Pergamon

Tetrahedron Vol. 50, No. 46, pp. 13261-13268, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00841-8

Absolute Stereochemistry of Petroformynes, High Molecular Polyacetylenes from the Marine Sponge *Petrosia ficiformis*

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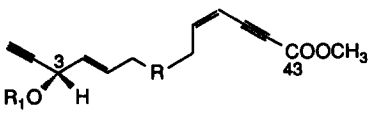
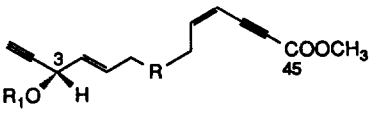
Abstract: The absolute stereochemistry of some petroformynes (1-7), characteristic metabolites of the Mediterranean sponge *Petrosia ficiformis* displaying terminal 1-yn-3-ol-4-ene moieties, has been elucidated by applying high field ^{1}H-NMR to Mosher method. Esterification of *Petrosia* polyacetylenes with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride yielded the corresponding (*S*)- and (*R*)-MTPA esters. Careful NMR measurements led to assign the *S* absolute stereochemistry at all the chiral centers of petroformynes. The *R* absolute stereochemistry of 3-hydroxydocosa-4(*E*), 15(*E*)-dien-1-yne (**8**), previously established on the basis of a questionable extention of the application of the exciton chirality method, is confirmed by applying advanced Mosher method. The structures of petroformyne-5 (7) and petroformyne-8 (11) are now supported by additional evidence.

Chemical studies of the relationship between the Mediterranean red sponge *Petrosia ficiformis* Poiret and its predator, the nudibranch *Peltodoris atromaculata* Bergh, showed[2] in both organisms the same mixture of unusual polyacetylenes characterized by long unbranched alkyl chains. Studies[3] of a white *Petrosia* variety of the sponge, living in dark caves and, because of this, devoid of the red coloured symbiotic alga *Aphanocapsa feldmanni*,[4] gave other polyacetylenes related to, but different from, those of the red variety. All the metabolites were only partially characterized and, erroneously, the length of the chain of these metabolites was suggested, by gas chromatographic analysis, to be included from 46 to 55 carbons. Further studies[5-7] of *P. ficiformis* led to the structural characterization of petroformynes 1-9 all displaying linear alkyl chains with 46 carbons, whereas petroformyne-A (1) and -B (2) exhibited chains with 43 and 45 carbons, respectively. However, the configuration at the chiral centers remained to be determined. Because of this, we have reinvestigated the *Petrosia* polyacetylenes, aiming at elucidating their absolute stereochemistry. The knowledge of the stereochemical details of petroformynes could be useful to gain a further understanding of the structural prerequisities that favour the bioactivity of sponge polyacetylenes. In fact, many biological activities, such as antimicrobial, cytotoxic, and antimitotic etc., are reported for linear polyacetylenes.[7,8]

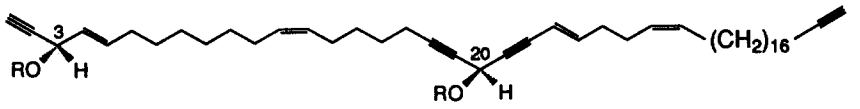
We will now report the determination of the absolute configuration of compounds **1-8** by applying high-field ^{1}H-NMR to Mosher method[9,10] with α-methoxy-α-trifluoromethylphenyl acetic (MTPA) esters, as recently proposed and successfully applied to determine the absolute stereochemistry of a series of both marine terpenoids[11-14] and acetogenins[15] all having chiral carbinol centers.



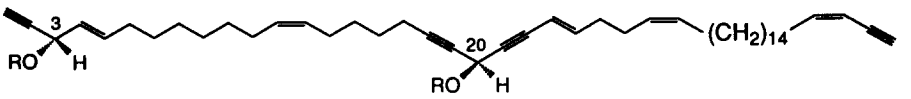
1 : R_1 = H ; R = $C_{31}H_{56}$
1S : R_1 = (*S*)-MTPA, R = $C_{31}H_{56}$
1R : R_1 = (*R*)-MTPA, R = $C_{31}H_{56}$



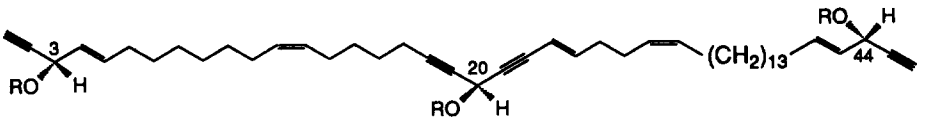
2 : R_1 = H ; R = $C_{33}H_{60}$
2S : R_1 = (*S*)-MTPA, R = $C_{33}H_{60}$
2R : R_1 = (*R*)-MTPA, R = $C_{33}H_{60}$



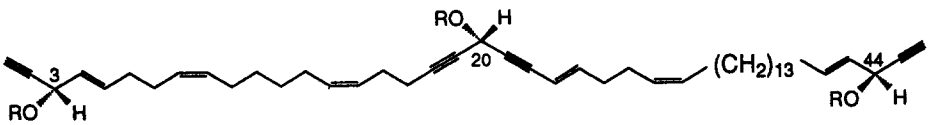
3 : R = H **3S** : R = (*S*)-MTPA **3R** : R = (*R*)-MTPA



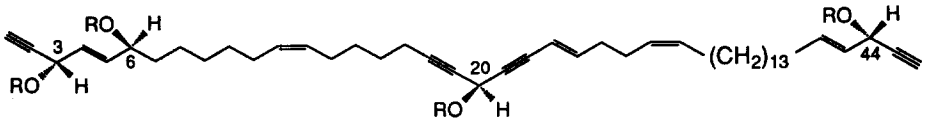
4 : R = H **4S** : R = (*S*)-MTPA **4R** : R = (*R*)-MTPA



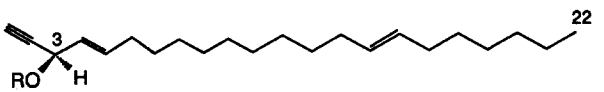
5 : R = H **5S** : R = (*S*)-MTPA **5R** : R = (*R*)-MTPA



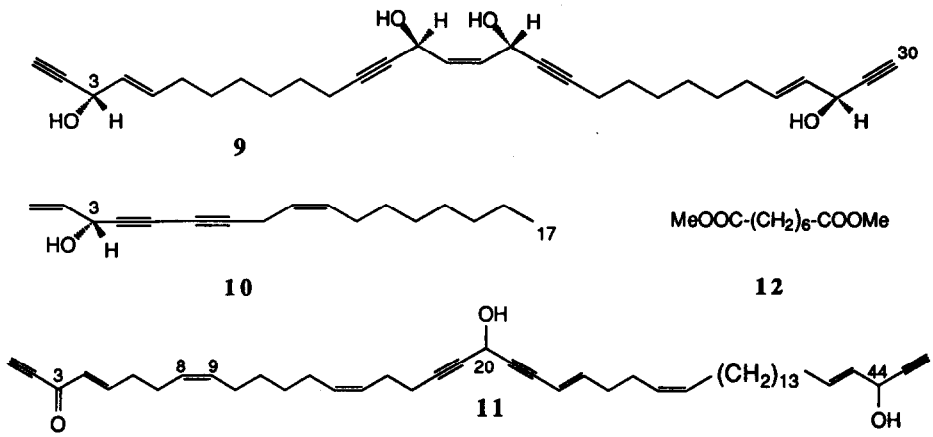
6 : R = H **6S** : R = (*S*)-MTPA **6R** : R = (*R*)-MTPA



7 : R = H **7S** : R = (*S*)-MTPA **7R** : R = (*R*)-MTPA



8 : R = H **8S** : R = (*S*)-MTPA **8R** : R = (*R*)-MTPA



The long unbranched alkyl chain acetylenic alcohols with either mono or bis terminal 1-yn-3-ol-4-ene moieties have been found in sponges belonging to the genera *Petrosia*,[7,8,16] *Siphonochalina*,[17] *Cribrochalina*,[18,19] *Xestospongia*[20] and, also, in molluscs[21] and seaweeds.[22] But, until now, the absolute stereochemistry has been reported only for **8**[19] and petrosynol (**9**)[8] by applying, in both cases, an extention of the exciton chirality method[23] as suggested[24] for panoxynol. But, recently,[25] the absolute stereochemistry of panoxynol (**10**) has been revised by applying advanced Mosher method. The authors[25] suggested that the application of the exciton chirality method is questionable when the asymmetric carbon is flanked by two chromophores. However, the absolute stereochemistry of petrosynol (**9**) has been recently[16] confirmed by applying Mosher method. We also have tried to apply the exciton chirality method to petroformynes, but the CD profiles of the *p*-bromobenzoates were difficult to rationalize, most likely for the interference of the conjugated chromophores present in all molecules. Before studying the stereochemistry of petroformynes, we have analysed, as model compound, the alcohol **8** isolated from the sponge *Cribrochalina vasculum*[18] by applying Mosher method. The *R* stereochemistry previously[19] suggested on the basis of the positive CD profile of the *p*-bromobenzoate of **8** was confirmed. Therefore, we have analysed the stereochemistry of polyacetylenes **1**-**7**. For a better clearness, we will report the studies of petroformynes in order of increasing stereochemical complexity, starting from **1**, **2** with only one chiral center and arriving to **7** with four secondary hydroxy groups.

Isolation of petroformynes from *P. ficiformis*

Both *P. ficiformis* varieties (the red and the white) living in the Mediterranean Sea were studied. On the day of collection the sponge was thoroughly extracted with Me_2CO. The Et_2O soluble fraction from the Me_2CO extract was chromatographed on silica gel column. The polyacetylene fractions were further purified by means of reversed phase HPLC.[7] Petroformyne-A (**1**), -1 (**5**), -2 (**6**), and -5 (**7**) were the most abundant metabolites from the red variety together with minor amounts of petroformyne-B (**2**), -3 (**3**) and -4 (**4**). Petroformynes 6-9 and other related metabolites, actually under investigation, were also isolated. The same chemical composition was recovered by analysing the digestive glands of a population of the nudibranch *P. atromaculata* living upon the sponge. The white variety of *P. ficiformis* contains petroformyne-3 (**3**), -4 (**4**) together with a series of minor components that include petroformyne-6 and -7.[7] The reanalysis of the ^{1}H-NMR spectra of petroformynes 1-9 led to revise the structure of petroformyne-8 (**11**), previously reported[7] without the $\Delta^{8,9}$ double bond. Both H-1 and H_2-6 of **11**, in comparison with those of model compounds (e.g. petroformyne-

7[7]) without $\Delta^{8,9}$ double bond, were downfield shifted (H-1 from δ 3.21 to δ 3.24; H_2-6 from δ 2.32 to δ 2.37) owing to the influence of the double bond at C-8. The positive FABMS peak at *m/z* 687 $(M+Na)^+$ supported the suggested structure.

Stereochemistry of 3-hydroxydocosa-4(*E*), 15(*E*)-dien-1-yne

The (*S*)- and (*R*)-MTPA esters of the alcohol **8** were prepared by treatment of **8** with (*R*)- and (*S*)-MTPA chloride in dry pyridine at room temperature, respectively. Assignment of the ^{1}H-NMR signals of the esters (**8S** = **8***S*-ester, **8R** = **8***R*-ester) was achieved by analysing 2D-COSY spectra. The $\Delta\delta$ (δ_S-δ_R) values of the protons near the oxygenated carbon (C-3) are summarized in the partial structure **a** (Fig. 1). The negative value (-20 Hz) recorded for the acetylenic proton (H-1) and the positive shifts observed for the olefinic protons (H-4, H-5) led, according to the MTPA determination rule[9-14], to assign the *R* absolute stereochemistry at C-3 in agreement with the result previously obtained by applying the exciton chirality method.[19]

Stereochemistry of petroformynes with one chiral center

Petroformyne-A (**1**) and -B (**2**) contain a secondary alcohol function at C-3 flanked, analogously with alcohol **8**, by a terminal acetylene and by a *trans* oriented disubstituted double bond. **1** and **2** were converted to (*S*) (**1S**, **2S**)- and (*R*) (**1R**, **2R**)-MTPA esters, respectively. All ^{1}H-NMR resonances of the esters were assigned by an extensive analysis of 1D and 2D NMR spectra. Some selected $\Delta\delta$ (δ_S-δ_R) values of **1** are depicted in the partial structure **b** (Fig. 1). Both **1** and **2** displayed very similar $\Delta\delta$ values. The comparison of these $\Delta\delta$ values with those reported for **8** revealed comparable, but with opposite sign, shifts for all the protons of the 1-yn-3-ol-4-ene fragment, so that the absolute stereochemistry at C-3 of **1** and **2** was suggested to be *S*.

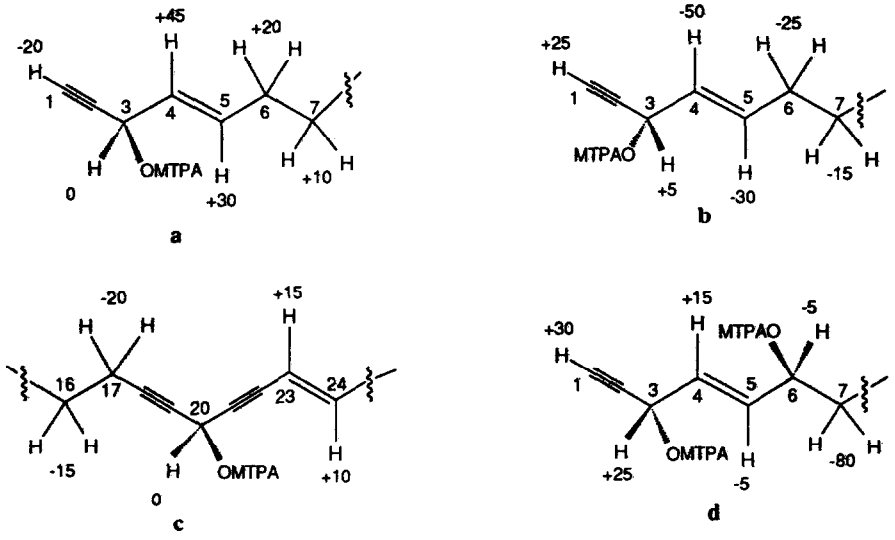


Fig. 1 $\Delta\delta$ values (Hz) obtained for the protons of the partial structures **a** (**8**), **b** (**1**), **c** (**3**) and **d** (**7**)

Table 1. Selected ^{1}H-NMR Chemical Shifts[a] for the MTPA Esters of Petroformynes 1-5 (3-7) and Δδ (δ_S-MTPA ester - δ_R-MTPA ester)[b]

H	Petroformyne-1			Petroformyne-2			Petroformyne-3			Petroformyne-4			Petroformyne-5		
	5S	5R	Δδ	6S	6R	Δδ	3S	3R	Δδ	4S	4R	Δδ	7S	7R	Δδ
1	2.62	2.58	+20	2.62	2.59	+15	2.62	2.58	+20	2.62	2.58	+20	2.65	2.59	+30
3	6.02	6.01	-5	6.01	6.01	0	6.02	6.02	0	6.01	6.01	0	6.06	6.01	+25
4	5.50	5.60	-50	5.51	5.61	-50	5.50	5.60	-50	5.50	5.60	-50	5.78	5.54	+15
5	5.99	6.06	-35	6.00	6.06	-30	5.99	6.05	-30	6.00	6.06	-30	5.92	5.93	-5
6	2.02	2.08	-30	2.10	2.14	-20	2.02	2.07	-25	2.03	2.08	-25	5.49	5.50	-5
7	1.35	1.38	-15	2.22	2.24	-10	1.35	1.38	-15	1.35	1.38	-15	1.54	1.70	-80
16	1.48	1.60	-10	2.11	2.12	-5	1.48	1.51	-15	1.47	1.49	-10	1.39	1.45	-30
17	2.19	2.22	-15	2.23	2.25	-10	2.18	2.22	-20	2.18	2.22	-20	2.14	2.21	-35
20	6.33	6.33	0	6.33	6.33	0	6.33	6.33	0	6.33	6.33	0	6.32	6.33	-5
23	5.50	5.4	+10	5.50	5.48	+10	5.50	5.47	+15	5.50	5.47	+15	5.50	5.47	+15
24	6.23	6.22	+5	6.23	6.22	+5	6.22	6.20	+10	6.23	6.21	+10	6.23	6.21	+10
40	1.35	1.38	-15	1.35	1.38	-15							1.35	1.38	-15
41	2.02	2.08	-30	2.03	2.08	-25							2.02	2.08	-30
42	5.99	6.06	-35	6.00	6.06	-30							6.01	6.07	-30
43	5.50	5.60	-50	5.51	5.61	-50							5.50	5.60	-50
44	6.02	6.01	+5	6.02	6.01	+5							6.02	6.02	0
46	2.62	2.58	+20	2.62	2.58	+20							2.62	2.58	+20

[a] 500 MHz; $CDCl_3$; δ values referred to $CHCl_3$(δ 7.26 ppm). [b] Δδ values are given in Hz.

Stereochemistry of petroformynes with two chiral centers

Petroformyne-3 (**3**) and -4 (**4**) are the most abundant metabolites in the white variety of *P. ficiformis* and, they are also present, even though in minor amounts, in the red variety. These two metabolites were characterized, analogously with **1** and **2**, by the terminal structural unit **b** (Fig. 1) but also by an additional secondary alcohol at C-20 (partial structure **c**, Fig. 1).

The(*S*)- and (*R*)-MTPA esters (3S, 4S, 3R, 4R) were prepared by treating **3** and **4** with (*R*)- and (*S*)-MTPA chloride in dry pyridine at room temperature, respectively. Some selected ^{1}H-NMR data and Δδ (δ_S-δ_R) values are listed in Table 1. The Δδ values observed for the signals of protons nearby the hydroxy group at C-3 of **3** and **4** were almost identical to those recorded for **1** and **2** (partial structure **b**, Fig. 1), indicating a *S* configuration at C-3. Analogously, the shifts observed for the protons near C-20 of **3** (partial structure **c**, Fig. 1) clearly supported a *S* absolute stereochemistry at C-20. In fact, positive Δδ shifts were recorded for the olefinic protons (H-23 and H-24), whereas negative effects were observed for the methylene protons (H_2-16 and H_2-17) near the triple bond. The Δδ values obtained for the partial structure **c** (Fig. 1) of **4** were almost identical to those of **3**.

Stereochemistry of petroformynes with three chiral centers

Petroformyne-1 (**5**) and -2 (**6**) are the most abundant metabolites from the red variety of *P. ficiformis*.

Conversely, they are completely absent in the white variety. Both compounds display three secondary hydroxy groups at C-3, C-20 and C-44, respectively. The absolute stereochemistry of **5** and **6** was established by applying the Mosher method, as mentioned above, to the MTPA esters **5S**, **5R**, **6S**, **6R**. The $\Delta\delta$ (δ_S-δ_R) values (see Table 1) recorded for the signals of the protons near C-3 and C-44 (partial structure **b**, Fig. 1) were almost identical to those observed in the ^{1}H-NMR spectra of the MTPA esters of **1**, **2**, **3** and **4**. On this basis, a *S* absolute configuration was suggested at both terminal carbinols. The third hydroxy group at C-20 displayed the same chemical environment as that of the C-20 hydroxy group in **3** and **4** (partial structure **c**, Fig. 1). The *S* absolute configuration at C-20 was determined by recording $\Delta\delta$ values for the protons at C-16, C-17, C-23, C-24 of the MTPA esters of **5** and **6** (**5S**, **5R**, **6S**, **6R**) which were similar to those detected for the MTPA esters of **3** and **4** (Table 1).

Stereochemistry of petroformynes with four chiral centers

Petroformyne-5 (**7**) is the only metabolite with four hydroxy groups isolated from *P. ficiformis* (red variety). It exhibits an additional hydroxy group at C-6 in comparison with petroformyne-1 (**5**). The structure of **7**, previously reported[7] as alternative to the formula with the hydroxy group at C-41, has been now firmly determined by gas chromatographic analysis of the methyl esters of the bicarboxylic acids obtained by oxidative ozonolysis of **7**, followed by methylation with CH_2N_2. The dimethyl ester of suberic acid (**12**) one of the major components among the ozonolysis products of petroformyne-1 (**5**), was completely absent in the mixture obtained from the ozonolysis of **7**, according to the localization of the additional hydroxy group at C-6 instead of C-41. Considering that **7** and **5** are closely related metabolites, probably, they should have the same absolute stereochemistry at C-3, C-20 and C-44. The (*S*)- and (*R*)-MTPA esters (**7S**, **7R**, respectively) of **7** exhibited $\Delta\delta$ shifts (see Table 1) that confirmed our proposition. In fact, both acetylenic (H-1, H-46) and olefinic (H-23, H-24) signals were downfield shifted in the *S*-MTPA ester (**7S**). Strangely, the ^{1}H-NMR $\Delta\delta$ values were irregularly distributed on the olefinic protons (H-4, H-5) between the two hydroxy groups (partial structure **d**, Fig. 1). One proton (H-4) showed a positive $\Delta\delta$ (+15 Hz). Conversely, the shift of the second proton (H-5) was negative (-5 Hz). These anomalies could be rationalized by the presence of the additional hydroxy group at C-6 that, probably, after MTPA esterification, induces effects opposite to those of the MTPA ester at C-3. The small values of the shifts were in agreement with this suggestion leading to the same *S* absolute stereochemistry at both chiral centers. The $\Delta\delta$ value recorded for the two protons at C-7 (-80 Hz) further supported the *S* configuration at C-6.

The scarcity of minor petroformynes 6-9[7] did not allow the preparation of MTPA esters in adequate amounts to confidently record ^{1}H-NMR spectra. But, considering that they are closely related to petroformynes 2-5 (**6**, **3**, **4**, **7**, respectively), most likely a *S* absolute stereochemistry can be suggested for all the secondary alcohol functions of these petroformynes. However, polyacetylene alcohols with opposite stereochemistry co-occur in the *Petrosia* sp. recently collected from Sukumo Bay (Japan).[16] Finally, the C-46 length of the alkyl chain of petroformynes 1-9 has been definitively confirmed by recording positive FABMS spectra with a matrix of *m*-nitrobenzyl alcohol as recently reported[26] for toxadocials, long alkyl chain (C-47, C-49) metabolites isolated from the sponge *Toxadocia cylindrica*. Probably, the unusual length of petroformynes could be explained by the coupling of two fragments that, as suggested for toxadocials,[26] might derive from linear fatty acids.

EXPERIMENTAL SECTION

General Procedures. The UV spectra were obtained on a Varian DMS 90 double beam spectrophotometer. ^{1}H-NMR spectra were recorded on a 500 MHz WM 500 Bruker spectrometer in $CDCl_3$ (δ 7.26 ppm). ^{1}H-NMR assignments were supported by using 2D COSY experiments. FABMS was recorded on a ZAB VG mass spectrometer using *m*-nitrobenzyl alcohol (positive ion mode) as matrix. Optical rotations were measured on a JASCO DIP-370 digital polarimeter in $CHCl_3$.Circular dichroism spectra were recorded on a JASCO J-710 spectropolarimeter in 95% EtOH. Reversed phase HPLC purifications were performed on a Waters liquid chromatograph by using UVIDEC-100-III as detector. Both analytical [5μ, 4.6 mm (I.D.) x 25 cm] and semi-preparative [5μ, 10 mm (I.D.) x 25 cm] columns were SPHERISORB-S5 ODS2. Gas chromatographic analysis was performed on a Carlo Erba HRCG-4500 instrument equipped with a flame ionization detector. The column was OV-1 [0.25 mm (I.D.) x 25 m]. Commercial Merck Si gel 60 (70-230 mesh ASTM) was used for column chromatography. Merck precoated Si gel plates were used for TLC.

Collection of animal material. The sponge *P. ficiformis* (the red and the white) and the nudibranch *P. atromaculata* were collected in the Bay of Naples (Italy) by SCUBA-diving at depth of 10-15m. Voucher specimens are available for inspection at the ICMIB. The fresh sponge of both varieties (each 0.5 kg, dry weight after extraction) was immediately extracted with Me_2CO. Mantle and viscera of the nudibranch (12 specimens) after dissection were separately extracted with Me_2CO.

Isolation of petroformynes. The isolation of petroformyne-A (**1**), -B (**2**), and -1-5 (**5**, **6**, **3**, **4**, **7**, respectively) from the Me_2CO extract of the sponge *P. ficiformis* was performed as previously reported[2-3]. This yielded **1** (24.8 mg), **5** (24.3 mg), **6** (31.5 mg), **7** (20.6 mg) together with minor amounts of **2** (4.5 mg), **3** (0.5 mg), **4** (1.0 mg), petroformyne-6 (1.2 mg), -7 (1.4 mg), -8 (**11**) (2.3 mg) and -9 (5.6 mg) from the red variety, whereas **3** (18.1 mg), **4** (20.8 mg), petroformyne-6 (2.0 mg), -7 (2.8 mg) together with other related metabolites were obtained from the white variety.

FABMS spectra of some petroformynes. Petroformyne-1(**5**): $(M+Na)^+$ *m/z* 691; petroformyne-2 (**6**): $(M+Na)^+$ *m/z* 689; petroformyne-3 (**3**): $(M+Na)^+$ *m/z* 677; petroformyne-4 (**4**): $(M+Na)^+$ *m/z* 675; petroformyne-5 (**7**): $(M+Na)^+$ *m/z* 707; petroformyne-6: $(M+Na)^+$ *m/z* 675; petroformyne-7: $(M+Na)^+$ *m/z* 673; petroformyne-8 (**11**): $(M+Na)^+$ *m/z* 687; petroformyne-9: $(M+Na)^+$ *m/z* 705.

Preparation of (*S*)-MTPA esters. (*S*)-MTPA esters of **1-8** were prepared by treating the polyacetylenes (2-10 mg) with (*R*)-(-)-MTPA chloride (0.05-0.1 ml) in dry pyridine (0.5 ml) for about 16 hours under stirring at room temperature. The esters were purified by chromatography in a Pasteur pipette (SiO_2; petroleum/Et_2O), obtaining **1S-8S**.

Preparation of (*R*)-MTPA esters. The (*R*)-MTPA esters of **1-8** (**1R-8R**) were prepared, as described above, by reaction with (*S*)-(+)-MTPA chloride.

1S: (8.6 mg); $[\alpha]_D^{21}$-12.1° (c 0.76, $CHCl_3$).^{1}H-NMR($CDCl_3$) δ(ppm): 2.63(1H, d, *J*=2.0 Hz, H-1), 6.02(1H, bd, *J*=6.0 Hz, H-3), 5.50(1H, dd, *J*=15.0, 6.0 Hz, H-4), 6.01(1H, dt, *J*=15.0, 7.4 Hz, H-5), 2.02(2H, dt, *J*=7.4 Hz, H-6), 1.35(2H, m, H-7).

1R: (10.1 mg); $[\alpha]_D^{21}$+20.1° (c 1.0, $CHCl_3$). ^{1}H-NMR($CDCl_3$) δ(ppm): 2.58(1H, d, *J*=2.0 Hz, H-1), 6.01(1H, bd, *J*=6.0 Hz, H-3), 5.60(1H, dd, *J*=15.0, 6.0 Hz, H-4), 6.07(1H, dt, *J*=15.0, 7.4 Hz, H-5), 2.07(2H, dt, *J*=7.4 Hz, H-6), 1.38(2H, m, H-7).

TLC, SiO_2, petroleum ether/Et_2O 9:1, R_f 0.40 for both esters (**1S** and **1R**).

2S: (0.5 mg); ^{1}H-NMR($CDCl_3$) δ(ppm): see **1S**.

2R: (0.6 mg); ^{1}H-NMR($CDCl_3$) δ(ppm): see **1R**.

TLC, SiO_2, petroleum ether/Et_2O 9:1, R_f 0.41 for both esters (**2S** and **2R**).

3S: (8.3 mg); $[\alpha]_D^{21}$-70.2° (c 0.83, $CHCl_3$). ^{1}H-NMR($CDCl_3$): see Table 1.

3R: (4.5 mg); $[\alpha]_D^{21}$+51° (c 0.45, $CHCl_3$). ^{1}H-NMR($CDCl_3$): see Table 1.

TLC, SiO_2, petroleum ether/Et_2O 9:1, R_f 0.47 for both esters (**3S** and **3R**).

4S: (0.5 mg); ^{1}H-NMR($CDCl_3$): see Table 1.

4R: (0.6 mg); ^{1}H-NMR($CDCl_3$): see Table 1.

TLC, SiO_2, petroleum ether/Et_2O 9:1, R_f 0.42 for both esters (**4S** and **4R**).

5S: (7.3 mg); $[\alpha]_D^{21}$-26.9° (c 0.28, $CHCl_3$).^{1}H-NMR($CDCl_3$): see Table 1.

5R: (8.4 mg); $[\alpha]_D^{21}$+56.6° (c 0.71, $CHCl_3$).^{1}H-NMR($CDCl_3$): see Table 1.

TLC, SiO_2, petroleum ether/Et_2O 9:1, R_f 0.47 for both esters (**5S** and **5R**).

6S: (5.0 mg); $[\alpha]_D^{21}$-3.3° (c 0.20, $CHCl_3$).^{1}H-NMR($CDCl_3$): see Table 1.

6R: (6.4 mg); $[\alpha]_D^{21}$+13.3° (c 0.59, $CHCl_3$).^{1}H-NMR($CDCl_3$): see Table 1.
TLC, SiO_2, petroleum ether/Et_2O 7:3, *Rf* 0.46 for both esters (**6S** and **6R**).

7S: (12.6 mg); $[\alpha]_D^{21}$-12.1° (c 0.54, $CHCl_3$).^{1}H-NMR($CDCl_3$): see Table 1.

7R: (11.7 mg); $[\alpha]_D^{21}$+26.2° (c 1.0, $CHCl_3$). ^{1}H-NMR($CDCl_3$): see Table 1.
TLC, SiO_2, petroleum ether/Et_2O 7:3, *Rf* 0.42 for both esters (**7S** and **7R**).

8S: (4.2 mg); $[\alpha]_D^{21}$-34° (c 0.38, $CHCl_3$). ^{1}H-NMR($CDCl_3$) δ(ppm): 2.58(1H, d, *J*=2.0 Hz, H-1), 6.01(1H, dd, *J*=6.0, 2.0 Hz, H-3), 5.60(1H, dd, *J*=15.0, 6.0 Hz, H-4), 6.07(1H, dt, *J*=15.0, 7.0 Hz, H-5), 2.08(2H, dt, *J*=7.0 Hz, H-6), 1.37(2H, m, H-7).

8R: (3.5 mg); $[\alpha]_D^{21}$+23.7° (c 0.30, $CHCl_3$). ^{1}H-NMR($CDCl_3$) δ(ppm): 2.62(1H, d, *J*=2.0 Hz, H-1), 6.01(1H, dd, *J*=6.0, 2.0 Hz, H-3), 5.51(1H, dd, *J*=15.0, 6.0 Hz, H-4), 6.01(1H, dt, *J*=15.0, 7.0 Hz, H-5), 2.04(2H, dt, *J*=7.0 Hz, H-6), 1.35(2H, m, H-7).
TLC, SiO_2, petroleum ether/Et_2O 9:1, *Rf* 0.62 for both esters (**8S** and **8R**).

Ozonolysis of compounds 5 and 7. The compounds **5** and **7** (each 4 mg) were dissolved in 10 ml of CH_2Cl_2. O_3 was added to the solution. The reaction was continued for 5 minutes at -78°C. The CH_2Cl_2 was removed under reduced pressure and then the ozonolysis products were dissolved in 7 ml of distilled water. 4 drops H_2O_2 (30%) were added to the solution followed by reflux at 100°C for 1 hour, then cooled and extracted with Et_2O (10 ml x 3). The Et_2O solution was concentrated to about 5 ml and fresh prepared CH_2N_2 was added to this solution. This solution was allowed to sit for 10 minutes at room temperature and was submitted to gas cromatography. The analysis of ozonolysis products of **5** and **7** was carried out using a procedure of gradient increasing temperature from 80°C to 240°C (3°C/ minutes). The retention time of **12** was 18'02".

Acknowledgments. The authors gratefully acknowledge Prof. E. Fattorusso (University of Naples) for kindly providing alcohol **8**. Dr. G. Villani and Dr. E. Mollo are also acknowledged for the collection of *P. ficiformis*. The authors are also grateful to Mr. G. Scognamiglio, Mr. F. Castelluccio and Mr. R. Turco for their valuable technical help. The NMR and the mass spectra were respectively obtained from the "ICMIB-NMR Service" and from the "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli". The staff of both services is gratefully acknowledged. This research was supported by the EEC project "Marine Science and Technology MAST II" Contract MAS2-CT91-0004. Dr. Y. Guo thanks the Ministry of Foreign Affairs of Italy for the financial support in the frame of the Italian-Chinese Scientific and Technological Cooperation. VII program-Basic Science-"Chemical mediators in marine invertebrates".

REFERENCES AND NOTES

1. On leave at ICMIB from the Nanjing Institute of Materia Medica, 210009 Nanjing, China.
2. Castiello, D.; Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *Tetrahedron Lett.* **1980**, *21*, 5047-5050.
3. Cimino, G.; Crispino, A.; De Rosa, S.; De Stefano, S.; Sodano, G. *Experientia* **1981**, *37*, 924-926.
4. Sarà, M.; Liaci, L. *Boll. Zool.* **1964**, *31*, 55-60.
5. Cimino, G.; De Giulio, A.; De Rosa, S.; De Stefano, S.; Sodano, G. *J. Nat. Prod.* **1985**, *48*, 22-27.
6. Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *Tetrahedron Lett.* **1989**, *30*, 3563-3566.
7. Cimino, G.; De Giulio, A.; De Rosa, S.; Di Marzo, V. *J. Nat. Prod.* **1990**, *53*, 345-353.
8. Fusetani, N.; Shiragaki, T.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1987**, *28*, 4313-4314.
9. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512-515.
10. Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143-2148.
11. Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731-4734.
12. Ohtani, I.; Kusumi, T.; Ishtsuka, O. M.; Kakisawa, H. *Tetrahedron Lett.* **1989**, *30*, 3147-3150.
13. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296-1298.
14. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092-4097.
15. Rieser, M. J.; Hui, Y.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, R. *J. Am. Chem. Soc.* **1992**, *114*, 10203-10213.
16. Ochi, M.; Ariki, S.; Tatsukawa, A.; Kotsuki, H.; Fukuyama, Y.; Shibata, K. *Chem. Lett.* **1994**, 89-92.
17. Rotem, M.; Kashman, Y. *Tetrahedron Lett.* **1979**, *20*, 3193-3196.
18. Gunasekera, S.; Faircloth, G. T. *J. Org. Chem.* **1990**, *56*, 6223-6225.
19. Aiello, A.; Fattorusso, E.; Menna, M. *J. Nat. Prod.* **1992**, *55*, 1275-1280.
20. Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1989**, *29*, 2037-2040.
21. Walker, R. P.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 1475-1477.
22. Paul, V. J.; Fenical, W. *Tetrahedron Lett.* **1980**, *21*, 3327-3332.
23. Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. *J. Am. Chem. Soc.* **1982**, *104*, 3775-3776.
24. Shim, S. C.; Koh, H. Y.; Chang, S. *Tetrahedron Lett.* **1985**, *26*, 5775-5776.
25. Matthew, W. B.; Yali, F. H.; Join, H. C.; Michael, R. B. *Tetrahedron Lett.* **1994**, *35*, 993-994.
26. Nakao, Y.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 11183-11188.

(*Received in UK* 15 *August* 1994; *revised* 26 *September* 1994; *accepted* 30 *September* 1994)