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Gabriele M. König, Anthony D. Wright, and Otto Sticher

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FOUR NEW ANTIBACTERIAL SESTERTERPENES FROM A MARINE SPONGE OF THE GENUS *LUFFARIELLA*

GABRIELE M. KÖNIG, ANTHONY D. WRIGHT, and OTTO STICHER

Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, CH-8092, Zürich, Switzerland

ABSTRACT.—The structures of four new sesterterpenoid compounds **1–4** isolated from a marine sponge of the genus *Luffariella*, collected from The Great Barrier Reef, Australia, have been determined by analysis of their ^{1}H-nmr, ^{13}C-nmr, ir, uv, and eims spectral data. The new metabolites **1–4** were found to co-occur with the compounds manoalide, secomanoalide, *Z*-neomanoalide [**5**], and *E*-neomanoalide.

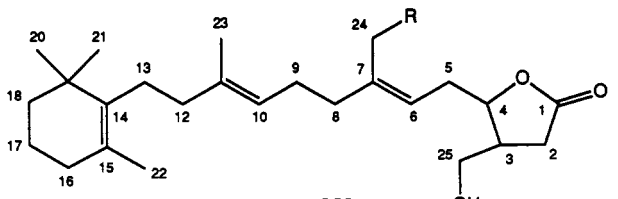
In our continuing search for new biologically active natural compounds we have embarked upon an investigation of several marine sponges collected from the northern region of The Great Barrier Reef, Australia. Marine sponges of the family Thorectidae are known to contain sesterterpene metabolites that possess interesting biological properties including antibacterial (1) and anti-inflammatory (2) activities. Our chemical investigation of a sponge belonging to the genus *Luffariella*, whose CH_2Cl_2 extract showed antibacterial and molluscicidal activities, yielded eight closely related sesterterpenoid metabolites. In this report we describe the structure elucidation of the four new natural products **1–4** from this sponge.

DISCUSSION

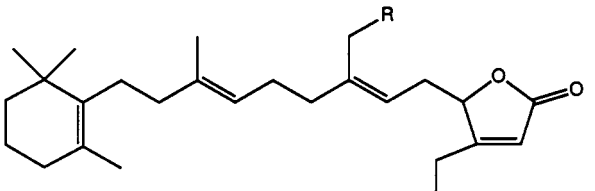
The CH_2Cl_2 solubles from the sponge were chromatographed over Si gel to yield fourteen fractions. Fractions 1–6 were predominantly manoalide (1); fraction 7 was predominantly secomanoalide (3); and fractions 8 and 9 contained *Z*-neomanoalide [**5**] and *E*-neomanoalide (3) as well as four new natural products, **1–4**.

Compound **1** was found to have the molecular formula $C_{25}H_{40}O_4$ by ms. Four of the six degrees of unsaturation implied by the molecular formula of **1** were taken up in three carbon-carbon double bonds and one carbon-oxygen double bond; the molecule was thus bicyclic. From the ^{13}C-nmr and ir spectroscopic data the presence of two primary alcohol functions, one of which was allylic [60.2 (t), 63.2 (t) ppm, 3390 cm^{-1}], and a γ lactone [176.1 (s), 1755 cm^{-1}] function was implied; the latter accounting for one of the rings within **1**. From the protons of the methylene group adjacent to the carbonyl of the lactone [C-2, δ 2.61 (dd, $J = 8.0$, 16.2 Hz), 2.39 (dd, $J = 7.7$, 16.2 Hz)] coupling was evident to the methine proton at C-3 [δ 2.51 (m)]. This latter proton in turn coupled to both of the protons of the hydroxymethyl group [C-25, δ 3.74 (dd, $J =$ 4.8, 10.8 Hz), 3.64 (dd, $J = 6.5$, 10.8 Hz)], which were geminally coupled, and to the proton at C-4. The C-4 proton was further coupled to the two methylene protons at C-5, which in turn coupled to the C-6 olefinic proton [δ 5.40 (br dd, $J = 7.8$, 7.9 Hz)]. This proton-proton intercoupling pattern clearly delineated the C-1 to C-7 portion of **1**. The remaining fragment of **1** was quickly recognized as a typical $C_{16}H_{27}$ alkylated cyclohexenyl grouping commonly found in sesterterpenes derived from marine sponges (1,3). The remaining hydroxymethyl function was thus positioned at C-7. With all of this information it became clear that **1** was the 2,3-dihydro derivative of *Z*-neomanoalide [**5**] (3). The two chiral centers within **1**, C-3 and C-4, could not be assigned unambiguously; even though the proton-proton coupling and 2D-NOESY spectral data of **1** were very good. Compound **1** is *Z*-2,3-dihydroneomanoalide.

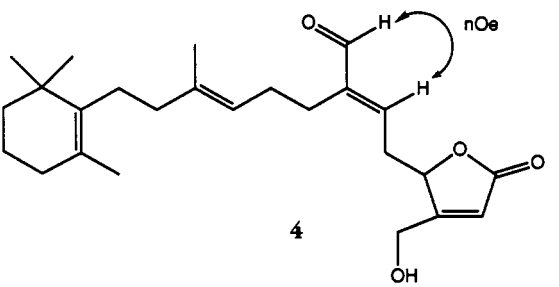
Compound **2** had the molecular formula $C_{27}H_{42}O_5$. The ^{1}H-nmr and the ^{13}C-nmr spectra of **1** and **2** appeared almost identical. Indeed the only differences between the two were the result of **2** being a monoacetoxyl derivative of **1**. The position of the



1 R=OH
2 R=OAc



3 R=OAc
5 R=OH



4

acetoxyl group was clearly at C-24 as the corresponding proton resonances were now at δ 4.54 (d, J = 12.2 Hz), 4.72 (d, J = 12.2 Hz) compared with δ 4.07 (d, J = 12.1 Hz), 4.17 (d, J = 12.1 Hz) in **1**. Compound **2** is *Z*-24-acetoxy-2,3-dihydroneomanoalide.

By ms compound **3** was shown to have the molecular formula $C_{27}H_{40}O_5$. Direct comparison of its ^{1}H-nmr and ^{13}C-nmr data (Experimental and Table 1) with those for **1**, **2**, and *Z*-neomanoalide [**5**] (3) indicated **3** to be the 2,3-dehydro derivative of **2**; it is thus the 7-acetoxymethyl derivative of *Z*-neomanoalide [**5**]. Compound **3** is therefore *Z*-24-acetoxyneomanoalide.

The final sesterterpene to be fully characterized from this sponge sample was **4**. From accurate mass measurement, **4** had the molecular formula $C_{25}H_{36}O_4$. The ^{13}C- and ^{1}H-nmr characteristics of **4** were very similar to those for the three previously discussed terpenes, with **4** being most similar to **3**. The major differences between the ^{13}C- and ^{1}H-nmr spectral data for **3** and **4** were the presence of resonances for an α,β-unsaturated aldehyde moiety in **4** [δ 9.39, 194.2 (d)] and the corresponding absence of ^{1}H- and ^{13}C-nmr resonances for the acetoxymethyl moiety of **3**. The stereochemistry of the $\Delta^{6,7}$ double bond was *E* on the basis of the nOe observed between the C-24 proton and the C-6 olefinic proton. Compound **4** is *E*-neomanoalide-24-al. Once the structure of **4** had been confirmed, it was found that it and its *Z* isomer had been produced by de Silva and Scheuer (3) by the treatment of the corresponding primary alcohol with pyridinium chlorochromate in CH_2Cl_2 at room temperature. This is, however, the first report of **4** as a natural product.

Antibacterial activities of **1**–**4** were investigated by tlc bioautographic tests using *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus* as test organisms. All four compounds (see Experimental) exhibited significant antibacterial activities.

TABLE 1. ^{13}C-nmr (75.5 MHz, $CDCl_3$) Data for Compounds **1–5**.

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	176.1 s	176.0 s	172.3[a] s	171.5 s	172.4[a] s
C-2	31.7[a] t	31.5[a] t	116.0 d	116.8 d	115.7[b] d
C-3	41.4 d	42.1 d	171.3[a] s	169.6 s	173.1[a] s
C-4	82.8 d	82.6 d	81.7 d	80.4 d	82.0 d
C-5	32.6[a] t	32.8[a] t	30.6 t	31.7 d	30.2 t
C-6	121.7 d	122.7 d	122.5[b] d	145.1 d	119.7[b] d
C-7	142.8 s	137.3 s	138.1 s	146.1 s	143.4 s
C-8	35.8 t	35.2 t	35.3 t	24.6 t	35.4 t
C-9	26.7 t	27.9 t	27.9 t	27.8 t	27.8 t
C-10	123.0 d	124.3 d	122.6[b] d	122.3 d	122.9 d
C-11	136.9 s	136.9 s	136.9[c] s	137.6 s	136.8[c] s
C-12	39.8 t	39.9 t	39.8 t	39.8 t	39.8 t
C-13	27.9 t	26.5 t	26.6 t	26.7 t	26.7 t
C-14	137.1 s	137.1 s	137.1[c] s	137.0 s	137.1[c] s
C-15	127.0 s	127.0 s	127.0 s	127.1 s	126.9 s
C-16	32.8 t	32.8 t	32.7 t	32.8 t	32.6 t
C-17	19.5 t	19.6 t	19.5 t	19.6 t	19.4 t
C-18	40.3 t	40.3 t	40.2 t	40.2 t	40.1 t
C-19	35.0 s	35.0 s	35.0 s	35.0 s	34.9 s
C-20	28.6 q	28.6 q	28.6 q	28.6 q	28.4 q
C-21	28.6 q	28.6 q	28.6 q	28.6 q	28.4 q
C-22	19.8 q	19.8 q	19.8 q	19.9 q	19.4 q
C-23	16.1 q	16.1 q	16.1 q	16.1 q	16.1 q
C-24	60.2 t	61.9 t	61.8 t	194.2 d	60.0 t
C-25	63.2 t	62.8 t	58.6 t	58.7 t	58.4 t
C-26		21.0 q	21.0 q		
C-27		171.3 s	171.2 s		

[a–c]Assignments may be interchanged.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General procedures were as described by König *et al.* (4).

SPONGE MATERIAL.—All sponge materials were collected by divers, using scuba, from Pelorus Island, Queensland, Australia. The animals were all collected during September 1990 and deep frozen. A voucher specimen is deposited with the Museum of Tropical Queensland, 74-84 Flinders Street, Townsville Q4810, Australia; museum number G25002.

The specimen was collected from under a ledge on coral substrata at 4–10 meters depth, north side of Pelorus Island, 18°34′S 146°29′E, Great Barrier Reef, Australia. The sponge is large, 1.5 × 1.5 meters in extent, and spreading. The color was brown to dark yellow and became dark brown after preservation in alcohol. The surface is unarmoured and conulose. The skeleton of the sponge is a reticulation of primary, secondary, and tertiary fibers. The thick primary fibers and thinner secondary fibers are laminated in cross section, and the tertiary fibers are thinner than either of the former. There are some sandgrains in the primary fibers but none apparent in the secondary or tertiary fiber. The presence of tertiary fibers is characteristic for this genus.

EXTRACTION AND ISOLATION.—Deep frozen sponge tissue was freeze-dried. Dry tissue (405 g) was extracted with CH_2Cl_2 (2.5 liters) and then with MeOH (2 liters). From both extracts the CH_2Cl_2 solubles (13.0 g) were taken, combined, and chromatographed over silica with petroleum ether containing increasing proportions of EtOAc as eluent; fourteen fractions of approximately 80 ml each were obtained. Tlc and ^{1}H-nmr analysis of fractons 1–6 indicated them to be almost pure manoalide (>1 g), while fraction 7 was predominantly secomanoalide (>0.5 g). Hplc separation of combined fractions 8 and 9, over normal phase silica with EtOAc as eluent afforded three pure compounds: **1**, **5** (60 mg, 0.016%), and *E*-neomanoalide (95 mg, 0.024%).

Z-2,3-Dihydroneomanoalide [**1**].—Compound **1** (8.7 mg, 0.002%): a clear oil; $[\alpha]^{25}D$ +2.5° (c = 0.44, $CHCl_3$); ir ν max 3390, 2910, 1755, 1200, 1010, 755 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 0.98 (s, 3H, H-20), 0.98 (s, 3H, H-21), 1.41 (m, 2H, H-18), 1.57 (m, 2H, H-17), 1.59 (s, 3H, H-22), 1.64 (br s, 3H, H-23), 1.90 (dd, 2H, J = 6.3, 6.3 Hz, H-16), 2.02 (m, 2H, H-13), 2.02 (m, 2H, H-12), 2.16 (m, 2H, H-9), 2.16 (m, 2H, H-8), 2.39 (dd, 1H, J = 7.7, 16.2 Hz, H-2), 2.51 (m, 1H, H-3), 2.57 (m, 2H, H-5), 2.61 (dd, 1H, J = 8.0, 16.2 Hz, H-2), 3.64 (dd, 1H, J = 6.5, 10.8 Hz, H-25), 3.74 (dd, 1H, J = 4.8, 10.8 Hz, H-25), 4.07 (d, 1H, J = 12.1 Hz, H-24), 4.17 (d, 1H, J = 12.1 Hz, H-24), 4.44 (ddd, 1H, J = 5.2, 6.0, 6.0 Hz, H-4), 5.13 (br dd, 1H, J = 6.3, 6.3 Hz, H-10), 5.40 (br dd, 1H, J = 7.8, 7.9 Hz, H-6); ^{13}C nmr see Table 1; hreims found m/z 404.2922 ($C_{25}H_{40}O_4$ requires 404.2928); eims m/z (% rel. int.) $[M]^+$ 404 (1), 386 (1), 372 (1), 258 (16), 177 (10), 137 (100), 95 (80).

Further hplc separation of combined fractions 8 and 9 was carried out over normal phase silica, this time with CH_2Cl_2-MeOH (24:1) as eluent. A further three pure compounds were subsequently isolated and characterized: **2**, **3**, and **4**.

Z-24-Acetoxy-2,3-dihydroneomanoalide [**2**].—Compound **2** (2.2 mg, 0.0005%): clear oil, $[\alpha]^{25}D$ +11.0° (c = 0.1, $CHCl_3$); ir ν max 3420, 2910, 1770, 1735, 1370, 1230 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$) δ 0.99 (s, 3H, H-20), 0.99 (s, 3H, H-21), 1.42 (m, 2H, H-18), 1.56 (m, 2H, H-17), 1.60 (s, 3H, H-22), 1.64 (br s, 3H, H-23), 1.90 (dd, 2H, J = 6.3, 6.3 Hz, H-16), 2.02 (m, 2H, H-13), 2.02 (m, 2H, H-12), 2.07 (s, 3H, H-26), 2.13 (m, 2H, H-9), 2.16 (m, 2H, H-8), 2.18 (m, 1H, OH), 2.42 (m, 1H, H-3), 2.49 (m, 1H, H-5), 2.49 (m, 1H, H-2), 2.62 (m, 1H, H-2), 2.68 (m, 1H, H-5), 3.66 (ddd, 1H, J = 5.0, 5.2, 11.1 Hz, H-25), 3.76 (ddd, 1H, J = 3.7, 4.0, 11.1 Hz, H-25), 4.38 (ddd, 1H, J = 4.5, 6.5, 6.7 Hz, H-4), 4.54 (d, 1H, J = 12.2 Hz, H-24), 4.72 (d, 1H, J = 12.2 Hz, H-24), 5.11 (m, 1H, H-10), 5.51 (dd, 1H, J = 7.6, 7.6 Hz, H-6); ^{13}C nmr see Table 1; hreims found m/z 446.3043 ($C_{27}H_{42}O_5$ requires 446.3033); eims m/z (% rel. int.) $[M]^+$ 446 (4), 386 (5), 371 (3), 262 (9), 203 (6), 177 (9), 137 (100), 95 (45).

Z-24-Acetoxyneomanoalide [**3**].—Compound **3** (15.3 mg, 0.004%): clear oil; $[\alpha]^{25}D$ −16.4° (c = 0.14, $CHCl_3$); ir ν max 3400, 2920, 1740, 1230 cm^{-1}; uv λ max (EtOH) 212 nm (ε 13900); ^1H nmr (300 MHz, $CDCl_3$) δ 0.99 (s, 3H, H-20), 0.99 (s, 3H, H-21), 1.41 (m, 2H, H-18), 1.56 (m, 2H, H-17), 1.60 (s, 3H, H-22), 1.63 (br s, 3H, H-23), 1.90 (dd, 2H, J = 6.3, 6.3 Hz, H-16), 2.02 (m, 2H, H-13), 2.02 (m, 2H, H-12), 2.07 (s, 3H, H-26), 2.10 (m, 2H, H-9), 2.10 (m, 2H, H-8), 2.48 (ddd, 1H, J = 6.9, 7.3, 15.3, H-5), 2.59 (dd, 1H, J = 5.4, 5.9 Hz, OH), 2.84 (ddd, 1H, J = 4.3, 7.5, 15.3 Hz, H-5), 4.45 (br dd, 1H, J = 5.4, 16.8 Hz, H-25), 4.56 (br dd, 1H, J = 5.9, 16.8 Hz, H-25), 4.58 (d, 1H, J = 12.4 Hz, H-24), 4.63 (d, 1H, J = 12.4 Hz, H-24), 5.04 (ddd, 1H, J = 1.6, 4.3, 6.9 Hz, H-4), 5.09 (m, 1H, H-10), 5.38 (dd, 1H, J = 7.4, 7.4 Hz, H-6), 6.01 (br s, 1H, H-2); ^{13}C nmr see Table 1; hreims found m/z 444.2882 ($C_{27}H_{40}O_5$ requires 444.2877); eims m/z (% rel. int.) $[M]^+$ 444 (4), 369 (1), 309 (2), 260 (6), 217 (6), 205 (5), 137 (100), 95 (80).

E-*Neomanoalide-24-al* [**4**].—Compound **4** (1.8 mg, 0.0004%): clear oil; $[\alpha]^{25}D$ −10.6° (c = 0.18, $CHCl_3$); ir and uv as previously reported (3); ^1H nmr (300 MHz, $CDCl_3$) δ 0.98 (s, 3H, H-20), 0.98 (s, 3H, H-21), 1.40 (m, 2H, H-18), 1.55 (m, 2H, H-17), 1.59 (s, 3H, H-22), 1.62 (s, 3H, H-23), 1.90 (dd, 2H, J = 6.3, 6.3 Hz, H-16), 2.00 (m, 2H, H-13), 2.00 (m, 2H, H-12), 2.07 (m, 2H, H-9), 2.30 (m, 2H, H-8), 2.69 (ddd, 1H, J = 7.0, 7.1, 16.1 Hz, H-5), 3.06 (ddd, 1H, J = 4.2, 7.2, 16.1 Hz, H-5), 4.51 (br d, 1H, H-25), 4.61 (br d, 1H, H-25), 5.10 (ddd, 1H, J = 1.0, 7.1, 7.2 Hz, H-10), 5.18 (ddd, 1H, J = 1.6, 4.3, 6.6 Hz, H-4), 6.09 (br s, 1H, H-2), 6.41 (dd, 1H, J = 7.1, 7.2 Hz, H-6), 9.39 (s, 1H, H-24); ^{13}C nmr see Table 1; eims m/z (% rel. int.) $[M]^+$ 400 (2), 382 (2), 273 (2), 177 (8), 149 (14), 137 (100), 95 (60).

BIOASSAYS.—Compound **1** showed antibacterial activities towards *E. coli* (ATCC 25922) and *M. luteus* (ATCC 9341) at 1 μg and 5 μg, respectively. Compounds **2**, **3**, and **4** against *B. subtilis* and *M. luteus* (ATCC 9341) at 3 μg and 11 μg; 8 μg and 2 μg, and 4 μg and 4 μg, respectively. Manoalide showed a molluscicidal activity towards *Biomphalaria glabrata* at 1.5 ppm. All tests were performed as described by Baumgartener *et al.* (5).

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