Tasnemoxides A−C, New Cytotoxic Cyclic Norsesterterpene Peroxides from the Red Sea Sponge *Diacarnus erythraenus*

Diaa T. A. Youssef*

Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

Received September 8, 2003

Tasnemoxides A−C (**1**−**3**), three new cytotoxic cyclic norsestertepene peroxides, were isolated from the Red Sea sponge *Diacarnus erythraenus*, together with the known compound sigmosceptrellin B (**4**). The structural determination of the isolated compounds was based on extensive 1D and 2D NMR studies and mass spectral determinations. Compounds **1**−**3** showed moderate cytotoxicity against three cancer cell lines.

Sesterterpenes isolated from marine organisms are often modified by the loss or addition of one or more carbon units. Norsesterterpene peroxides are characterized by a 2-substituted propionic acid or methyl propionate group attached to a 1,2-dioxane ring at the C-3 position, as exemplified by **1**−**4** (Figure 1). Norterpene peroxides included compounds possessing acyclic,[1] monocyclic,[2] and bicyclic[2] carbon skeletons. The genus *Diacarnus* (family Podospongiidae, order Poecilosclerida)[3] is known to be a rich source of terpene peroxides.[1,2,4,5] Such metabolites have been found in both marine and terrestrial organisms. Interest has been focused on such metabolites by natural products chemists because of their diverse biological properties, which include antimalarial,[4,5] antimicrobial,[6−9] sea urchin egg cell-division inhibitory,[10] antiviral,[5,11] ichthyotoxic,[12,13] antioxoplasmosis,[5] and cytotoxic.[1,2,14−17]

In continuation of our ongoing search for drug leads from Red Sea marine invertebrates we have investigated the Red Sea sponge *Diacarnus erythraenus*. Previous work on the sponge resulted in the isolation of many biologically active norterpene peroxides.[1,5]

The frozen sponge was extracted with a mixture of MeOH/CH_2Cl_2 (1:1), and the combined extracts were dried and the residue partitioned between 90% aqueous MeOH and *n*-hexanes, and 60% MeOH and CH_2Cl_2. The cytotoxic *n*-hexanes fraction was subjected to size exclusion chromatography on Sephadex LH-20, flash ODS column, and normal- and reversed-phase HPLC purification to afford tasnemoxides A−C (**1**−**3**) together with sigmosceptrellin B (**4**).[12,13]

Tasnemoxide A (**1**) was isolated as a colorless oil with a molecular formula of $C_{24}H_{40}O_5$ as deduced from HRFABMS (*m*/*z* 431.2773, $[M + Na]^+$). The molecular formula requires five degrees of unsaturation including a carboxylic acid (δ 178.0), a disubstituted double bond (δ 121.6, 140.8), and an endoperoxide ring (δ 81.0, 80.2). The remaining two degrees of unsaturation are accounted for by a 2,2,6-trimethylcyclohexyl moiety attached to C-10 through the ethylene fragment C-11/C-12.

The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra together with HMQC data revealed resonances for six methyls, eight methylenes, four methines, and six quaternary carbons. Interpretation of the $^1H-^1H$ COSY and HMQC experiments allowed the assembly of the structural fragments C-1/C-5, C-7/C-9, C-11/C-12, and C-15/C-17. The coupling constant of $J = 15.6$ Hz between H-8 and H-9 supported the *E* configuration at C-8/C-9. Further COSY correlation between H_2-7 and H-8 was observed.

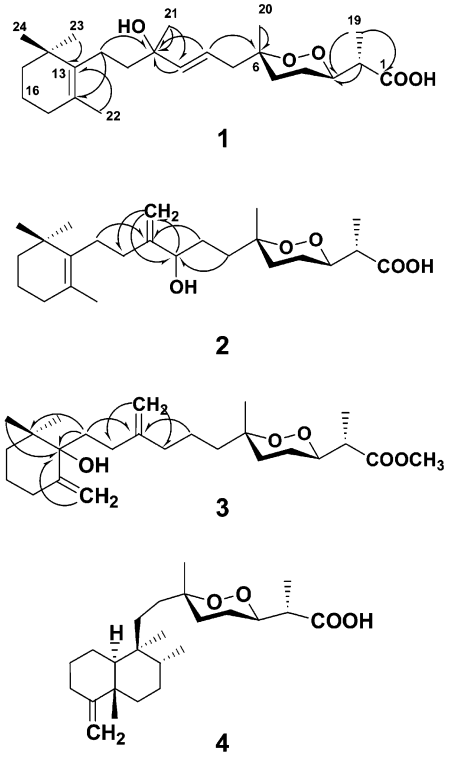

Figure 1. Structures of **1**−**4** and significant HMBC correlations of **1**−**3**.

Assignment and connectivity of the subunits of **1** were supported by an HMBC experiment. The placement of the OH and methyl moieties at C-10 was supported by HMBC cross-peaks of H-8/C-10, H-9/C-10, H_3-21/C-9, H_3-21/C-10, and H_2-12/C-10 (Figure 1). Similarly the attachment of the trimethylated cyclohexyl moiety to C-12 was supported by HMBC correlations and is illustrated in Figure 1.

Applying the established empirical rules by Capon and MacLeod,[6] the chemical shift of the C-6 methyl (δ 21.2, s, H_3-20) indicated an axial orientation, the chemical shift of the methyl signal at C-2 (δ 1.28 d, H_3-19) requires a C-2/C-3-*threo* configuration, and the coupling constant of H-3 (δ 4.15, ddd, $J_{3,4ax} = 9.5$ Hz) established an axial H-3.[6]

Tasnemoxide B (**2**) was isolated as a colorless oil. It possesses the same molecular formula of **1** as deduced from HRFABMS ($C_{24}H_{40}O_5$, *m*/*z* 431.2773, $[M + Na]^+$). Inter-

* To whom correspondence should be addressed. Tel: (+20)64-381-593. Fax: (+20)64-355-741. E-mail: youssefdiaa@hotmail.com.

10.1021/np0340192 CCC: $27.50
Published on Web 12/25/2003

Table 1. ^{1}H NMR Data for Tasnemoxides A (**1**), B (**2**), and C (**3**) ($CDCl_3$)

atom no.	**1** δ_H (mult., *J*/Hz)	**2** δ_H (mult., *J*/Hz)	**3** δ_H (mult., *J*/Hz)
2	2.69 (m)	2.68 (m)	2.65 (m)
3	4.15 (ddd, 9.5, 7.5, 3.0)	4.14 (ddd, 9.6, 7.5, 3.0)	4.09 (m)
4	1.77 (m)	1.77 (m)	1.70 (m)
5	1.66 (m)	1.65 (m)	1.63 (m)
7	2.22 (m), 2.28 (m)	1.60 (m)	1.62 (m)
8	5.57 (dd, 15.6, 5.7)	1.55 (m), 1.67 (m)	1.52 (m)
9	5.16 (d, 15.6)	4.08 (m)	2.00 (m)
11	1.58 (m)	2.00 (m), 2.11 (m)	2.03 (m)
12	1.99 (m)	2.14 (m)	1.57 (m)
15	1.88 (t, 6.3)	1.90 (t, 6.4)	2.32 (m), 1.95 (m)
16	1.57 (m)	1.56 (m)	2.34 (m), 1.98 (m)
17	1.40 (m)	1.41 (m)	1.52 (m)
19	1.28 (d, 7.0)	1.28 (d, 6.9)	1.23 (d, 6.9)
20	1.26 (s)	1.27 (s)	1.24 (s)
21	1.29 (s)	5.03 (s), 4.91 (s)	5.03 (s), 5.01 (s)
22	1.56 (s)	1.59 (s)	4.97 (s), 4.85 (s)
23	0.96 (s)	0.99 (s)	0.97 (s)
24	0.96 (s)	0.99 (s)	0.87 (s)
OCH_3			3.68 (s)

Table 2. ^{13}C NMR Data for Tasnemoxides A (**1**), B (**2**), and C (**3**) ($CDCl_3$)

atom no.	**1** δ_C (mult.)	**2** δ_C (mult.)	**3** δ_C (mult.)
1	178.0 (C)	177.7 (C)	174.2 (C)
2	42.7 (CH)	42.6 (CH)	42.9 (CH)
3	81.0 (CH)	80.9 (CH)	81.3 (CH)
4	23.3 (CH_2)	23.3 (CH_2)	23.4 (CH_2)
5	31.6 (CH_2)	31.8 (CH_2)	30.1 (CH_2)
6	80.2 (C)	80.1 (C)	79.7 (C)
7	42.6 (CH_2)	42.5 (CH_2)	39.4 (CH_2)
8	121.6 (CH)	28.9 (CH_2)	24.0 (CH_2)
9	140.8 (CH)	75.6 (CH)	26.2 (CH_2)
10	73.3 (C)	152.4 (C)	155.5 (C)
11	42.8 (CH_2)	31.7 (CH_2)	31.1 (CH_2)
12	23.0 (CH_2)	27.4 (CH_2)	24.8 (CH_2)
13	136.6 (C)	136.5 (C)	90.1 (C)
14	127.1 (C)	127.4 (C)	147.8 (C)
15	32.7 (CH_2)	32.7 (CH_2)	34.0 (CH_2)
16	19.7 (CH_2)	19.8 (CH_2)	19.0 (CH_2)
17	39.8 (CH_2)	39.9 (CH_2)	39.9 (CH_2)
18	35.1 (C)	34.9 (C)	42.7 (C)
19	13.2 (CH_3)	13.2 (CH_3)	13.5 (CH_3)
20	21.0 (CH_3)	20.8 (CH_3)	20.9 (CH_3)
21	27.7 (CH_3)	109.3 (CH_2)	114.9 (CH_2)
22	19.5 (CH_3)	19.5 (CH_3)	108.7 (CH_2)
23	28.6 (CH_3)	28.5 (CH_3)	22.7 (CH_3)
24	28.6 (CH_3)	28.5 (CH_3)	22.0 (CH_3)
OCH_3			51.8 (CH_3)

preting the 1D and 2D (COSY, HMQC, HMBC) NMR data of **2** showed its close similarity to those of **1**. The only difference was the disappearance of the signals of the olefinic moiety at C-8/C-9, the methyl group at C-10, and the oxygenated quaternary carbon C-10 and appearance of new signals for an oxymethine and a geminal disubstituted double bond in the NMR spectra of **2**. This was supported by HMQC signals at δ 4.08 (m, H-9)/75.6 (d, C-9), 5.03 (s), 4.91 (s)/109.3 (t, C-21) and 152.4 (s, C-10) (Tables 1 and 2). The assignment and placement of the OH at C-9 and the geminal disubstituted double bond between C-10 and C-21 were unequivocally secured from HMBC correlations of H_2-7/C-9, H_2-8/C-10, H_2-11/C-10, H_2-21/C-9, and H_2-21/C-11. Similarly to **1**, and by applying the empirical rules,[6] compound **2** showed the same relative stereochemistry at C-2, C-3, and C-6.

Tasnemoxide C (**3**) was purified as a colorless oil and analyzed for $C_{25}H_{42}O_5$ as determined from HRFABMS ($C_{25}H_{42}O_5$, *m/z* 445.2937, $[M + Na]^+$). Its ^{1}H NMR spectrum (Table 1) showed resonances for a methyl ester (δ 3.68, s), a secondary methyl (δ 1.23, d), three tertiary methyls (δ1.24, 0.97, and 0.87, each s), seven methylenes, two exomethylenes (δ 5.03, 5.01, 4.97, and 4.85, each s), an oxymethine (δ 4.09), and an alkylmethine (δ 2.65). Interpretation of the ^{13}C NMR data (Table 2), together with COSY and HMQC experiments, led to the assembly of the fragments C-1/C-5, C-7/C-9, C-11/C-12, and C-15/C-17. The molecular formula of **3** needs five degrees of unsaturation including the carboxylic ester, two exomethylenes, and the endoperoxide ring. The existence of two methyl singlets (δ 0.97 and 0.87, each s), an oxygenated quaternary carbon (δ 89.2, C-13), and terpene biogenetic considerations suggested one remaining ring was present as a 1-hydroxy-2,2-dimethyl-6-methylenecyclohexyl moiety tethered to C-10 by an ethylene fragment (C-11/C-12), thus completing the degrees of unsaturation. This substructure has appeared in other norterpene peroxides.[1,2] The connectivity and the assignment of the structural subunits of **3** were secured from HMBC correlations and are illustrated in Figure 1. Tasnemoxide C possesses the same relative stereochemistry at C-2, C-3, and C-6 as in **1** and **2**, which was indicated by chemical shift values of C-6, H_3-19, and the coupling constant of H-3.[6]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco-DIP-700 using CH_2Cl_2 at 20 °C at the sodium D line (589 nm). IR spectra were recorded on a Perkin-Elmer 1310/84 spectrometer. NMR spectra were determined on a Varian Unity 300 instrument (300 MHz for ^{1}H and 75 MHz for ^{13}C NMR). Homonuclear ^{1}H connectivities were determined by using the 2D double-quantum-filtered COSY. One-bond heteronuclear ^{1}H$-^{13}$C connectivities were determined by a 2D proton-detected HMQC experiment, and two- and three-bond ^{1}H$-^{13}$C connectivities were determined by a 2D proton-detected HMBC experiment. HRFABMS were determined on a Finnigan MAT-312 using 3-NBA as matrix.

Animal Material. The sponge material was collected by hands using scuba at a depth of 17 m off Hurghada at the Red Sea, Egypt, in January 2003. A description of the sponge has been previously reported.[1] A voucher specimen was deposited in our Red Sea invertebrates collection at the Department of Pharmacognosy, Faculty of Pharmacy, under the registration code number DY-38.

Extraction and Isolation. The frozen sponge materials (150 g, wet wt) were extracted three times (3 × 500 mL) with a mixture of MeOH/CH_2Cl_2 (1:1) at room temperature. The combined organic extracts were concentrated under reduced pressure, suspended in 500 mL of MeOH/H_2O (9:1), and extracted with *n*-hexanes (3 × 300 mL) to give 1.1 g of *n*-hexanes residue. The remaining methanolic layer was diluted with H_2O to 3:2 MeOH/H_2O and then extracted with CH_2Cl_2 (3 × 300 mL) to afford 535 mg of CH_2Cl_2 extract. The cytotoxic hexane residue was subjected to size exclusion chromatography on a Sephadex LH-20 column equilibrated with CH_2Cl_2/MeOH (1:1). Fractions of 5 mL were collected and monitored by TLC. Similar fractions were combined together to give five major fractions. Fractions 3 and 5 showed cytotoxicity to the three cell lines at $IC_{50} = 2.0$ μg/mL. Fraction 3 (215 mg) was subjected to an ODS flash column eluted with 70% H_2O in MeCN through 100% MeCN. The fraction eluted with 20% H_2O in MeCN was cytotoxic, with $IC_{50} = 1.0$ μg/mL. The residue (25.3 mg) was finally purified on a semipreparative SiO_2 HPLC column using 5% 2-propanol in hexanes to give **1** (3.0 mg) and **2** (2.5 mg). Fraction 5 (70 mg) was purified on a semipreparative C_{18} HPLC column using 90% MeCN to give compounds **3** (1.0 mg) and **4** (27 mg).

Cytotoxicity Testing. Cytotoxicity assays (IC_{50}, μg/mL) were carried out against three types of cancer cells including murine leukemia (P-388; ATCC: CCL 46), human lung carcinoma (A-549; ATCC: CCL 8), and human colon carcinoma (HT-29; ATCC: HTB 38). A dilution assay limit corresponding to 1 μg/mL was set as a cutoff value for further in vitro screening. Compounds **1**−**3** showed activity of $IC_{50} > 1$ μg/mL against the three types of cells. Such activity is not of sufficient interest to pursue such compounds in in vivo studies.

Tasnemoxide A (1): colorless oil (3.0 mg, 2×10^{-3}%, based on wet wt); $[\alpha]^{25}_D$ +21.8° (*c* 0.55, CH_2Cl_2); IR (film) ν_{max} 3445, 2937, 1712, 1450, 1376, 1238, 1201, 1170, 1011 cm^{-1}; NMR data, see Tables 1 and 2; HRFABMS *m/z* 431.2773 (calcd for $C_{24}H_{40}O_6Na$ $[M + Na]^+$, 431.2771).

Tasnemoxide B (2): colorless oil (2.5 mg, 1.6×10^{-3}%, based on wet wt); $[\alpha]^{25}_D$ +62.5° (*c* 0.4, CH_2Cl_2); IR (film) ν_{max} 3442, 2940, 1714, 1455, 1370, 1253, 1213, 1009, 883 cm^{-1}; NMR data, see Tables 1 and 2; HRFABMS *m/z* 431.2773 (calcd for $C_{24}H_{40}O_6Na$ $[M + Na]^+$, 431.2771).

Tasnemoxide C (3): colorless oil (1.0 mg, 0.6×10^{-3} %, based on wet wt); $[\alpha]^{25}_D$ +76.0° (*c* 0.3, CH_2Cl_2); IR (film) ν_{max} 3450, 2935, 1738, 1455, 1372, 1251, 1190, 1152, 1051 cm^{-1}; NMR data, see Tables 1 and 2; HRFABMS *m/z* 445.2937 (calcd for $C_{25}H_{42}O_5Na$, $[M + Na]^+$, 445.2930).

Acknowledgment. This research was supported by the Committee on Scientific and Technological Cooperation of the Organization of Islamic Conference, Islamabad, Pakistan, and the International Foundation for Science, Stockholm, Sweden, through a grant (F/3116-1) to D.Y. We would like to thank the Personnel at the Egyptian Environmental Agency Affairs (EEAA) and the Red Sea Protectorate of Egypt for the collection permission and the kind support during the collection of the sponge material. We thank V. Brecht of the Albert-Ludwigs-Universität, Freiburg, Germany, for the NMR and MS measurements. Our thanks goes also to PharmaMar for cytotoxicity testing.

References and Notes

(1) Youssef, D. T. A.; Yoshida, W. Y.; Kelly, M.; Scheuer, P. J. *J. Nat. Prod*. **2001**, *64*, 1332−1335.
(2) Sperry, S.; Valeriote, F. A.; Corbett, T., H.; Crews, P. *J. Nat. Prod*. **1998**, *61*, 241−247.
(3) Kelly, M.; Samaai, T. In *Systema Porifera: A Guide to the Classification of Sponges*; Hooper, J. N. A., Soest van, R. W. M., Willenz, P., Ed.; Kluwer Academic/Plenum Publishers: New York, 2002; Vol. 1, pp 694−702.
(4) D'Ambrosio, M.; Guerriero, A.; Deharo, E.; Debitus, C.; Munoz, V.; Pietra, F. *Helv. Chim. Acta* **1998**, *81*, 1285−1292.
(5) El Sayed, K. A.; Hamann, M. T.; Hashish, N. E.; Shier, W. T.; Kelly, M.; Khan, A. A. *J. Nat. Prod*. **2001**, *64*, 522−524.
(6) Capon, R. J.; MacLeod, J. K. *Tetrahedron* **1985**, *41*, 3391−3404.
(7) Faulkner, D. J.; He, H.; Lu, H. S. M.; Clardy, J. *J. Org. Chem*. **1991**, *56*, 2112−2115.
(8) Capon, R. J. *J. Nat. Prod*. **1991**, *54*, 190−195.
(9) Sokoloff, S.; Halevy, S.; Usieli, V.; Colorni, A.; Sarel, S. *Experientia* **1982**, *38*, 337−338.
(10) Manes, L. V.; Bakus, G. J.; Crews, P. *Tetrahedron Lett*. **1984**, *25*, 931−934.
(11) Tanaka, J.; Higa, T.; Suwanborirux, K.; Kokpol, U.; Bernardinelli, G.; Jefford, C. W. *J. Org. Chem*. **1993**, *58*, 2999−3002.
(12) Albericci, M.; Collart-Lempereur, M.; Braekman, J. C.; Daloze, D.; Tursch, B. Declercq, J.-P.; Germain, G.; Van Meerssche, M. *Tetrahedron Lett*. **1979**, 2687−2690.
(13) Albericci, M.; Braekman. J. C.; Daloze, D.; Tursch, B. *Tetrahedron* **1982**, *38*, 1881−1890.
(14) Harrison, B.; Crews, P. *J. Nat. Prod*. **1998**, *61*, 1033−1037.
(15) Rudi, A.; Kashman., Y. *J. Nat. Prod*. **1993**, *56*, 1827−1830.
(16) Rudi, A.; Talpir, R.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *J. Nat. Prod*. **1993**, *56*, 2178−2182.
(17) Gunasekera, S. P.; Gunasekera, M.; Gunawadana, G. P.; McCarthy, P.; Burres, N. *J. Nat. Prod*. **1990**, *53*, 669−674.

NP0340192