Notes

Cyclic Peroxides Derived from the Marine Sponge *Plakortis simplex*

Michael Holzwarth,[†] Jean-Michel Trendel,[‡] Pierre Albrecht,[‡] Armin Maier,[§] and Walter Michaelis*[,†]

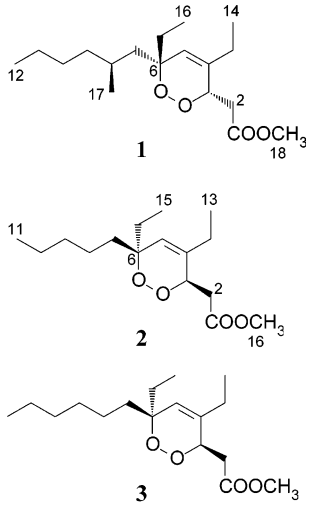
Institut für Biogeochemie und Meereschemie, Universität Hamburg, Bundesstrasse 55, D-20146 Hamburg, Germany, Laboratoire de Géochimie Bioorganique, Université Louis Pasteur, ECPM, 25 rue Becquerel, F-67200 Strasbourg, France, and Oncotest GmbH, Am Flughafen 12-14, D-79108 Freiburg, Germany

Received October 19, 2004

Two new cyclic peroxides, **2** and **3**, were isolated from a sample of the Norwegian sponge *Plakortis simplex*. Their structures including relative stereochemistry were elucidated by interpretation of MS and NMR data. Compound **3** exhibited moderate in vitro activity against six solid human tumor cell lines with IC_{50} values in the range 7−15 μg/mL.

Sponges of the family Plakinidae are known to be a source of numerous biologically active metabolites.[1] The structures of these natural products include members of different compound classes such as lactones,[2,3] alkaloids,[4] glycolipids,[5] and others. Another prominent family is formed by the cyclic peroxides and structurally related compounds,[6] many of which possess a six-membered ring structure within a substituted fatty acid backbone.[7−10] This class of compounds is represented by structure **1**, which has been described previously from *Plakortis* sp.[11] and *Plakortis* aff. *angulospiculatus*.[12] Peroxides such as these have proven to be potent agents with various activities such as antifungal,[13] antibiotic,[14] cytotoxic,[9,15] antiplasmodial,[16,17] or cardiac SR-Ca^{2+}-pumping ATPase activating effects.[18] In our search for new biologically active metabolites of boreal sponges, we purified two new cyclic peroxides, **2** and **3**, from a sample of *Plakortis simplex* (order Homosclerophorida, family Plakinidae). The current paper presents the isolation, structure elucidation, and cytotoxicity test results of these compounds.

The sponge sample was collected in summer of 1999 at the Norwegian Sula Ridge Reef from a water depth of 280 m. The collection was performed using a manned submersible. A MeOH/CH_2Cl_2 extract was dried and chromatographed over a silica gel column. Further purification of one fraction by HPLC on a RP-18 column using CH_3CN/H_2O as mobile phase led to the isolation of metabolites **2** and **3**. The two compounds showed no molecular ion peak by positive EIMS (70 eV) analysis, but did show $[M + NH_4]^+$ ions as base peaks at *m/z* 302 for **2** and *m/z* 316 for **3** by CIMS using ammonia as reactant gas. Pseudomolecular ion peaks $[M + H]^+$ were also observed by HRFABMS at *m/z* 285.2058 (285.2060 calculated for $C_{16}H_{29}O_4$) and *m/z* 299.2218 (299.2217 calculated for $C_{17}H_{31}O_4$), enabling assignment of the molecular formulas as $C_{16}H_{28}O_4$ and $C_{17}H_{30}O_4$ for **2** and **3**, respectively. The EIMS of **2** exhibits ions at *m/z* 266 and 255 corresponding to the loss of water and to the loss of an ethyl group, respectively. Likewise, **3** shows ions at *m/z* 280 and 269. The mass differences of 14 amu in these fragment ions, as well as in their molecular weights, indicate that the structures **2** and **3** vary only by one methylene group. The ^1H NMR spectrum of **2** (Table 1) shows signals for 28 protons, including three methyl triplets, one methyl singlet (δ 3.72, ester methyl), and two downfield signals, one at δ 5.51 ascribable to a vinylic proton and the other (δ 4.62) consistent with a proton located on an oxygen-bearing carbon atom. ^{13}C and DEPT NMR spectra reveal the presence of seven methylene as well as six methyl and methine carbons. Moreover, three quaternary carbons can be observed, one single-bonded to an oxygen (δ 82.4), one olefinic (δ 137.6), and one carboxylic (δ 171.6). A strong heteronuclear coupling between the last mentioned carbon (C-1) with the protons of the methyl singlet (H-16), observed in the long-range $^1H-^{13}C$ ($^{2,3}J_{CH}$) correlation data from an HMBC experiment (Table 2), indicates the presence of a methyl ester group. $^1H-^1H$ COSY (Table 2) and HSQC ($^1J_{CH}$) spectra revealed the proton connectivity pattern, which, together with the HMBC data, allowed establishment of the carbon framework. Thus, the backbone consists of an undec-4-enoic acid unit, which bears two ethyl groups at the C-4 and C-6 positions. Two carbon atoms, C-3 (δ 76.6) and C-6 (δ 82.4), are linked to heteroatoms, which must form an epidioxy



* To whom correspondence should be addressed. Tel: +49-40-42838-5001. Fax: +49-40-42838-6347. E-mail: michaelis@geowiss.uni-hamburg.de.
† Universität Hamburg.
‡ Université Louis Pasteur, Strasbourg.
§ Oncotest GmbH, Freiburg.

10.1021/np049665v CCC: $30.25
Published on Web 04/22/2005

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**, **2**, and **3** in $CDCl_3$

	1[a]		**2**		**3**	
atom	δ_C[b]	δ_H[b]	δ_C[b]	δ_H[b] (multiplicity; J[c])	δ_C[b]	δ_H[b] (multiplicity; J[c])
1	171.6		171.6		171.7	
2	37.2	2.88	36.9	2.88 (dd; 16.0, 9.5)	37.0	2.88 (dd; 16.0, 9.5)
		2.55		2.56 (dd; 16.0, 3.0)		2.56 (dd; 16.0, 3.0)
3	76.6	4.59	76.6	4.62 (m)	76.7	4.61 (m)
4	137.6		137.6		137.7	
5	125.2	5.48	124.7	5.51 (ddd; 1.5, 1.5, 1.5)	124.8	5.51 (ddd; 1.5, 1.5, 1.5)
6	83.5		82.4		82.5	
7	42.4	1.45	34.8	1.46 (m)	34.9	1.46 (m)
		1.36				
8	28.4	1.43	22.9	1.15−1.35	23.4	1.15−1.35
9	38.3	1.22	32.2	1.15−1.35	29.8	1.15−1.35
10	29.3	1.22	22.4	1.15−1.35	31.6	1.15−1.35
11	22.9	1.22	14.0	0.87 (t; 7.0)	22.7	1.15−1.35
12	14.1	0.86	24.9	2.01 (m)	14.1	0.88 (t; 7.0)
13	24.9	1.99	11.7	1.08 (t; 7.5)	25.0	2.01 (m)
14	11.8	1.06	29.6	1.76 (dq; 14.0, 7.5)	11.8	1.08 (t; 7.5)
				1.62 (dq; 14.0, 7.5)		
15	30.9	1.73	7.9	0.89 (t; 7.5)	29.8	1.76 (dq; 14.0, 7.5)
		1.63				1.62 (dq; 14.0, 7.5)
16	8.2	0.87	51.8	3.72 (s)	8.0	0.89 (t; 7.5)
17	22.2	0.88			52.0	3.72 (s)
18	51.9	3.70				

[a] See ref 12. [b] δ in ppm relative to TMS. [c] J given to the nearest 0.5 Hz.

Table 2. H,H COSY and HMBC NMR Correlations of **2** in $CDCl_3$

atom	H,H COSY[a]	HMBC[b]
2	3	1,3,4
3	2,5	1,2,4,5,12
5	3,12	3,4,6,7,12
7	[8,9,10][c]	5,6,8,9
[8,9,10][c]	7,11	7,8,9,10,11
11	[8,9,10][c]	9,10
12	5,13	3,4,5,13
13	12	4,12
14	15	5,6,7,15
15	14	6,14
16		1

[a] Correlations are given from the proton designated in the left column to the protons listed. [b] Correlations are given from the proton designated in the left column to the carbons listed. [c] Correlations from or to one of the given protons in brackets showing overlapping ^{1}H NMR resonances.

bridge and consequently a six-membered ring, due to the elemental composition and the unsaturation equivalents.

Relative chirality (*RS*) at C-3 and C-6 follows from NOESY data, i.e., correlations observed for H-3 with H-15 and one of the C-14 protons as well as between the most deshielded C-2 proton and one or more of the other protons located at C-8, C-9, or C-10. Details of absolute stereochemistry could not be determined, since the limited amounts of **2** and **3** isolated were employed for biological tests.

As suggested by EIMS and supported by ^{13}C NMR spectra (Table 1), compound **3** differs from **2** only in the presence of an additional methylene group. ^{1}H NMR spectra of both products (Table 1) are nearly superimposable, except for the 1.15−1.35 ppm region. On the basis of 2D NMR correlations, exactly the same structural features were evident in both compounds. Consequently, the backbone of **3** was established as a dodec-4-enoic acid unit. Comparison of ^{1}H and ^{13}C NMR data from **2** and **3** with those described for **1**[12] (Table 1) shows excellent agreement, except for the signals caused by atoms of, and close to, the side chain branching of **1**. On the basis of these results, the structures of **2** and **3** were assigned as shown. Substance **3** differs from the closest known compound **1** in the lack of the methyl branch at C-8.

Table 3. Antitumor Activity of **2** and **3** in Human Tumor Cell Lines

	IC_{50}[a]	
tumor cell line	**2**	**3**
GXF 251L	>10.0	8.3
LXF 529L	>10.0	7.7
MAXF 401NL	>10.0	14.9[b]
MEXF 642NL	>10.0	7.2
RXF 486L	>10.0	14.4[b]
UXF 1138L	>10.0	14.1[b]

[a] IC_{50} in μg/mL. [b] Value extrapolated.

Peroxide **3** exhibited moderate cytotoxicity toward tumor cells, whereas peroxide **2** did not inhibit proliferation in any of the tumor cell lines tested up to a concentration of 10 μg/mL. Peroxide **3** selectively inhibited proliferation in gastric cancer (GXF 251L, IC_{50} = 8.3 μg/mL), non-small cell lung cancer (LXFL 529L, IC_{50} = 7.7 μg/mL), and melanoma (MEXF 462NL, IC_{50} = 7.2 μg/mL) cell lines (Table 3). Mammary (MAXF 401NL, IC_{50} = 14.9 μg/mL), renal (RXF 486L, IC_{50} = 14.4 μg/mL), and uterin (UXF 1138L, IC_{50} = 14.1 μg/mL) cancer cell lines were slightly less sensitive.

Experimental Section

General Experimental Procedures. 1D (^{1}H, ^{13}C, DEPT 135) and 2D (^{1}H−^{1}H COSY and NOESY; ^{1}H−^{13}C HSQC and HMBC) NMR spectra were taken on a Bruker ARX-500 spectrometer operating at observation frequencies of 500 and 125 MHz for ^{1}H and ^{13}C nuclei, respectively. Data were recorded at 300 K in $CDCl_3$ using the standard Bruker software package. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane using the ^{13}C and the residual ^{1}H signals of the solvent as secondary references (δ 77.0 and 7.26, respectively). Coupling constants (J values) are given to the nearest 0.5 Hz. All mass spectra were obtained on a Micromass Autospec spectrometer. 3-Nitrobenzyl alcohol was used as matrix and PEG 400 as reference for HRFABMS. Liquid chromatography was performed over silica gel (Merck Kieselgel 60, 63−200 μm).

Sample Collection. During the research cruise POS 254 with the *RV Poseidon* in July 1999, the sponge was collected at the Sula Ridge Reef (64°04′50″ N/08°01′40″ E), Norwegian Shelf. The sampling was performed at a water depth of 280 m

and a water temperature of ~8 °C by the manned submersible JAGO, which was equipped with a remote-controlled gripper arm. The light brown sponge was encrusting on dead coral material located at the peripheral region of the coral reef. The cylindrical formation of coral rubble and sponge specimen was ~10 cm in diameter and ~15 cm in length. The thickness of the coral-coating sponge was between 1 and 2 cm. The specimen was immediately frozen and kept at −20 °C until analysis. A voucher specimen (POS 31VII99 22J1-20) has been deposited at the Institut für Biogeochemie und Meereschemie, Universität of Hamburg.

Extraction and Isolation. A 10.2 g (wet weight) sample of the sponge was separated from remaining coral rubble, fragmented into small pieces, and homogenized. It was exhaustively extracted with a mixture of MeOH and CH_2Cl_2 (1:1, v/v). The solvent was evaporated under reduced pressure to yield 82 mg (0.8% of wet weight) of a pale yellowish oil. This oil was adsorbed on 0.5 g of Si gel and separated on a 10 × 1 cm Si gel column. A sequence of eluents with increasing polarity from *n*-hexane (1 × 75 mL) through CH_2Cl_2 (3 × 50 mL) and EtOAc (1 × 75 mL) to MeOH (1 × 100 mL) was used to produce six fractions. The third fraction overall (the second CH_2Cl_2 fraction) was further purified by reversed-phase HPLC using a C-18 column (Merck LiChrosphere RP18, 5 μm, 250 × 10 mm). Multiple injections with 30% H_2O in CH_3CN (isocratic, 4 mL/min) yielded 1.3 mg of **2** (0.01% of wet weight) and 1.1 mg of **3** (0.01% of wet weight).

Antitumor Test. Effects of the test compounds on the proliferation of human tumor cells were determined in an assay, where human tumor cell lines growing as monolayers were treated with the test compounds and surviving cells were stained with a fluorescent dye. Details of the test procedure have been described elsewhere.[19] Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice. Doubling times of the cell lines ranged between 24 h ± 1 h (MEXF 462NL) and 35 h ± 2 h (MAXF 401NL). Antitumor efficacies were described by inhibitory concentrations (i.e., IC_{50}), reflecting concentration-dependent cytotoxicity. Extrapolated IC values are given if the exact value could not be determined within the test range, and if linear regression of existing T/C values would result in IC values within a range of 3-fold the highest test concentration. In the case of resistant cell lines, exhibiting no activities, IC values are expressed to be greater than the highest test concentration.

Methyl (3*R,6*S**,*Z*)-4,6-diethyl-3,6-epidioxyundeca-4-enoate (2):** ^1H and ^{13}C NMR data, see Table 1; EIMS no molecular ion, fragment ions at *m/z* 266 $[M - H_2O]^+$ (2), 255 (39), 237 (20), 213 (17), 195 (32), 181 (100), 163 (49), 139 (67), 125 (38), 111 (32), 99 (32); CIMS *m/z* 302 $[M + NH_4]^+$ (100), 285 (10), 267 (16), 239 (19), 197 (28); HRFABMS *m/z* 285.2058 $[M + H]^+$ (calcd for $C_{16}H_{29}O_4$, 285.2060).

Methyl (3*R,6*S**,*Z*)-4,6-diethyl-3,6-epidioxydodeca-4-enoate (3):** ^1H and ^{13}C NMR data, see Table 1; EIMS no molecular ion, fragment ions at *m/z* 280 $[M - H_2O]^+$ (1), 269 (29), 251 (20), 219 (18), 195 (83), 177 (75), 159 (32), 149 (38), 139 (100), 125 (49), 111 (43); CIMS *m/z* 316 $[M + NH_4]^+$ (100), 299 (12), 281 (13), 253 (18), 197 (55); HRFABMS *m/z* 299.2218 $[M + H]^+$ (calcd for $C_{17}H_{31}O_4$, 299.2217).

Acknowledgment. The authors gratefully thank T. Pape for preparation of the sponge during the sampling cruise, F. Hoffmann for taxonomic determination, and R. Graff for assistance in NMR experiments. This paper represents publication no. 31 of the research program BOSMAN (03F0256 A). Financial support was provided by the Bundesministerium für Bildung und Forschung (BMBF).

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP049665V