Journal of Natural Products
Vol. 53, No. 3, pp. 669-674, May-Jun 1990

TWO NEW BIOACTIVE CYCLIC PEROXIDES FROM THE MARINE SPONGE *PLAKORTIS ANGULOSPICULATUS*

SARATH P. GUNASEKERA,* MALIKA GUNASEKERA, GEEWANANDA P. GUNAWARDANA, PETER MCCARTHY, and NEAL BURRES

Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Fort Pierce, Florida 34946

ABSTRACT.—Two new antifungal and cytotoxic cyclic-peroxide-containing acids, **1** and **3**, were isolated from a marine sponge, *Plakortis angulospiculatus*, which was collected by scuba off the coast of Venezuela. The structures were elucidated through spectral and chemical analysis of the corresponding methyl esters, **2** and **4**. The acids are potent cytoxic and antifungal agents although their esters are only cytotoxic.

In addition to the common steroidal peroxides (1), numerous other cyclic peroxides have been reported from marine organisms (2,3). Most are derived from sponges and are carboxy-containing products of terpenoid (4–7) or polyketide (8–12) biosynthesis. Examples are the branched chain esters, plakortin (9) and epiplakortin (11); the free acid, plakortic acid (12); and the straight-chain metabolites, e.g., chondrillin (5) and the related compounds from *Plakortis lita* (13). These compounds exhibit antimicrobial activity (7, 9–12), ichthyotoxicity (5), and cytotoxicity (6,12). We report here details of the isolation and structure determination of two related and biologically active metabolites **1** and **3** and their chemically derived methyl esters **2** and **4** from a sponge, *Plakortis angulospiculatus* (Carter) of the family Plakinidae.

The frozen sponge was homogenized with MeOH and then with EtOAc. The MeOH extract was partitioned between H_2O and EtOAc. The EtOAc-soluble layers were combined to yield a crude extract that was subjected to Si gel chromatography. A portion of a biologically active fraction was purified by reversed-phase hplc (C_{18}, 10% H_2O/MeCN) to yield two bioactive acids **1** and **3** (Table 1). The remainder of this fraction was treated with excess CH_2N_2 in Et_2O to give the corresponding methyl esters. The methyl ester fraction was separated by hplc (Si, 1.5% EtOAc-heptane) to give the corresponding methyl esters **2** and **4**.

The ester **2** ($[\alpha]_D$ −89°) yielded the molecular formula $C_{22}H_{38}O_4$ by hrfabms. Its ir spectrum indicated the presence of an ester group (1732 cm^{-1}) and the absence of hydroxyl groups. The ^{13}C-nmr spectrum (Table 2) contained a carbonyl signal observed at δ 171.7 (s), a methoxy methyl signal observed at δ 51.9 (q), two signals for oxygen-bearing carbon atoms observed at δ 83.5 (s) and 76.5 (d), two signals attributed to a trisubstituted olefin observed at δ 137.5 (s) and 125.1 (d), two signals attributed to a disubstituted olefin observed at δ 133.2 (d) and 132.1 (d), and four signals for five methyl groups observed at δ 21.3, 14.3, 11.7 (2 × Me), and 8.2. Three of the four req-

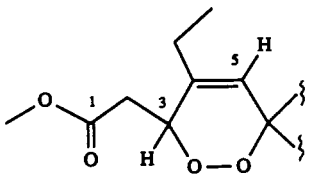
TABLE 1. Biological Activity of Compounds **1–4**.

Compound	Cytotoxicity	Antimicrobial Activity
	Murine leukemia P-388 IC_{50} (μg/ml)	*Candida albicans* MIC (μg/ml)
Acid **1**	0.3	1.6
Acid **3**	0.2	1.6
Ester **2**	0.9	>50
Ester **4**	0.2	>50

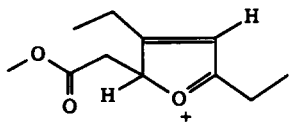
uisite degrees of unsaturation are accounted for in the ^{13}C-nmr data; the compound must contain one ring. The ^{1}H-nmr spectrum confirmed the presence of a trisubstituted olefin [δ 5.47 (dt, 1H, J = 1.6, 1.4 Hz)] and a methyl ester [δ 3.70 (s, 3H)]. The olefinic proton showed allylic coupling (J = 1.6 Hz) to an oxycarbyl-methine signal observed at δ 4.57, which in turn was coupled to two geminally coupled signals observed at δ 2.51 (1H, dd, J = 16.0, 2.8 Hz) and 2.87 (1H, dd, J = 16.0, 9.5 Hz). These signals are assigned to the α-methylene protons of the ester moiety. The same olefinic proton showed additional allylic couplings (J = 1.4 Hz) to the methylene protons of an ethyl group [δ 1.97 (2H, m) and 1.06 (3H, t)]. Absence of other cross peaks for the same olefinic proton in the COSY spectrum confirmed that the adjacent carbons are fully substituted. Thus the only two quaternary carbon atoms in the ^{13}C spectrum observed at δ 137.5 and 83.5 can be assigned to C-4 and C-6, respectively. The HMBC experiment (14) of the corresponding acid **1** (Table 2) revealed cross peaks between signals observed at δ 4.55 and 124.9 and at δ 5.46 and 76.5, which allowed the remaining oxygen-bearing carbon to be placed at C-3. Absence of any other free hydroxyl groups in the molecule suggested the partial structure **A**.

The mass spectrum gave the base peak at *m/z* 197 ($C_{11}H_{17}O_3$), which corresponds to a fragment ion that contains the carbonyl terminus. Absence of any deshielded methyl singlets and the presence of deshielded methylene protons (H-17a and H-17b), which are vicinally coupled to a methyl group, argued for the attachment of an ethyl group to C-6. This suggested the partial structure **B** for the mass fragment *m/z* 197.

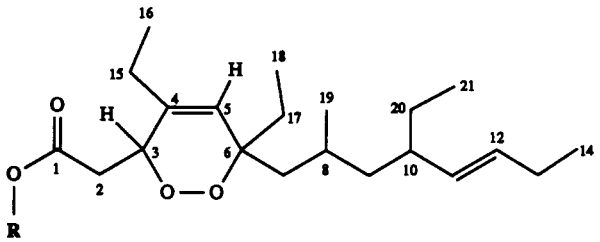
The remaining methyl groups appeared in the ^{1}H-nmr spectrum as one doublet and two triplets. Biosynthetically, the branching could be on C-8 or C-10 (11). The ^{1}H-



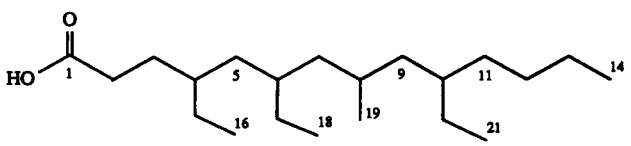
A



B



1 R=H
2 R=Me
3 R=H, 11,12 dihydro
4 R=Me, 11,12 dihydro



5

TABLE 2. ^{1}H- (360 MHz) and ^{13}C- (90.5 MHz) nmr Data[a] in $CDCl_3$.

Position	Compound				
	1			2	
	δH	δC	HMBC(^{1}H)	δH	δC
1		176.6[b] (s)[c]			171.7[b] (s)[c]
2	2.86 (dd, 16.0, 9.5)	35.3 (t)		2.87 (dd, 16.0, 9.5)	37.1 (t)
	2.53 (dd, 16.0, 2.8)		3H	2.51 (dd, 16.0, 2.8)	
3	4.55 (ddd, 9.5, 2.8, 1.6)	76.5 (s)	5H, 15H	4.57 (ddd, 9.5, 2.8, 1.6)	76.5 (d)
4		137.5 (s)	3H, 5H, 15H, 16H		137.5 (s)
5	5.46 (dt, 1.6, 1.4)	124.9 (d)	3H, 7H, 15H, 17H, 18H	5.47 (dt, 1.6, 1.4)	125.1 (d)
6		83.6 (s)	5H, 7H, 8H, 17H, 18H		83.5 (s)
7	1.38 (d, 5.4)	43.3 (t)	5H, 17H	1.38 (d, 5.4)	43.3 (t)
8	1.50 (m)	25.9 (d)	9H, 19H	1.52 (m)	25.9 (d)
9	1.39 (m)	28.9 (t)		1.38 (m)	28.9 (t)
	1.15 (m)		11H, 19H	1.15 (m)	
10	1.80 (m)	42.3 (d)	11H, 12H, 20H, 21H	1.81 (m)	42.3 (d)
11	4.95 (ddt, 15.4, 9.2, 1.2)	133.2 (d)	13H, 20H	4.95 (ddt, 15.4, 9.2, 1.2)	133.2 (d)
12	5.35 (dt, 15.4, 6.4)	132.1 (d)	13H, 14H	5.35 (dt, 15.4, 6.4)	132.1 (d)
13	1.98 (m)	25.7 (t)	11H, 12H, 14H	1.97 (m)	25.7 (t)
14	0.94 (t, 7.5)	14.2 (q)	12H, 13H	0.93 (t, 7.5)	14.3 (q)
15	1.98 (m)	24.8 (t)	3H, 15H, 16H	1.97 (m)	24.9 (t)
16	1.06 (t, 7.4)	11.7 (q)	15H	1.06 (t, 7.4)	11.7 (q)
17	1.69 (dq, 15.0, 7.5)	30.9 (t)		1.70 (dq, 15.0, 7.5)	30.9 (t)
	1.58 (dq, 15.0, 7.5)		7H, 18H	1.57 (dq, 15.0, 7.5)	
18	0.86 (t, 7.5)	8.1 (q)	17H	0.86 (t, 7.5)	8.2 (q)
19	0.83 (d, 6.7)	21.3 (q)	7H, 9H	0.83 (t, 6.7)	21.3 (q)
20	1.28 (m)	44.5 (t)		1.28 (dq, 6.5, 7.4)	44.5 (t)
	1.13 (m)		9H, 11H	1.13 (dq, 6.5, 7.4)	
21	0.79 (t, 7.4)	11.6 (q)		0.79 (t, 7.4)	11.7 (q)
22				3.70 (s)	51.9 (q)

[a]Table entries are chemical shift, ppm from solvent (multiplicity, *J* in Hz).
[b]One-bond CH correlations were observed by HMQC experiment.
[c]Bruker's DEPT pulse sequence.

nmr spectrum indicated a trans disubstituted double bond [δ 4.95 (J = 15.4, 9.2, 1.2 Hz), 5.35 (J = 15.4, 6.4 Hz)] and three allylic protons [δ 1.81 (1H) and 1.97 (2H)], implying branching at one of the allylic positions. A ^{1}H-^{1}H COSY spectrum indicated coupling between the two allylic protons at δ 1.97 with a methyl group at δ 0.93. This argued for the attachment of one of the two remaining ethyl groups at C-12. This also established the double bond at C-11 and C-12. The *E* configuration was assigned to the double bond based on the large coupling constant between the two olefinic protons (J = 15.4 Hz). A difference double resonance experiment indicated that the remaining allylic proton observed at δ 1.81 is coupled to a CH_2 group [δ 1.28 (H), 1.13 (H)], which in turn was vicinally coupled to an Me group (δ 0.79). Thus, the remaining ethyl group must be attached to C-10. Irradiation of the remaining methyl doublet in a difference double resonance experiment simplified the multiplet for the proton at C-8, which is observed at δ 1.50 in the free acid **1**, to a triplet of triplet. These data argue for the attachment of the remaining methyl group at C-8. This deduction was confirmed by correlations observed in the HMBC experiment (Table 2). Accordingly, the structure for the methyl ester was assigned as **2**.

Methylation of the purified acid **1** with excess CH_2N_2 in Et_2O furnished the methyl ester, which was identical in all respects to **2** and thus confirmed the structure of **1**.

The ester **4** ([α]D −90°) had the molecular formula $C_{22}H_{40}O_4$, suggesting the monosaturated analogue of **2**. The ir spectrum indicated the presence of an ester group (1727 cm^{-1}) and absence of any hydroxyl groups. Observed in the low-field region of the ^{1}H-nmr spectrum were two signals at δ 4.58 (1H, ddd, J = 9.5, 2.8, 1.6 Hz) and 5.47 (1H, dt, J = 1.6, 1.4 Hz) for the protons at C-3 and C-5, respectively. In contrast to **2**, the absence of any other low-field signals established that the side chain is saturated. The ^{13}C-nmr spectrum suggested two olefinic signals, which, by comparison with the spectrum of **2**, led to the assignment of these two olefinic carbons at C-4 and C-5. The ^{1}H-nmr spectrum of **4** indicated four methyl triplets and one methyl doublet, similar to the spectrum of compound **2**. These data suggested that **4** is a dihydro analogue of **2**. Hydrogenation of **1** and **3** separately in the presence of 10% Pd/C as a catalyst followed by purification gave a mixture of C-4 epimers of the acid **5** as the major product. Comparison of the ^{1}H- and ^{13}C-nmr data and the mass spectral data confirmed the structural relationship between **1** and **3**. Methylation of purified dihydro free acid **3** with CH_2N_2 in Et_2O furnished a methyl ester whose ^{1}H- and ^{13}C-nmr spectra were identical to those of **4** and thus established the structure **3** for the dihydro free acid. Difference nOe experiments on **2** did not give conclusive evidence for the relative stereochemistry of the substituents attached to the ring containing the peroxide moiety.

Acids **1** and **3** both showed activity against *Candida albicans* (Table 1). Activity was also observed against *Aspergillus nidulans* and *Bacillus subtilis* when assayed by the disc diffusion method. No activity was seen against *Escherichia coli*. The esters **2** and **4** were inactive against all of the microorganisms tested. Cytotoxicity against P-388 murine leukemia was observed at similar levels for compounds **1–4** (Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. Nmr spectra were recorded on a Bruker instrument operating at 360 MHz for ^{1}H and 90.5 MHz for ^{13}C. The high resolution mass spectra were obtained on a VG ZAB-2SE mass spectrometer at University of Illinois at Urbana-Champaign. Optical rotations were measured with a Jasco DIP 360 digital polarimeter.

COLLECTION AND EXTRACTION.—The sponge *P. angulospiculatus* was collected off the coast of Venezuela at a depth of 30 m in January 1988, and immediately frozen. A voucher specimen is deposited in the

Indian River Coastal Museum of the Harbor Branch Oceanographic Institution. The freshly thawed sponge (103 g wet wt) was extracted with MeOH and then exhaustively with EtOAc. The MeOH extract was concentrated in vacuo on an H_2O bath maintained at 35°. The resulting extract was partitioned between EtOAc and H_2O. The EtOAc-partitioned fraction was combined with the other EtOAc extracts, and the solvent was removed in vacuo on an H_2O bath to obtain a brown gum (0.53 g, 0.5% wet wt). The EtOAc-soluble fraction (0.50 g) was chromatographed on Si gel (Kieselgel 60H) using CH_2Cl_2 and CH_2Cl_2/MeOH step gradient and monitored by antifungal assay. Further chromatography using hplc (absorbosphere C_{18}, 5 μm, 250 × 10 mm, 10% H_2O/MeCN) of a portion of the antifungal active fraction furnished the acid **1** (0.01% yield wet wt) and acid **3** (0.002%). The remainder of the active fraction was treated with excess CH_2N_2 and purified by hplc (absorbosphere Si, 5 μm, 250 × 10 mm, 1.5% EtOAc/heptane) to yield esters **2** and **4**.

Acid **1**.—$[\alpha]_D$ −34.6° (c = 0.2, $CHCl_3$); ir ($CHCl_3$) 3650, 3510, 2980, 2920, 1710, 1450, 1380, 1290, 972 cm^{-1}; lreims *m/z* (rel. int.) $[M - CH_2COOH]^+$ 293 (0.4%), 277 (0.5), 263 (0.7), 249 (2), 233 (3), 221 (1), 207 (2), 199 (5), 195 (10), 193 (2), 167 (12), 151 (12).

Ester **2**.—$[\alpha]_D$ −89° (c = 0.1, $CHCl_3$); ir ($CHCl_3$) 2920, 1732, 1351, 1160, 974 cm^{-1}; hrfabms *m/z* 367.2847, Δ 0.1 mmu for $C_{22}H_{39}O_4$ $[M + 1]^+$; lrfabms *m/z* (rel. int.) 367 (10), 349 (22), 321 (51), 309 (12), 291 (3), 279 (22), 209 (23), 197 (100).

Acid **3**.—$[\alpha]_D$ −36.0° (c = 0.16, $CHCl_3$); ir ($CHCl_3$) 3500, 2978, 2960, 1705, 1580, 1432, 1378, 1285 cm^{-1}; ^1H nmr ($CDCl_3$ + 1 drop CD_3OD) δ 0.78 (3H, t, J = 7.1 Hz), 0.85 (9H, m), 1.03 (3H, t, J = 7.3 Hz), 1.97 (2H, m), 2.65 (1H, m), 2.79 (1H, m), 4.56 (1H, m), 5.47 (1H, s); ^{13}C nmr ($CDCl_3$) δ 176.6 (s), 138.5 (s), 124.1 (d), 83.7 (s), 77.3 (d), 43.4 (t), 42.9 (t), 36.2 (t), 36.2 (d), 32.6 (t), 31.1 (t), 28.6 (t), 26.3 (t), 25.9 (d), 24.8 (t), 23.2 (t), 22.4 (q), 14.1 (q), 11.6 (2q), 10.8 (q); lreims *m/z* (rel. int.) $[M - C_2H_5]^+$ 325 (10), 292 (3), 263 (12), 247 (10), 199 (8), 195 (6), 151 (11), 139 (48), 137 (62), 125 (26), 109 (22), 83 (35), 71 (35), 56 (100).

Ester **4**.—$[\alpha]_D$ −90.7° (c = 0.04, MeOH); ir ($CHCl_3$) 2955, 2920, 1727, 1425, 1235, 1170, 922, cm^{-1}; ^1H nmr (C_6D_6) δ 0.85 (3H, t, J = 7.4 Hz), 0.88 (3H, t, J = 7.2 Hz), 0.89 (3H, t, J = 7.2 Hz), 0.95 (3H, t, J = 7.4 Hz), 1.03 (3H, d, J = 6.3 Hz), 1.99 (2H, m), 2.42 (1H, dd, J = 16.0, 2.8 Hz), 3.02 (1H, dd, J = 16.0, 9.5 Hz), 3.32 (3H, s), 4.76 (1H, ddd, J = 9.5, 2.8, 1.6 Hz), 5.35 (1H, dt, J = 1.6, 1.4 Hz); ^{13}C nmr (CD_3OD) δ 172.8 (s), 139.5 (s), 126.1 (d), 85.0 (s), 77.9 (d), 52.4 (q), 44.4 (t), 44.3 (t), 38.2 (t), 37.6 (d), 33.7 (t), 32.0 (t), 29.6 (t), 27.5 (t), 27.0 (d), 25.8 (t), 24.2 (t), 22.8 (q), 14.4 (q), 12.1 (q), 11.2 (q), 8.4 (q); hrfabms *m/z* 323.2598, Δ 1.2 mmu for $C_{20}H_{35}O_3$ $[M - C_2H_5 - O]^+$; lrfabms *m/z* (rel. int.) $[M + Na]^+$ 391 (5), $[M + 1]^+$ 369 (6), 351 (36), 335 (15), 323 (42), 295 (28), 279 (17), 263 (7), 329 (5), 205 (22), 197 (100), 152 (90).

CATALYTIC HYDROGENATION OF THE ACIDS **1** AND **3**.—Acid **1** (3.0 mg) in absolute EtOH (5.0 ml) was treated in a Parr apparatus for 12 h at room temperature with a catalytic amount of 10% Pd on charcoal, under a pressure of 20 atm H_2. After filtration and evaporation, the crude product was purified by Si Sep-Pak using 2% MeOH/CH_2Cl_2 to give the C-4 epimeric mixture of acid **5** as the major product (1.0 mg, colorless oil): ^1H nmr ($CDCl_3$) δ 0.81 (3H, t, J = 7.3 Hz), 0.83 (3H, t, J = 7.3 Hz), 0.85 (3H, t, J = 7.2 Hz), 0.86 (3H, d, J = 6.7 Hz), 0.93 (3H, t, J = 7.3 Hz), 2.35 (1H, dt, J = 16.0, 9.5 Hz), 2.45 (1H, dt, J = 16.0, 9.5 Hz); ^{13}C nmr ($CDCl_3$) δ 176.5 (s), 47.5 (d), 46.7 (d), 43.4 (d), 42.6 (d), 36.5 (t), 33.5 (t), 32.5 (t), 30.8 (t), 29.4 (t), 28.6 (t), 27.3 (t), 26.6 (t), 25.9 (t), 23.2 (t), 22.2 (t), 14.2 (q), 10.9 (q), 10.6 (q), 8.4 (q), 8.3 (q); hrcims (isobutane) *m/z* 325.3084, Δ 0.8 mmu for $C_{21}H_{41}O_2$ $[M + 1]^+$; lrcims (isobutane) *m/z* (rel. int.) $[M + 1]^+$ 325 (100), 311 (51), 227 (24), 213 (61), 199 (48), 185 (32), 169 (83), 139 (48).

Acid **3** (3.0 mg) was hydrogenated under the same conditions and purified to give the C-4 epimeric acid as the major product (1.2 mg, colorless oil). It was shown to be identical to acid **5** by comparison of ^1H and ^{13}C nmr and mass spectral fragmentation patterns.

BIOLOGICAL ASSAYS.—Cytotoxic activity against the P-388 murine leukemia cell line was determined by the method of Alley *et al.* (15). MICs were determined for *C. albicans* (ATCC 44506) by a standard microdilution broth method (16) using Sabouraud dextrose broth as growth medium. Activities against other microorganisms were determined by the disc diffusion method (17). Zones of growth inhibition were determined from paper discs containing 20 μg of the test compound.

ACKNOWLEDGMENTS

We thank Richard Milberg, University of Illinois, Urbana, for mass spectral data. We are also grateful to Dr. O.J. McConnell for useful discussions, Dr. S. Pomponi for collection and identification of the sponge, J. Hunter for the antitumor assay, and T. Peterson and J. Johnson for antimicrobial assays. This is Harbor Branch Oceanographic Institution Contribution No. 757.

LITERATURE CITED

1. A.A.L. Gunatilaka, Y. Gopichand, F.J. Schmitz, and C. Djerassi, *J. Org. Chem.*, **46**, 3860 (1981).
2. D.J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984), and references cited therein.
3. D.J. Faulkner, *Nat. Prod. Rep.*, **4**, 539 (1987), and references cited therein.
4. Y. Kashman and M. Rotem, *Tetrahedron Lett.*, 1707 (1979).
5. A. Albericci, J.C. Breakman, D. Daloze, and B. Tursch, *Tetrahedron*, **38**, 1881 (1982).
6. L.V. Manes, G.J. Bakus, and P. Crews, *Tetrahedron Lett.*, **25**, 931 (1984).
7. R.J. Capon and J.K. MacLeod, *Tetrahedron*, **41**, 3391 (1985).
8. R.J. Wells, *Tetrahedron Lett.*, 2637 (1976).
9. M.D. Higgs and D.J. Faulkner, *J. Org. Chem.*, **43**, 3454 (1978).
10. D.B. Stierle and D.J. Faulkner, *J. Org. Chem.*, **44**, 964 (1979).
11. D.B. Stierle and D.J. Faulkner, *J. Org. Chem.*, **45**, 3396 (1980).
12. D.W. Phillipson and K.L. Rinehart Jr., *J. Am. Chem. Soc.*, **105**, 7735 (1983).
13. S. Sakemi and T. Higa, *Tetrahedron*, **43**, 263 (1987).
14. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
15. M.C. Alley, D.A. Scudieso, A. Monks, M.L. Hulsey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, and M.R. Boyd, *Cancer Res.*, **48**, 589 (1988).
16. R.N. Jones, A.L. Barry, T.L. Gavan, and J.A. Washington II, in: "Manual of Clinical Microbiology," 4th ed. Ed. by E.H. Lennette, A. Balows, W.J. Hausler, and H.J. Shadomy, American Society for Microbiology, Washington, DC, 1985, p. 972.
17. R.J. White, W.M. Maiese and M. Greenstein, in: "Manual of Industrial Microbiology and Biotechnology." Ed. by A.L. Demain and N.A. Solomon, American Society for Microbiology, Washington, DC, 1986, p. 24.

Received 4 December 1989