93 t, 2.6	68.0	3.06 d, 1.8			
-OMe				3.79 s		3.80 s

[a]Assignments were deduced from the analysis of mono- and two-dimensional nmr spectra.

[b]All signals for unreported methylene protons contributed to a large signal at δ 1.26, or to CH_2 between two double bonds (in **11**, 2H, dd, 2.79 ppm) or to CH_2 near isolated double bonds (δ 2.02).

[c]Signals assigned to quaternary acetylene carbons were observed between δ 77.8 and 85.6, while the methylene values not reported resonated between δ 29.8 and 29.0. Finally, signals for unreported olefinic carbons were due to isolated double bonds and resonated between δ 129.8 and 130.1.

[d–h]Values in the same column with the same superscript may be interchanged.



(Table 2), its reduction with $NaBH_4$, and considerations analogous to the ones described above suggested structure **7** for this metabolite, named petroformyne 7.

^{1}H-nmr spectra of C5 (see Experimental) showed the presence of the sets of signals due to the partial structures **a**, **b**, and **d**. It was therefore obvious to suggest structures **8a** and **8b** deriving from the oxidation of only one of the terminal alcoholic functions present in **1**. Analogously, the ^{1}H-nmr spectra of E2 revealed the presence of the partial structures **b**, **c**, and **d**, suggesting the oxidation of the terminus of petroformyne 5 [**5a** or **5b**] with only one of the hydroxy functions, thus generating either one of structures **9a** or **9b**. Again, reduction of fractions C5 and E2 with $NaBH_4$ and chromatographic identity of the alcohols produced with **1** and **5a/5b**, respectively, supported these findings.

The two methyl esters F2 and F5 both displayed the nmr resonances easily assignable to the partical structures **a** and $-CH_2-CH=CH-C\equiv C-COOMe$ [**e**], where the con-

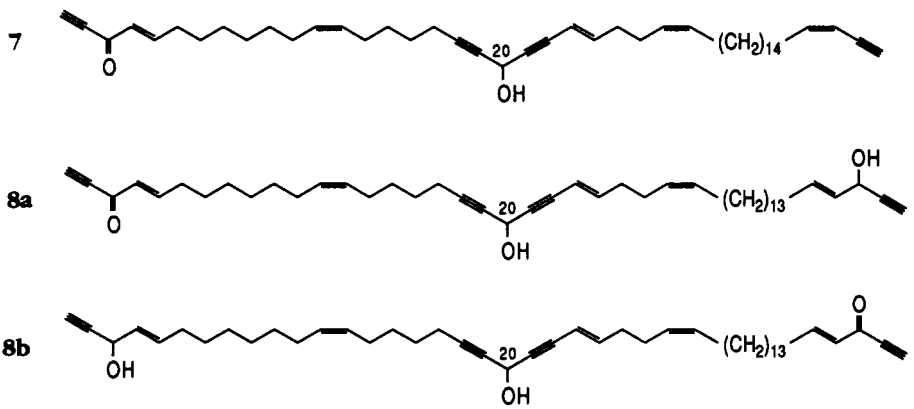
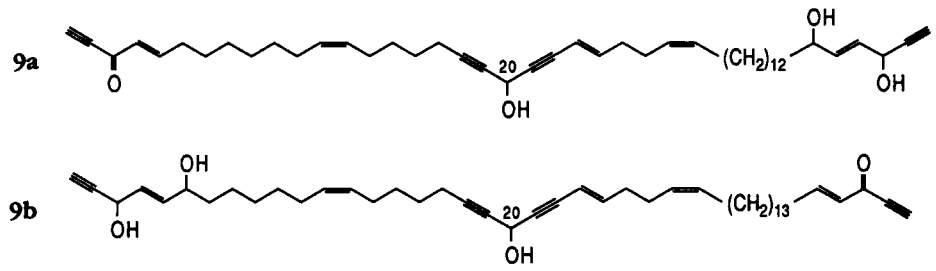


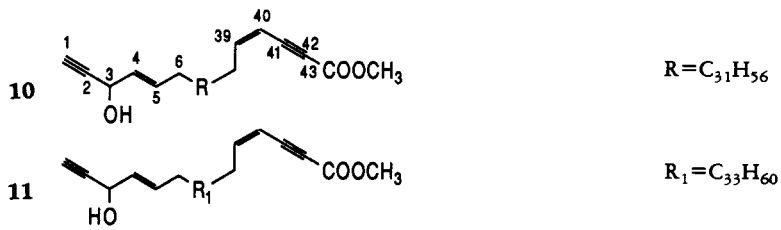
TABLE 3. Relevant Correlations Observed in the ^{1}H-^{1}H COSY Spectrum of **6** (see also Table 2).

δ^1H	δ^1H correlated
7.25 (H-5)	6.20 (H-4), 2.32 (H-6)
6.20 (H-4)	7.25 (H-5), 2.32 (H-6)
6.20 (H-24)	5.52 (H-23), 2.16 (H-25)
5.52 (H-23)	6.20 (H-24)
5.32–5.38 (H-12, -13, -27, -28)	2.02 (H-11, H-14, H-29), 2.14 (H-26)
5.20 (H-20)	5.52 (H-23), 2.23 (H-17)
2.32 (H-6)	5.20 (H-20), 1.53 (H-16)
2.14–2.16 (H-25, H-26)	6.20 (H-24), 5.52 (H-23), 5.32–5.38 (H-27, H-28)
2.15 (H-44)	1.53 (H-43), 1.93 (H-46)
1.93 (H-46)	2.15 (H-44)
1.52–1.53 (H-7, -16, -43)	1.38 (H-8, H-15, H-42)



figurations for the double bonds (*Z* in **e** and *E* in **a**) were deduced from the coupling constants (10.8 and 15.0 Hz, respectively) shown by the two couples of olefinic protons at δ 5.54–6.25 and δ 5.60–5.90. The chemical shifts for the protons in **a** were basically identical to the ones relative to the same partial structure in compounds **1–5** and **8** (Table 2). In both compounds a multiplet at δ 2.38 due to the allylic protons in structure **e** was also present. The ^{1}H-nmr spectrum of the more abundant compound (F2, 8 mg), named petroformyne A, was completed by a strong signal at δ 5.40 (6 olefinic protons), a singlet at δ 3.7 (3H, -COOCH_3), and finally by large signals centered at δ 2.02 (allylic protons) and at δ 1.26 (methylenes in saturated alkyl chains). These data suggested for petroformyne A a linear structure characterized by the terminal units **a** and **e** and containing three more unsaturations within the straight alkyl chain. COSY experiments and ^{13}C-nmr spectra confirmed these assignments (Table 2). The length of the alkyl chain was determined using eims analysis. This led to the molecular ion peaks at *m/z* 646, accompanied by fragment ions at −32 and −60 mass units. These data suggest the structure **10**, where the position of the double bonds could not be determined by degradative ozonolysis because of the scarcity of this metabolite.

The compound F5, named petroformyne B [**11**], showed spectral data closely related to those of **10**. However, the presence of a double doublet (J = 6.0, 6.0 Hz) at δ 2.79 in the ^{1}H-nmr spectrum suggested the presence of a bisvinylic methylene. As for **10**, the length of the straight alkyl chain was ascertained by eims ([M]$^+$, *m/z* 674).



The brine shrimp (*A. salina*) lethality assay was repeated on the pure compounds, although the antitumor potato disc assay (8) and the assay for the inhibition of sea urchin egg development (9), whose detection limits and assay conditions require more material in order to perform the tests, were only conducted on the most abundant compounds, **1–4**. The results of these assays are reported in Table 4. Compounds **1–4** inhibited the full development of sea urchin fertilized eggs which, at concentrations ranging from 10 to 50 ppm, never reached the final stage (pluteum). Only the trihydroxypolyacetylenes **1** and **2** exhibited the greatest antitumor activity.

All compounds tested with *A. salina* were found to be extremely toxic at concentrations ranging from 0.002 to 0.12 ppm. Therefore, to the best of our knowledge, these are among the most potent substances ever reported in this assay. The most active compounds were usually the most polar (8) although methylation of the carboxylic acids

TABLE 4. Biological Activities of Petroformynes.

Compound	Brine Shrimp Assay (LC_{50}, μg/ml)	Sea Urchin Egg Assay (LC_{50}, μg/ml)	Antitumor Potato Disc Assay (% of inhibition, 0.5 mg/ml)
1 (D3)	0.014	10	35
2 (D4)	0.009	1	15
3 (B1)	0.003	10	49
4 (B2)	0.0075	50	43
5a or **5b** (E3)	0.004	N.T.[a]	N.T.
6 (A2)	0.12	N.T.	N.T.
7 (A1)	0.04	N.T.	N.T.
Mixture of **8a** and **8b** (C5)	0.015	N.T.	N.T.
9a or **9b** (E2)	0.05	N.T.	N.T.
10 (F2)	0.002	N.T.	N.T
11 (F3)	0.0025	N.T.	N.T.

[a]N.T. = not tested.

does not appear to affect their biological activity. The potency exhibited by the alcohols was usually one order of magnitude higher than that observed for the corresponding ketones. The extreme care used in the one-day extraction of the fresh sponge and the selectivity of the oxidation of the alcohol groups rules out the possibility of these novel metabolites being of artifactual nature. Therefore, oxidation of the *Petrosia* polyacetylenes might represent an endogenous mechanism aimed at reducing their toxic activity. The latter is probably due mainly to the acetylenic function, while the hydroxy group only increases this type of biological activity. Further studies are now required to establish the function in vivo and the exact pharmacological properties of the *P. fciformis* polyacetylenes as well as to answer the intriguing questions arising from the biosynthesis of their extremely long alkyl chain.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded on a Perkin-Elmer Infracord 257 instrument, and the uv spectra were obtained on a Varian DMS 90 double beam spectrophotometer. ^{1}H- and ^{13}C-nmr spectra analyses were performed on a 500 MHz WM 500 Bruker Spectrometer in $CDCl_3$ using TMS as zero. Hplc purifications were performed on a Waters liquid chromatograph equipped with a model 660 solvent programmer and M-45 solvent delivery system, using both analytical and semipreparative μ-Bondapak C-18 columns. Mass spectra were recorded with an AEI MS30 mass spectrometer. Commercial Merck Si gel 60 (70–230 mesh ASTM) was used for cc. 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.

ANIMAL MATERIAL.—*P. fciformis* was collected in the Bay of Naples, Italy. Voucher specimens are available for inspection at the Institute.

ANIMAL EXTRACTION.—Fresh material (1 kg dry wt after extraction) was extracted with Me_2CO (10 liters); after concentration, the aqueous residue was extracted with Et_2O (3 × 200 ml). The combined ethereal extracts were taken to dryness, yielding an oily residue (7 g).

FRACTIONATION OF THE EXTRACT.—The isolation of petroformynes is shown in Table 1. The numbers 1–5 combined with the letters A–F indicate the increasing retention time in reversed-phase hplc purification procedure.

ISOLATION OF COMPOUNDS A1, A2, C5, AND E3.—Preparative hplc purification of fraction A was carried out using an isocratic elution with MeOH-H_2O (95:5). This yielded A1 (2 mg) and A2 (7 mg). Fraction C5 was the most abundant peak (2 mg) in the hplc fractionation of C, conducted with an isocratic elution using MeOH-H_2O (85:15). Finally E2 (3 mg) was separated from E3 (25 mg) using a gradient elution from MeOH-H_2O (8:2) to MeOH in 1 h. Flow rate for all separations was 2 ml/min.

ISOLATION OF F2 AND F5.—The acid fraction eluted from *Si gel* chromatography was methylated with CH_2N_2 (15 min at room temperature in MeOH) and submitted to preparative reversed-phase hplc using an isocratic elution with MeOH-$CHCl_3$-H_2O (85:10:5). Fractions F2 and F5 were the two most abundant peaks among 7 components (8 mg and 2 mg, respectively).

REDUCTION WITH $NaBH_4$.—Compounds A1, A2, C5, and E2 (0.5 mg each) were dissolved in 2 ml of absolute EtOH. An excess of $NaBH_4$ was added to the solution and the reaction was continued under stirring for 3 h at room temperature. After stopping the reaction by adding 2 ml of MeOH, the mixture was dried, redissolved in MeOH, and submitted to hplc.

SIGNIFICANT SPECTRAL DATA FOR PETROFORMYNE 5 (E3).—Two alternative structures (**5a** and **5b**) are proposed; assignments with an asterisk indicate **5b**.—^{1}H-nmr δ 2.56 (J = 2 Hz, H-1 or H-46*), 2.58 (1H, d, J = 2 Hz, H-46 or H-1*), 4.83 (1H, d, J = 3.5 Hz, H-3 or H-44*), 4.86 (1H, d, J = 6 Hz, H-44 or H-3*), 5.82 (1H, dd, J = 16, 6 Hz, H-43 or H-4*), 5.96 (1H, dd, J = 16, 6.4 Hz, H-42 or H-5*), 4.16 (1H, dt, J = 6.4, 6.4 Hz, H-41 or H-6*), 2.07 (2H, m, H-6 or H-41*), 5.61 (1H, dd, J = 16, 3.5 Hz, H-4 or H-43*), 5.92 (1H, dt, J = 16, 8 Hz, H-5 or H-42*), 1.58 (2H, m, H-40 or H-7*), 2.02 (6H, methylenes near isolated double bonds), 5.32–5.38 (4H, isolated double bonds), 1.52 (2H, m, H-16), 2.24 (2H, dt, J = 7, 1.5 Hz, H-17), 5.20 (1H, bs, H-20), 5.52 (1H, d, J = 15.6 Hz, H-23), 6.20 (1H, dt, J = 15.6, 6.2 Hz, H-24), 2.16 (4H, m, H-25 and H-26), 1.34–1.36 (8H, methylenes), 1.29–1.26 (protons of isolated methylene); ^{13}C-nmr δ 73.8 (C-1 or C-46*), 74.3 (C-46 or C-1*), 62.8 (C-3 or C-44*), 134.5 (C-4 or C-43*), 128.7 (C-5 or C-42*), 31.8 (C-6 or C-41*), 28.6 (C-7 or C-40*), 62.1 (C-44 or C-3*), 135.9 (C-43 or C-4*), 129.1 (C-42 or C-5*), 71.7 (C-41 or C-6*), 36.8 (C-40 or C-7*), 27.2 (C-11, C-14 and C-29), 128.3 (C-12), 131.0 (C-13), 18.5 (C-17), 52.8 (C-20), 109.1 (C-23), 145.7 (C-24), 33.1 (C-25), 129.7 (C-27), 130.2 (C-28), 76.7, 77.0, 77.2, 78.0, 79.1, 82.8 (-C = C- carbons), 29.0–29.8 (methylene carbons). Other spectral data were as previously reported (2).

OTHER SIGNIFICANT SPECTRAL DATA FOR PETROFORMYNE 6 [**6**] AND PETROFORMYNE 7 [**7**].—Ir (liquid film) ν max approximately at 3295, 2205, 2110, 1645 cm^{-1}; uv λ max (MeOH) 228 nm (ε = 14500) for A1 [**7**] and 232 nm (ε = 15600) for A2 [**6**]; [α]D + 3.0 and + 2.9 respectively for A1 and A2 (c = 2%, $CHCl_3$); eims peaks for A2 *m/z* 607 (30%), 455 (30%), 149 (15%), 341 (10%), 313 (5%), 207 (10%); ^{1}H nmr and ^{13}C nmr see Table 2.

OTHER SIGNIFICANT SPECTRAL DATA FOR PETROFORMYNE A [**10**] AND PETROFORMYNE B [**11**] (F2 AND F5).—Uv (MeOH) λ max 248 nm (ε = 8400) and 245 nm (ε = 4500), respectively, for F5 and F2; [α]D + 5.0 and + 4.5 (c = 2%, $CHCl_3$) for F2 and F5, respectively; eims F2 peaks at *m/z* 646 (50%), 614 (20%), 586 (20%); low resolution eims F5 peaks were at *m/z* 674 (45%), 642 (20%), 614 (20%).

SIGNIFICANT SPECTRAL DATA FOR E2.—Alternative structures **9a** and **9b** are proposed; assignments with an asterisk refer to structure **9b**. ^{1}H-nmr δ 2.58 (1H, d, J = 2 Hz, H-46 or H-1*), 3.22 (1H, s, H-1 or H-46*), 6.20 (1H, d, J = 15.8 Hz, H-4 or H-43*), 7.29 (1H, ddd, J = 15.8, 7.3, 7.3 Hz, H-5 or H-42*), 2.32 (1H, dt, J = 7.3, 7.1 Hz, H-6 or H-41*), 1.52 (4H, m, H-7 or H-40* and H-16), 4.91 (1H, d, J = 5.4 Hz, H-44 or H-3*), 5.82 (1H, dd, J = 15.4, 5.4 Hz, H-43 or H-4*), 5.96 (1H, dd, J = 15.4, 6.0 Hz, H-42 or H-5*), 4.18 (1H, bdt, J = 6, 6 Hz, H-41 or H-6*), 1.54 (2H, m, H-40 or H-7*), 1.35 (8H, m, methylenic protons), 2.02 (6H, methylenes near isolated double bonds), 5.32–5.38 (4H, isolated double bonds), 1.40 (2H, m, H-15), 2.23 (2H, dt, J = 7, 1.5 Hz, H-17), 5.20 (1H, bs, H-20), 5.51 (1H, d, J = 15.6 Hz, H-23), 2.14 (4H, m, H-25 and H-26), 1.29–1.26 (protons of isolated methylenes), 6.20 (1H, dt, J = 15.6, 6.2 Hz, H-24); ir (liquid film) ν max approximately 3295, 2205, 2110, 1645 cm^{-1}; uv λ max (MeOH) 226 nm (ε = 12000); [α]D + 13.0 (c = 2%, $CHCl_3$).

SIGNIFICANT SPECTRAL DATA FOR C5.—C5 is probably a mixture of compounds **8a** and **8b** because a couple of ^{1}H-nmr signals with the same shape were observed at δ 5.20 (bs) and δ 4.83 (d). Assignments with an asterisk refer to **8b**. ^{1}H-nmr δ 2.56 (1H, d, J = 2.2 Hz, H-46 or H-1*), 4.83 and 4.84 (1H, d, J = 5 Hz, H-44 or H-3*), 5.63 (1H, dd, J = 15.1, 5 Hz, H-42 or H-4*), 5.90 (1H, dt, J = 15.1, 7 Hz, H-42 or H-5*), 2.08 (2H, m, H-41 or H-6*), 3.24 (1H, s, H-1 or H-46*), 6.21 (2H, t, J = 15.7 Hz, H-4 or H-43* and H-24, dt, J = 15.3, 5 Hz), 7.26 (1H, ddd, J = 15.7, 7 Hz, H-5 or H-42*), 2.37 (2H, dt, J = 7, 7 Hz, H-6 or H-41*), 1.35 (6H, H-10, H-15, H-30), 2.02 (6H, methylenes near isolated double bonds), 5.33–5.37 (4H, protons of isolated double bonds), 2.23 (2H, dt, J = 7.3, 1.5 Hz, H-17), 5.19 and 5.21 (1H each, bs, H-20), 5.54 (1H, d, J = 15.7 Hz, H-23), 2.16 (4H, m, H-25 and H-26), 1.26–1.29 (methylene protons); ir (liquid film) as for A1, A2, and E2. Uv λ max (MeOH) at 228 nm (ε = 14000).

BIOLOGICAL ASSAYS.—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.* (11). Briefly, for each dose tested survivor shrimps were counted after 24 h and data were statistically analyzed by the

Finney program (12) which yields LC_{50} values with 95% confidence intervals. The fertilized sea urchin egg assay was performed in EtOH (1% final volume). About 1000 fertilized eggs for each Petri capsule were suspended in 20 ml of purified natural sea water. Five capsules were used for each dose and examined for 48 h (the time necessary for controls to reach the pluteum stage). Finally, the potato disc antitumor (10) assay was performed using 2 mg of each compound first dissolved in 0.5 ml of DMSO and then diluted in 1.5 ml of sterile H_2O and 2 ml of *Agrobacterium tumefaciens* suspension. Sterile potato discs ($n = 25$, $\varnothing = 1.8$ cm, $h = 0.5$ cm) were used for each compound and 25 µl of the suspension as added to each disc. Tumors were counted after 16–18 days and data were compared with control discs.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. Branno for her help during the sea urchin egg assay. Eims data were provided by "Servizio di Spettrometria di Massa del CNR e dell'Universitá di Napoli," and the assistance of the staff is gratefully acknowledged. The authors are grateful for the precious technical help provided by Mr. F. Castelluccio, Mr. A. Crispino, and Mr. R. Turco.

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Received 28 July 1989