Four New C 1,2-Dioxene-polyketides from the Sponge Plakortis aff. simplex

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FOUR NEW C_{16} 1,2-DIOXENE-POLYKETIDES FROM THE SPONGE *PLAKORTIS* AFF. *SIMPLEX*

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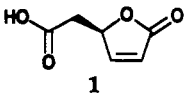
ABSTRACT.—Two new cyclic peroxide-containing polyketide C_{16} acids **2** and **4** with their methyl esters **3** and **5** have been isolated from the Indo-Pacific marine sponge *Plakortis* aff. *simplex*. Both **2** and **3** are proposed to contain a single 1,2-dioxene ring, while **4** and **5** incorporate two 1,2-dioxene rings. The structures were elucidated mainly through 1D and 2D nmr spectral analysis. The methyl esters **3** and **5** were found to be active against cultured P-388 murine leukemia cells.

In search of new biologically active substances from marine organisms, we have isolated four new oxygen heterocycles, compounds **2–5**, and substituted butenolide **1** from the sponge *Plakortis* aff. *simplex* (Schulze, class Demospongiae, order Homosclerophorida, family Plakinidae) collected in Sodwana Bay,



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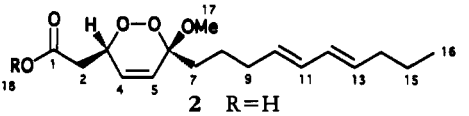
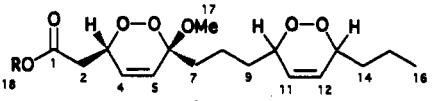
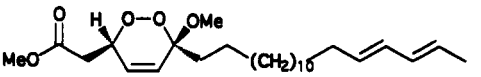


2 R=H
3 R=Me



4 R=H
5 R=Me



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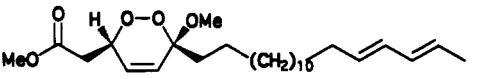

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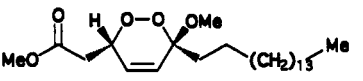

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South Africa. The isolation of compounds **2–5** is in agreement with the known ability of sponges of the genus *Plakortis* to produce oxygenated polyketides, including cyclic peroxides and other related compounds (1–8). Structure elucidation of these compounds was assisted by comparison of spectral data with those of the metabolites of *Xestospongia* sp. reported by Quinoa *et al.* (9), namely methyl 5-butenolidyl acetate, xestin A [**6**], and xestin B [**7**], and with chondrillin [**8**] reported by Wells (10) from the sponge *Chondrilla* sp.

Compound **1**, $C_6H_6O_4$, on interpretation of its spectral data, was found to be 2-oxo-2,5-dihydrofuran-5-acetic acid [**1**]. The methyl ester of this butenolide acid was earlier reported by Quinoa *et al.* (9), and indeed methylation of **1** with CH_2N_2 afforded the known methyl ester.

Compounds **1**, **2**, and **4** are very unstable even under N_2 atmosphere in the dark at low temperature, presumably due to an acidic auto-catalyzed decomposition. These compounds were also very unstable to normal phase chromatography. Therefore, purification was accomplished by solvent partitioning (aqueous MeOH against hexane, CCl_4, and $CHCl_3$) followed by repeated reversed-phase chromatographies.

For compound **3**, a molecular formula of $C_{18}H_{28}O_5$ could be established by hreims ($[M]^+$ 324.1942) and further confirmed by nmr data. Comprehensive 1D and 2D nmr studies, summarized in Table 1, enabled the determination of the complete structure of **3**.

A COSY experiment (Table 1) determined the two halves of the molecule, namely a C_{10} *E,E*-diene moiety,-$(CH_2)_3$ CH=CH-CH=CH-CH_2CH_2Me, and a C_8 segment,-C(OMe)(O-)CH=CH-CH(O-) CH_2CO_2Me, both accounting for all the C atoms of the molecule. Next, the $[M-32]^+$ (*m/z* 292) fragment in the mass spectrum [loss of O_2, as known for cyclic peroxides (9)] suggested a substituted 1,2-dioxene, thus establishing the structure of **3**.

TABLE 1. Nmr Data of Compound **3** (90 and 500 MHz).

Position	δ_C	δ_H[a]	δ_H	COSY
	($CDCl_3$)	($CDCl_3$)	(C_6D_6)	(H to H)
1	169.9 s			
2	36.3 t	2.48 dd (16.5 6.5)	2.07	2′, 3, 18[c]
		2.59 dd (16.5, 8)	2.30	2, 3, 18
3	73.5 d	4.97 dd t (8, 6.5, 2)	4.95	2, 2′, 4, 5
4	130.5 d	6.10 dd (10.5, 1.5)	5.67	3, 5
5	126.8 d	5.81 d (10.5, 2)	5.50	3, 4
6	101.0 s			
7	34.5 t	1.66 m	1.66	8
8	22.5 t	1.42 m	1.48	7, 9
9	32.5 t	2.01 m	1.97	8, 10, 11
10	132.7 d	5.55 m[b]	5.50[b]	9, 11
11	131.6 d[b]	5.96 br d (13.5)	6.05	9, 10
12	131.1 d[b]	5.96 br d (13.5)	6.05	13, 14
13	131.1 d	5.48 m[b]	5.45[b]	12, 14
14	34.4 t	2.01 m	1.97	12, 13, 15
15	22.5 t	1.38 m	1.33	16, 14
16	13.7 q	0.87 t (7)	0.84	15
17	51.4 q	3.37 s	3.22	
18	52.0 q	3.70 s	3.31	2, 2′

[a] *J* values are given in Hz

[b] Interchangeable

[c] A clear correlation between H-2 and the Me group (^5J) was observed in the regular COSY-45 experiment.

Compound **3** is the lower C_{16} homologue of xestin A [**6**], and it possesses the same stereochemistry of the dioxene heterocycle based on comprehensive nmr studies of the chemical shift values and coupling constants by Quinoa *et al.* (9) (Table 2). Furthermore, an nOe between H-3 and H-7 (1%) confirmed unequivocally the cis configuration of H-3 and H-7 (4). Compound **3** was readily obtained from **2** by methylation with CH_2N_2. Therefore compound **2** is the carboxylic acid counterpart of **3**.

Compound **5**, $C_{18}H_{28}O_7$, was obtained as a yellow-greenish oil in yields up to 0.02% (dry wt). The hreims of **5** gave as highest peak a fragment at *m/z* 324.1937 (5%), $C_{18}H_{28}O_5$. From the nmr data, summarized in Table 3, it was evident that compound **5** possesses the same substituted 1,2-dioxene moiety as compounds **2** and **3** but differs in the second functionality: the diene of **2** and **3** is replaced in **5** by a second 1,2-dioxene ring. According to the ^{13}C-nmr data, (four sp^3 oxygen-bearing C atoms, a CO_2 Me, and an OMe group), the *m/z* 324 fragment in the ms, has to be $[M-32]^+$. Loss of 32 mu (O_2), already encountered in the ms of compound **3**, is also observed for a second time in the ms of compound **5**, *m/z* 292, $[M-32-32]^+$ (4%), and it has to be responsible for the absence of the molecular ion peak of the latter compound. The complexity of H-10 and H-13 and the partial overlapping of the neighboring H_2-9 and H_2-14 groups prevented the determination of the stereochemistry of the second 1,2-dioxene ring in **5**. To the best of our knowledge, this is the first report of a didioxene polyketide of marine origin.

Compound **4** was very unstable and could not be obtained in a pure state; however, its existence in the sponge was confirmed by methylation of semi-purified fraction of **4** with CH_2N_2 to afford compound **5**.

Both compounds **3** and **5** are cytotoxic to P388 murine leukemia cells and gave $IC_{50}<0.1$ μg/ml. Compounds **2** and **4** were expected to be more potent (3), but regrettably they were too unstable to be tested.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. Low resolution mass spectra were recorded in a Finnigan-4021 mass spectrometer and hrms on a VG70 VSEQ instrument. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker ARX-500 spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter.

ISOLATION OF COMPOUNDS **1–5**.—A sample of the sponge was collected in Sodwana Bay, South Africa. The *Plakortis simplex* species is widespread in all tropical and subtropical seas; so far it was not reported from South Africa (no Plakinidae has been ever recorded for the area). All reports seem to concern closely related, but presumably separate, species which are poorly separated morphologically. A voucher specimen is deposited at the Zoological Department of Tel Aviv Univeristy (TASA-54). The freeze-dried sponge (15 g) was extracted with EtOAc (×3) to afford a brown gum

TABLE 2. Nmr Comparison of Compounds **2**, **3**, and **5–7** (500 MHz).[a]

Position	Compound				
	2	**3**	**5**	**6**[b]	**7**[b]
3	4.88, $J_{3,4}$=1.4 $J_{3,5}$=2.1	4.97, $J_{3,4}$=1.5 $J_{3,5}$=2.0	5.00, $J_{3,4}$=1.5 $J_{3,5}$[c]	5.00, $J_{3,4}$=1.2, $J_{3,5}$=2.1	4.78 $J_{3,4}$=4.5, $J_{3,5}$=1.5,
4	6.06, $J_{4,5}$=10.2	6.10, $J_{4,5}$=10.5	6.10, $J_{4,5}$=10.0	6.11, $J_{4,5}$=10.2	6.11, $J_{4,5}$=10.2
5	5.72	5.81	5.81	5.84	5.85

[a]For these compounds the ^{13}C-nmr data for C-2,-3,-4,-5,-6 (δ c 36.3, 73.3, 130.5, 126.7, 101.0 ± 0.3 ppm, respectively) are essentially the same as those of **6**.

[b]Data for xestin A [**6**] and xestin B [**7**] are from Quinoa *et al.* (9).

[c]Could not be measured because of overlapping.

TABLE 3. Nmr Data of Compound **5** (125 and 500 MHz).

Position	δ_C	δ_H[a]	HMBC	COSY
	($CDCl_3$)	($CDCl_3$)	(H to C)	(H to H)
1	169.9 s			
2	36.4 t	2.50 dd (16, 16.5)	4	2′, 3, 18[b]
		2.60 dd (16, 7.5)	4	2, 3, 18
3	73.5 d	5.00 dd t (7.5, 6.5, 1.5)	2, 5	2, 2′ 4, 5
4	130.7 d	6.10 dd (10, 1.5)	3, 6	3, 5
5	126.7 d	5.81 m		3, 4
6	101.0 s			
7	35.0 t	1.65 m	5, 6, 8	8, 8′
8	19.6 t	1.40 m[c]	6, 7, 9, 10	7, 9, 9′
		1.50 m[c]	6, 7, 9, 10	7, 9, 9′
9	32.9 t	1.50 m[c]	7, 8, 10, 11	10
		1.65 m[c]	7, 8, 10, 11	10
10	77.9 d[d]	4.39 m[c]	8, 9	9, 9′, 11
11	128.0 d	5.83 m	10, 13	10
12	127.4 d	5.84 m	10, 13	13
13	78.1 d[d]	4.45 m[c]	14, 15	12, 14, 14′
14	34.8 t	1.45 m[c]	12, 13, 15	13
		1.60 m[c]	12, 13, 15, 16	13
15	18.5 t	1.38 m[c]	14, 16	16
		1.45 m[c]	14, 16	16
16	14.0 q	0.91 t (7)	14, 15	15, 15′
17	51.5 q	3.36 s		
18	52.3 q	3.68 s		2, 2′

[a]*J* values are given in Hz.
[b]A clear correlation between H-2 and the OMe group (^5J) was observed in the regular COSY-45 experiment.
[c]Chemical shifts of overlapping protons were determined from the HMQC experiments.
[d]Interchangeable.

(300 mg) (IC_{50} against P388, 2.5μg/ml). The EtOAc extract in aqueous MeOH was successively partitioned between hexane, CCl_4, and $CHCl_3$. The first two partitioned phases contained the methyl esters **3** and **5**, while the CCl_4 and $CHCl_3$ phases contained the free acids **1**, **2**, and **4**. Repeated reversed-phase chromatographies [10 um ODS column, MeOH-H_2O (4:6 to 9:1)] afforded **1** (15 mg), **2** (18 mg), **3** (5 mg), **4** (4 mg), and **5** (2 mg).

2-Oxo-2,5-dihydrofuran-5-acetic acid [**1**].—Oil: eims *m/z* $[M]^+$ 142 ($C_6H_6O_4$) (100), 125 (35); ^{1}H nmr (500 MHz, $CDCl_3$) δ 7.66 (dd, *J*=5.6, 1.4, H-4), 6.17 (dd, *J*=5.6, 1.7, H-3), 5.42 (ddt, *J*=1.7, 1.4, 6.8, H-5), 2.83 and 2.66 (ABX system, J_{AB}=16.4, J_{AX}=6.8, J_{BX}=6.8, H-6; 6′); ^{13}C nmr (125 MHz, $CDCl_3$) δ 173.5 s, 173.0 s, 155.0 d, 112.0 d, 79.0 d, 36.0 t. Methylation of **1** (5 mg) with CH_2N_2 in Et_2O (3 ml) afforded, after 18 h, the methyl ester (*m/z* 156), which was found to be identical in all respects with the reported ester (9).

6-Methoxy-3,6-peroxyhexadeca-4,10,12-trienoic acid [**2**].—Oil: $[\alpha]_D$ +60° (*c*=0.25, $CHCl_3$); ir ν max (neat) 3500–2500 br, 1710, 1460, 1400, 1290 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.06 (dd, *J*=10.2, 1.4, H-4), 5.89 (bd, *J*=10.5, H-11), 5.78 (bd, *J*=10.5, H-12), 5.72 (dd, *J*=10.2, 2.1, H-5), 5.48 (m, H-10), 5.45 (m, H-13), 4.88 (tdd, *J*=6.4, 1.6, 1.4, H-3), 3.25 (s, OMe), 2.56 and 2.45 (ABX system, *J*=16.4, 7.6, 6.4, H_2-2), 1.95 (m, H-9), 1.93 (m, H-14), 1.53 (m, H-8), 1.30 (m, H-7), 1.28 (m, H-15), 0.80 (t, *J*=6.7 Me); ^{13}C nmr δ 173.0 s, 132.5 d, 131.1 d, 130.9 d, 130.4 d, 130.2 d, 126.7 d, 101.1 s, 73.3 d, 51.8 q, 36.3 t, 34.5 t, 34.4 t, 32.4 t, 22.9 t, 22.3 t, 13.5 q. To compound **2** (2 mg) in Et_2O (3 ml) was added a solution of CH_2N_2 in Et_2O (1 ml). After 18 h, the solvent was removed to afford the ester **3**.

Methyl 6-methoxy-3,6-peroxyhexadeca-4,10,12-trienoate. [**3**].—Oil: $[\alpha]^{25}_D$ +52° (*c*=0.25, $CHCl_3$); ir ν max (neat) 3050, 1740, 1440, 1290, 1180, cm^{-1}; cims *m/z* $[MH]^+$ 325 (4), 292 (4), 276 (9), 233 (6), 137 (24), 136 (62), 123 (25), 109 (74); hreims *m/z* 324.1942 (calcd for $C_{18}H_{28}O_5$, 324.1929); ^{1}H and ^{13}C nmr see Table 1.

Methyl 6-methoxy-3,6,10,13-diperoxyhexadeca-11-enoate [**5**] *and acid* **4**.—Oil: $[\alpha]_D$ 0° (*c*=

0.1, $CHCl_3$); ir ν max (neat) 2950, 2918, 1735, 1457, 1275, 1180 cm^{-1}; cims *m/z* (%) $[M-32]^+$ 324 (5), 292 (9), 276 (10), 233 (10), 137 (20), 136 (30), 123 (10), 109 (60); hreims *m/z* 324.1937 (calcd for $C_{18}H_{28}O_5$ $[M-32]^+$ 324.1929; nmr see Table 2. Methylation of semipurified **4** with CH_2N_2 as described for **2** afforded methyl ester **5**. The nmr spectrum of crude **4** was essentially identical with that of **5** except for the missing OMe signal.

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