Journal of Natural Products
Vol. 53, No. 4, pp. 926-931, Jul-Aug 1990

PEROXY ALIPHATIC ESTERS FROM THE SPONGE *PLAKORTIS LITA*[1]

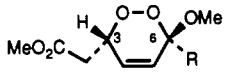
FLORECITA S. DE GUZMAN and FRANCIS J. SCHMITZ*

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019-0370

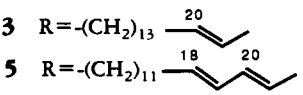
ABSTRACT.—Five new cyclic peroxy aliphatic esters **2–6** and three new butenolides **7–9** have been isolated from *Plakortis lita*, a sponge collected at Truk Island. The former are related to chondrillin [**1**], a known peroxy aliphatic ester. Structures and stereochemistry were determined by nmr spectroscopy.

An assortment of peroxy derivatives of natural products have been isolated from marine sources; the most common are sterol peroxides (1). The list also includes peroxy lactones, an ichthyotoxic peroxy cembranolide, a variety of peroxy terpenoids, and peroxy derivatives of aliphatic acids, both conventional straight chain ones and branched-chain propionate-based examples. The first of the straight-chain peroxy fatty acid esters to be reported was chondrillin [**1**] (2). Subsequently, similar peroxyesters varying in chain length from C-18 to C-24 have been recorded (3,4). As part of an ongoing search for bioactive compounds from marine sources, we have studied the organic extracts of an encrusting, maroon sponge, *Plakortis lita* De Laubenfels (Order Homosclerophorida) collected in Truk lagoon at depths of 4 to 15 feet. We report here the isolation of five new cyclic peroxy aliphatic esters and three new butenolides from this sponge together with chondrillin [**1**] and the known ester **10** (3). The butenolides appear to be further oxidation products of higher molecular weight peroxy aliphatic esters.

The peroxy esters and the butenolides were isolated as described in the Experimental section from specimens perserved by freezing. Chondrillin [**1**] was identified by comparison of its ^{1}H- and ^{13}C-nmr data with those reported by Wells (2). In agreement with the observation of Wells, the highest mass ions generally observed for chondrillin and the other peroxy esters described below were those corresponding to $[M-O_2]^+$ and $[M-(O_2+CO_2Me)]^+$.

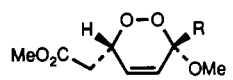


1 R=-$(CH_2)_{15}$-Me

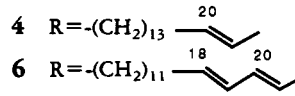


3 R=-$(CH_2)_{13}$-CH=CH-CH=CH-Me (20)

5 R=-$(CH_2)_{11}$-CH=CH-CH=CH-Me (18, 20)

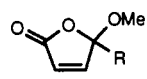


2 R=-$(CH_2)_{15}$-Me

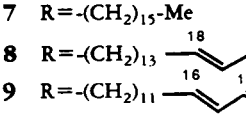


4 R=-$(CH_2)_{13}$-CH=CH-CH=CH-Me (20)

6 R=-$(CH_2)_{11}$-CH=CH-CH=CH-Me (18, 20)

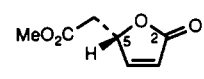


7 R=-$(CH_2)_{15}$-Me



8 R=-$(CH_2)_{13}$-CH=CH-CH=CH-Me (18)

9 R=-$(CH_2)_{11}$-CH=CH-CH=CH-CH=CH-Me (16, 18)



10

[1]Taken from the Ph.D. dissertation of Florecita S. DeGuzman, Department of Chemistry, University of Oklahoma, Norman, Oklahoma, 1989.

The cis arrangement of the alkyl substituents in **1** was confirmed by nOe experiments, and those are in agreement with the results of Sakemi *et al.* (4). Irradiation of the ketal methoxy signal at 3.37 ppm produced a 4.2% enhancement of the H-3 signal at 4.78 ppm, and irradiation of the H-2 signals at 2.92 and 2.61 ppm produced a 1.1% nOe on the 1.62 ppm methylene signal.

6-*epi*-Chondrillin [**2**] showed its highest mass ion at *m/z* 380.3280 ($C_{24}H_{44}O_3$, −2.7 ppm) corresponding to the same $[M-O_2]^+$ ion as observed for chondrillin. Decoupling revealed the same H-2 to H-5 spin system as is present in **1**, and all the ^{1}H- and ^{13}C-nmr data of **2**, except for H-2 and H-3, were nearly identical to those of **1**, which suggested that the two compounds were stereoisomers. Indeed, irradiation of the H-3 signal (5.00 ppm) of **2** induced a 3% nOe on the 1.67 ppm methylene signal, and irradiation of the methylene signals at 2.61 and 2.50 ppm (H-2) produced a 2.8% nOe on the methoxy signal at 3.38 ppm. Hence **2** has the trans disposition of alkyl substituents on the cyclic peroxide ring, making it the 6-*epi*- isomer of chondrillin.

Ester pairs **3/4** and **5/6** were proven to be cis/trans isomeric peroxy esters analogous to **1** and **2** by (a) comparison of ^{1}H- and ^{13}C-nmr data (Tables 1 and 2) (b) decoupling to confirm the H-2 to H-5 spin systems, and (c) determination of nOe's to confirm the relative stereochemistries as described above for **1** and **2**. Ester pairs **3/4** and **5/6** were determined to have, respectively, one and two additional double bonds compared to **1** and **2** by mass spectral analyses and ^{1}H- and ^{13}C-nmr data (Tables 1 and 2). Because the only methyl signals in the ^{1}H-nmr spectra of **3** and **4** were doublets in the vinyl region, 1.64 ppm, the position of the double bonds in the aliphatic chains was established as shown in the structures. The *E*-configuration of these double bonds follows from the chemical shifts of the terminal methyl carbons, 18.1 and 17.9 ppm (4,5).

For esters **5** and **6** the two double bonds in each of the aliphatic chains were found to be conjugated, inasmuch as only a vinyl methyl signal, 1.71, and one methylene signal, 2.02 ppm, were coupled to the olefinic signals. Uv absorption was observed at 217 nm for **5** indicative of conjugation but short of expectations based on Woodward's rules (6).

The formula of lactone **7** was confirmed by hrms analysis to be $C_{21}H_{38}O_3$, which implies three degrees of unsaturation. The ^{13}C-nmr spectrum confirmed there were only three non-sp^3 carbons, namely, one carbonyl carbon and two olefinic carbons; hence, **7** must contain one ring. This ring was formulated as a γ-methoxy-γ-alkyl substituted butenolide moiety on the basis of (a) ir absorption at 1724 cm^{-1}, (b) uv absorption at 206 nm, (c) an isolated, mutually coupled pair of doublet ^{1}H nmr signals at 6.21 and 7.20 ppm, (d) a ketal carbon singlet peak at 101.0 ppm, and (e) nmr signals for a methoxy group at 3.22 (s)/51.1 ppm. Because the only other ^{1}H-nmr signals consisted of a methyl triplet at 0.88 ppm and methylene signals at 1.89 (m, 2H) and 1.25 (br s) ppm, the remaining portion of **7** was identified as an *n*-C_{16} chain.

Lactones **8** and **9** also showed ir absorption at 1724 cm^{-1} and were characterized as analogues of **7** containing, respectively, one and two double bonds in the penultimate positions in the alkyl chain by comparison of ^{1}H- and ^{13}C-nmr data with that of **7** and **3–6** (Tables 1–3).

Lactone-ester **10** was identified by in-depth analysis of its spectral data, which matched those reported by Quinoa *et al.* (3) for 2-oxo-2,5-dihydrofuran-5-acetic acid methyl ester. However, the optical rotation measured for **10** was opposite (−96.1°) to that reported by Quinoa *et al.*; hence, the two isolates are antipodal.

Due to sample limitations and some decomposition, only chondrillin [**1**] and chondrilli-18*E*,20*E*-diene [**5**] were tested for cytotoxicity. Neither showed significant toxicity towards P388 leukemia cells, $ED_{50} > 10$ μg/ml. This contrasts with results re-

TABLE 1. ^{1}H-nmr Data of Chondrillins **1–6**.[a]

Proton	Compound					
	1	**3**	**5**	**2**	**4**	**6**
H-2	2.91 dd (16.2,8.1)	2.93 dd (16.2,8.1)	2.91 dd (16.1,8.1)	2.61 dd (15.9,7.5)	2.61 (16.2,7.2)	2.61 dd (16.2,7.5)
	2.60 dd (16.2,5.4)	2.62 dd (16.2,5.4)	2.61 dd (16.1,5.4)	2.50 dd (15.9,6.6)	2.50 dd (16.2,6.6)	2.54 dd (16.2,6.3)
H-3	4.78 m	4.80 m	4.78 m	5.00 m	50.1 br t	5.00 br t
H-4	6.17 dd (10.2,4.2)	6.19 dd (10.2,4.5)	6.18 dd (10.1,4.5)	6.12 dd (10.2,<1)	6.11 dd (10.2,1.2)	6.11 d (10.2,1.2)
H-5	5.86 dd (10.2,2.7)	5.87 dd (10.2,2.0)	5.86 dd (10.1,1.8)	5.84 dd (10.2,2.4)	5.84 dd (10.2,2.4)	5.84 dd (10.2,2.1)
H-7	1.62 m	1.64 m	1.63 m	1.64 m	1.64 m	1.63 m
H-8–16	1.23 br s	1.25 br s	1.24 br s	1.24 br s	1.25 br s	1.24 br s
H-17	1.23 br s	1.25 br s	2.02 q (6.9)	1.24 br s	1.25 br s	2.03 q (6.6)
H-18	1.23 br s	1.25 br s	5.54 m	1.24 br s	1.25 br s	5.56 m
H-19	1.23 br s	1.96 m	5.98 m	1.24 br s	1.96 br q	6.00 m
H-20	1.23 br s	5.41 m	5.98 m	1.24 br s	5.41 m	6.00 m
H-21	1.23 br s	5.41 m	5.54 m	1.24 br s	5.41 m	5.56 m
H-22	0.86 t (6.6)	1.64 m	1.71 d (6.5)	0.87 t (6.3)	1.64 m	1.72 m
Ketal OMe	3.38 s	3.40 s	3.38 s	3.38 s	3.39 s	3.35 s
Ester OMe	3.71 s	3.73 s	3.71 s	3.71 s	3.72 s	3.71 s

[a]300 MHz in $CDCl_3$. Coupling constants are given in parentheses.

TABLE 2. ^{13}C-nmr Data of Chondrillins **1–6**.[a]

Carbon	Compound					
	1	**3**	**5**	**2**	**4**	**6**
C-1	170.8	170.8	170.9	170.0	170.0	170.0
C-2	37.2	37.3	37.2	36.3	36.3	36.3
C-3	73.7	73.7	73.7	73.5	73.5	73.5
C-4	129.2	129.1	129.2	130.4	130.4	130.4
C-5	126.4	126.4	126.4	127.0	127.0	127.0
C-6	100.5	100.5	100.5	101.1	101.1	101.1
C-7	34.2	34.3	34.2	34.8	34.8	34.8
C-8–16	22.7–31.9	23.6–29.9	22.7–31.9	22.7–31.9	23.3–29.6	23.3–31.6
C-17	22.7–31.9	23.6–29.9	32.6	22.7–31.9	23.3–29.6	32.6
C-18	22.7–31.9	23.6–29.9	131.9	22.7–31.9	23.3–29.6	132.2[b]
C-19	22.7–31.9	32.7	131.7[b]	22.7–31.9	32.6	131.7[b]
C-20	22.7–31.9	131.6	130.2[b]	22.7–31.9	131.7	130.2[b]
C-21	22.7–31.9	124.5	126.3	22.7–31.9	124.5	126.6
C-22	14.1	18.1	18.0	14.1	17.9	17.9
Ketal OMe	50.9	51.0	51.0	51.3	51.3	51.3
Ester OMe	51.9	52.1	52.0	52.1	52.1	52.1

[a]75 MHz, $CDCl_3$.
[b]Interchangeable.

ported by Quinoa *et al.* (3) and Sakemi *et al.* (4), who reported IC_{50} values of 0.05–5 μg/ml for various cyclic peroxy aliphatic esters.

Sakemi *et al.* noted previously that peroxy aliphatic esters have been isolated from representatives of different orders of sponges and hence appear not to have any taxonomic significance. Interestingly, the *P. lita* from Truk Island investigated in this work yielded an array of C-22 peroxy esters while *P. lita* from Okinawa yielded chondrillin (C-22) as the major product and C-18 and C-20 minor peroxy esters.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained using a Varian XL-300

TABLE 3. ^{1}H and ^{13}C nmr Data of **7–9**.

Position	δ ^{1}H[a]			δ ^{13}C[b]	
	7	**8**	**9**	**7**	**8**
1	—	—	—	153.5	153.5
2	6.21 d (5.7)	6.22 d (5.7)	6.20 d (5.7)	124.8	124.8
3	7.20 d (5.7)	7.12 d (5.7)	7.10 d (5.7)	142.9	140.0
4	—	—	—	101.0	101.5
5	1.89 m	1.89 m	1.88 m	37.0	37.0
6–14	1.25 br s	1.24 br s	1.23 br s	22.7–31.9	23.5–32.6
15	1.25 br s	1.24 br s	2.02 q (6.6)	22.7–31.9	23.5–32.6
16	1.25 br s	1.24 br s	5.53 m	22.7–31.9	23.5–32.6
17	1.25 br s	1.94 m	5.98 m	22.7–31.9	23.5–32.6
18	1.25 br s	5.41 m	5.98 m	22.7–31.9	131.7
19	1.25 br s	5.41 m	5.53 m	22.7–31.9	124.5
20	0.88 t (6.3)	1.63 d (6.3)	1.71 d (6.3)	14.1	19.0
ketal OMe	3.22 s	3.22 s	3.20 s	51.1	51.1

[a]300 MHz, $CDCl_3$. Coupling constants given in parentheses.
[b]75.4 MHz, $CDCl_3$.

spectrometer (300 MHz for ^1H and 75.4 MHz for ^{13}C) in the solvents specified. Uv spectra were measured in dioxane on a Perkin-Elmer Lambda 3 uv/vis spectrophotometer. Ir spectra were taken on Bio-Rad FTS-7 Ft-ir or Perkin-Elmer 298 spectrophotometers. Fabms were obtained using a VG ZAB-E spectrometer. Low resolution mass spectra were obtained on a Hewlett-Packard 5985B quadrupole mass spectrometer. Optical rotations were determined using a Rudolph Autopol III automatic polarimeter in the solvents specified.

Flash chromatography columns were made using Merck Si gel 60H. Thin layer chromatograms were run on precoated Kodak chromatogram sheets with silica adsorbent and fluorescent indicator, or on Whatman MKC18F reversed-phase tlc plates. Alltech Econosphere C_{18} 5μ, 10 mm × 29.9 cm, and Alltech Econosphere silica 5μ, 10 mm × 30 cm, columns were used in the hplc separations. All solvents used in the extraction and separations were distilled prior to use.

ISOLATION.—Specimens of the dark maroon sponge *P. lita* were collected around Moen Island, Truk Lagoon, in 1984 and 1985 at depths of 4 to 15 feet and frozen shortly after collection. A voucher specimen has been deposited with the Oklahoma Museum of Natural History, University of Oklahoma. Material from the first collection was divided into two portions, one of which was freeze-dried, while the other was thawed, cut up, and extracted with MeOH, then MeOH-$CHCl_3$ (1:1) for one day each. The extracts were combined and concentrated to give 29.93 g of crude extract which was then partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was concentrated to give 4.77 g of extract, which was then partitioned between hexane and 10% aqueous MeOH. The hexane extract, upon concentration, gave 2.70 g of extract which was then chromatographed by flash chromatography on Si gel, step gradient of hexane with increasing amounts of $CHCl_3$. Like fractions (tlc analysis) were pooled. Fraction 5 was further resolved by using a Si gel column with 4% EtOAc in hexane to give, in order of elution, **7** (3 mg), **8** (1 mg), and **9** (2 mg). Fraction 7 was chromatographed using hplc, Si gel column, and 5% Me_2CO in hexane. A fraction rich in **6** (^1H nmr analysis) was purified further by C_{18} reversed-phase column, MeOH solvent, to give pure **6** (10 mg).

The 10% aqueous MeOH layer from above was diluted to give a 20% aqueous MeOH solution, and this was extracted with CCl_4. The aqueous layer was diluted again to 30% aqueous MeOH and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to give 0.7236 g of extract which was chromatographed on C_{18} RP using 10% aqueous MeOH, and one fraction therefrom was rechromatographed by hplc using Si gel with 10% Me_2CO in hexane to give pure **10** (15 mg).

The freeze-dried portion of sponge (245.5 g dry wt) was extracted with hexane to give 17.75 g of extract. This was chromatographed in a manner similar to that described for the hexane-soluble materials described above to give **1** (2 mg), **3** (8.5 mg), **4** (6 mg), **5** (21.5 mg), and **6** (8 mg).

Sponge material collected in 1985 (2.4 kg) was thawed, cut up, and extracted with MeOH for one day and then twice with MeOH-$CHCl_3$ (1:1). A portion of the MeOH/$CHCl_3$ extract (17.33 g) was concentrated and chromatographed first by flash chromatography (silica; hexane and then hexane with increasing amounts of $CHCl_3$), and then by Si gel (3% EtOAc in hexane). Fractions from the latter chromatography were further purified by reversed-phase hplc (C_{18}, 2% aqueous MeOH) to give **2** (11 mg), **4** (1 mg), **6** (5 mg), **7** (4 mg), and **8** (1 mg).

Chondrill-(20E)-ene [**3**].—Oil: ^1H nmr see Table 1; ^{13}C nmr see Table 2.

6-epi-*Chondrillin* [**2**].—White solid: ir (neat) ν max 1737 cm^{-1}; mp 42.5–43.5°; $[\alpha]_D$ +30.5 (c = 1.09, $CHCl_3$); ^1H nmr see Table 1; ^{13}C nmr see Table 2; hrms (70 ev) *m/z* (rel. int.) $[M-O_2]^+$ 380.3 (100.0), 112.9 (70.6); hrms $[M-O_2]^+$ 380.3280, $C_{24}H_{44}O_3$ (Δ −2.7 ppm).

Chondrilli-(18E,20E)-diene [**5**].—Light yellow oil: ir (neat) ν max 1720 cm^{-1}; $[\alpha]_D$ +35.2 (c = 0.21, $CHCl_3$); uv λ 217 nm; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hrms *m/z* (rel. int.) $[M]^+$ 408.2868 (1.7), $C_{24}H_{40}O_5$ (Δ −1.8 ppm), 113.0033 (100.0).

6-epi-*Chondrill-(20E)-ene* [**4**].—White solid: mp 34–34.5°; $[\alpha]_D$ +22.5 (c = 0.16, $CHCl_3$); ir (neat) ν max 1724 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; lrms (70 ev) *m/z* (rel. int.) $[M-O_2]^+$ 378.4 (54.4), 113.0 (100.0); hrms 378.3112, $C_{24}H_{42}O_3$ (Δ −5.8 ppm).

6-epi-*Chondrilli-(18E,20E)-diene* [**6**].—White solid: mp 28–29°; $[\alpha]_D$ +21.1 (c = 0.39; $CHCl_3$); ir (neat) ν max 1724 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; lrms (70 ev) *m/z* (rel. int.) $[M-O_2]^+$ 376.3 (10.5), 113.0 (100.0).

4-Methoxy-2-eicosen-4-olide [**7**].—White solid: mp 34.5–35.5°; $[\alpha]_D$ −13.9 (c = 0.43, CH_2Cl_2); ir (neat) ν max 1724 cm^{-1}; ^1H nmr see Table 3; ^{13}C nmr see Table 3; lrms (12 ev) *m/z* (rel. int.) $[M]^+$ 338.2 (6.1), 169.0 (12.6), 156.1 (40.3), 130.0 (10.4), $[M-C_{16}H_{33}]^+$ 113.0 (100.0); hrms 338.2816, $C_{21}H_{38}O_3$ (Δ −1.3 ppm).

4-Methoxy-(2,18E)-eicosadien-4-olide [**8**].—White solid: Ft-ir (neat) ν max 1724 cm^{-1}; $[\alpha]_D$ −13.7

($c = 0.71$, CH_2Cl_2); ^{1}H nmr see Table 3; ^{13}C nmr see Table 3; lrms (12 ev) *m/z* (rel. int.) $[M]^+$ 336.2 (13.9), $[M - C_{16}H_{31}]^+$ 113.0 (100.0), hrms 336.2650, $C_{21}H_{36}O_3$ (Δ 3.2 ppm).

*4-Methoxy-(2,16*E*,18*E*)-eicosatrien-4-olide* [**9**].—Ir (neat) ν max 1775 cm^{-1}; ^{1}H nmr see Table 3; lrms (12 ev) *m/z* (rel. int.) $[M]^+$ 334.3 (14.4), $[M - C_{16}H_{29}]^+$ 113.1 (100).

5-epi-*2-Oxo-2,5-dihydrofuran-5-acetic acid methyl ester* [**10**].—Light yellow oil: $[\alpha]_D$ −96.1 ($c = 1.05$, $CHCl_3$); ^{1}H nmr 7.59 (H-4, dd, 5.7, 1.5), 6.15 (H-3, dd, 5.7, 2.1), 5.39 (H-5, tt, 7.2, 2.1), 3.72 (OMe, s), 2.84 (H-6, dd, 16.5, 7.2), 2.64 (H-6′, dd, 16.5, 6.9); ^{13}C nmr 172.3 (C-7), 169.3 (C-2), 155.8 (C-4), 121.7 (C-3), 79.0 (C-5), 52.0 (OMe), 37.4 (C-6); lrms (70 ev) *m/z* (rel. int.) $[M]^+$ 156.1 (20.6), $[M - OMe]^+$ 125.1 (34.6), $[M - MeOH]^+$ 124.2 (47.9), $[M - H - CO_2Me]^+$ 96.1 (100.0), $[M - CH_2CO_2Me]^+$ 83.1 (96.8).

ACKNOWLEDGMENTS

This work was supported by Department of Commerce, NOAA Sea Grant Project NA86AA-D-SG-074. We thank Mr. Charles Arneson for assistance in specimen collection; the Department of Marine Resources, Moen, Truk, Federated States of Micronesia, for the use of their facilities; Dr W. Lichter, University of Miami, for cytotoxicity testing; and Dr. Pat Bergquist, University of Auckland, New Zealand for sponge identification. We gratefully acknowledge NSF Grant CHE 8113507 and the University of Oklahoma Research Fund for funds to purchase a high-field nmr spectrometer.

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988), and earlier reviews cited therein.
2. R.J. Wells, *Tetrahedron Lett.*, 2637 (1976).
3. E. Quinoa, E. Kho, L. Manes, and P. Crews, *J. Org. Chem.*, **51**, 4260 (1986).
4. S. Sakemi, T. Higa, U. Anthoni, and C. Christophersen, *Tetrahedron*, **43**, 263 (1987).
5. R.M. Silverstein, G.C. Bassler, and T.C. Morrill, "Spectrometric Identification of Organic Compounds," John Wiley and Sons, New York, 1981, p. 263.
6. R.M Silverstein, G.C. Bassler, and T.C. Morrill, "Spectrometric Identification of Organic Compounds," John Wiley and Sons, New York, 1981, p. 225.

Received 23 January 1990