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THREE NEW SESTERTERPENOID ANTIBIOTICS FROM THE MARINE SPONGE *LUFFARIELLA VARIABILIS* (POLEJAFF)

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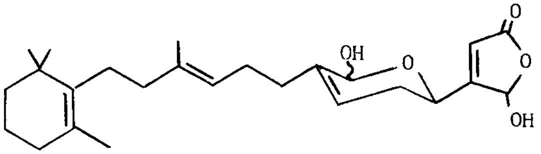
Abstract -- Structures of three new sesterterpenoid antibiotics 2, 3, and 4, isolated from a marine sponge have been determined by spectral analysis and chemical transformations.

We recently described the sesterterpenoid manoalide (1),[2] from the sponge *Luffariella variabilis*. We now report isolation and characterization of three additional related metabolites, 2, 3, and 4 from the same sponge, all exhibiting *in vitro* antibiotic activity. The methylene chloride extract of the freeze-dried sponge was chromatographed on Bio-Sil A, first with CH_2Cl_2/EtOAc (3:1) to elute sterols, 1 and 2; then with EtOAc/MeOH (1:1) to elute 3 and 4. Crude 2 was further purified on Bio-Sil A (CH_2Cl_2/EtOAc, 5:2) and by HPLC (Partisil, hexane/EtOAc, 1:1): colorless glass $[\alpha]_D$ +16.2 (c 0.99; $CHCl_3$), 0.03% from dry animal. Purification of 3 and 4 was achieved on Bio-Sil A (first with hexane/EtOAc, 1:9, then with EtOAc) and by HPLC (Partisil, EtOAc) to give 3, colorless glass, $[\alpha]_D$ -25.9 (c, 0.54; CH_2Cl_2) and 4, colorless glass, $[\alpha]_D$ -27.8 (c, 0.79; CH_2Cl_2) in 0.02% and 0.04%. All three compounds, as did 1, showed *in vitro* activity against Gram positive bacteria *Bacillus subtilis* and



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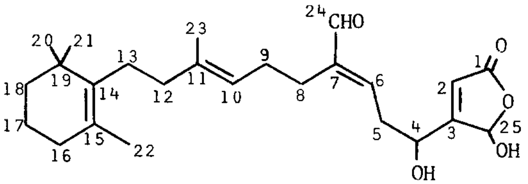

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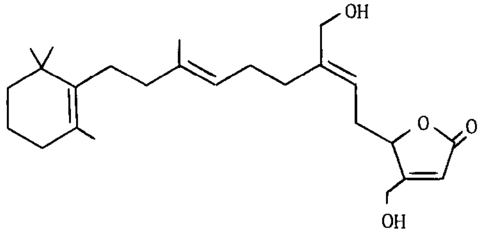

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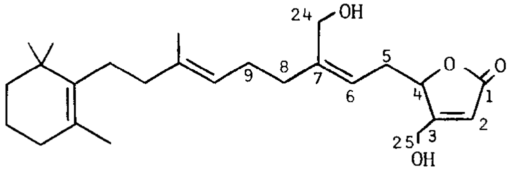

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Staphylococcus aureus, but were inactive against Escherichia coli, and Pseudomonas aeruginosa, and Candida albicans.

Compound **2** exhibited a parent mass spectral peak at m/z 416 (416.2587; calcd for $C_{25}H_{36}O_5$, 416.2563) and an intense M^+-H_2O peak at m/z 398, and is therefore an isomer of **1**. HRMS data for **3** and **4** (M^+: 402.2778; calcd for $C_{25}H_{38}O_4$, 402.2770) showed these two compounds to be isomers of each other. Mass spectrometry by the base peak at m/z 137 also showed that the three new compounds possessed identical $C_{10}H_{17}$ alkylated cyclohexenyl end groups. ^1H and ^{13}C (Table 1) NMR data confirmed this.[3]

Table 1. ^{13}C NMR ($CDCl_3$) Data of **1**, **2**, **3**, and **4**

C-No	**1**	**2**	**3**	**4**
1	172.3(s)	171.1(s)	172.5(s)*	172.4(s)*
2	117.7(d)	118.5(d)	115.9(d)≠	115.7(d)≠
3	169.1(s)	169.0(s)	173.2(s)*	173.1(s)*
4	63.3(d)	66.9(d)	81.8(d)	82.0(d)
5	33.1(t)∤	28.0(t)∤	30.1(t)	30.2(t)
6	121.1(d)	145.9(d)	117.1(d)≠	119.7(d)≠
7	137.7(s)*	148.3(s)	143.4(s)	143.4(s)
8	40.9(t)*∤	35.2(t)∤	28.4(t)	35.4(t)
9	28.5(t)	27.0(t)	27.7(t)	27.8(t)
10	123.6(d)*	122.4(d)	122.7(d)	122.9(d)
11	137.3(s)*	137.6(s)	137.0(s)	136.8(s)*
12	40.3(t)*	40.1(t)*	39.8(t)*	39.8(t)*
13	26.5(t)	24.7(t)	26.6(t)	26.7(t)
14	136.7(s)	137.1(s)	137.0(s)	137.1(s)*
15	127.3(s)	127.2(s)	127.0(s)	126.9(s)
16	33.1(t)	33.1(t)	32.9(t)	32.6(t)
17	20.1(t)	19.8(t)	19.5(t)	19.4(t)
18	40.3(t)*	40.4(t)*	40.2(t)*	40.1(t)*
19	35.2(s)	34.7(s)	34.9(s)	34.9(s)
20, 21	28.9(q)	28.8(q)	28.4(q)	28.4(q)
22	20.1(q)	20.1(q)	19.8(q)	19.4(q)
23	16.3(q)	16.3(q)	16.1(q)	16.1(])
24	91.7(d)	195.2(d)	66.0(t)	60.0(t)
25	99.1(d)	99.0(d)	58.5(t)	58.4(t)

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*interchangeable with closest values

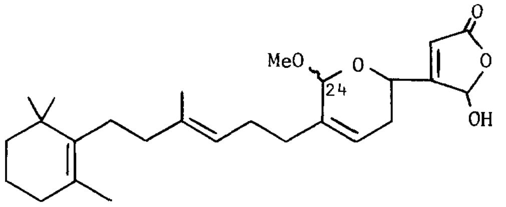
≠may be interchanged

∤may be interchanged

Spectral comparison of manoalide (**1**) with **2** made it apparent that **1** and **2** also had in common a δ-hydroxy-α,β-unsaturated butenolide: ^1H NMR ($CDCl_3$): δ 6.07 (br s H-2), 6.15 (br s

H-25); ^{13}C NMR: C-1, C-2, C-3, C-25, see Table 1; IR ($CHCl_3$) 1772 cm^{-1}. But the two compounds differed in that 2, conveniently named *seco*-manoalide, appeared to be the chain tautomer of the α,β-unsaturated δ-lactol of 1: ^{1}H NMR ($CDCl_3$): δ 9.3 (s, H-24), 6.56 (t, *J* = 6.5 Hz, H-6), 2.79 (dd, *J* = 6.5, 6.0, H_2-5), 4.78 (t, *J* = 6.0, H-4); UV (MeOH) 229.5 nm (18500); IR ($CHCl_3$) 1689 cm^{-1}. *E*-configuration of the C-6,7 olefin of 2 prevents ring closure between the C-24 aldehyde and the δ-OH at C-4. The ^{13}C resonance of C-23 (16.3 ppm) is diagnostic for *E*-configuration of the C-10,11 olefin. This methyl group would resonate at lower field in the *Z*-isomer.[4]

Structure 2 was confirmed by nearly quantitative (monitored by TLC) conversion of 2 to 1 (^{1}H NMR comparison) following UV (254 nm) irradiation in benzene. Moreover, when 2 was treated with excess 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid, manoalide-24-methyl ether (5, *m/z* 430), C-24 epimers, were formed [two equally intense ^{1}H NMR (C_6D_6) signals at δ 3.20 and 3.26]. Under the same conditions 1 was transformed to a single epimer of 5: one MeO-signal at δ 3.23 (C_6D_6).



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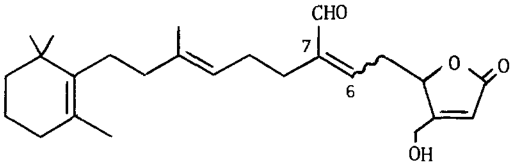

6 C-6,7 *E*

7 C-6,7 *Z*

Inspection of mass and ^{13}C NMR data of isomers 3 and 4, $C_{25}H_{38}O_4$, revealed that these compounds differed from the isomeric pair 1 and 2 only in the functionalized portion of the molecule. Two oxygen atoms in 3 and 4 are present as primary alcohols (C-24, C-25, Table 1). Both compounds form diacetates (M^+ 486) when reacted with Ac_2O/Py. The remaining two oxygen atoms of 3 and 4 make up an α,β-unsaturated butenolide [3: ν_{max} (CH_2Cl_2) 1760 cm^{-1}; λ_{max} (MeOH) 220 nm (6450); 4: ν_{max} (CH_2Cl_2) 1755 cm^{-1}; λ_{max} (MeOH) 222 nm (6250)]. The disubstituted nature of the butenolides (in contrast to monosubstitution in 1 and 2) may be seen from the ^{13}C NMR data for C-1, C-2, C-3, and C-4 (Table 1). Carbon resonances for C-6 and C-7 (Table 1) further show that 3 and 4 possess trisubstituted olefins that are geometrically isomeric. Based on ^{13}C NMR data, compound 3 must have *E* configuration (δ 28.4 for C-8, 66.0 for C-24) about the C-6,7 double bond, while 4 is the *Z* isomer (δ 35.4 for C-8, 60.0 for C-24).[5] Compounds 3 and 4, named (*E*)- and (*Z*)-neomanoalide, are formally derived from *seco*-manoalide (2) by ring-opening of the γ-hydroxybutenolide to the aldehyde-acid, reclosing of a new δ-lactone with the C-4 hydroxy group, and reduction of the free C-24 and C-25 aldehyde functions.

These structural assignments are further confirmed by pertinent ^{1}H NMR data (Table 2) and by oxidation of the neomanoalides to monoaldehydes. Treatment of 3 with pyridinium chlorochromate in CH_2Cl_2 at room temperature[6] furnished 6: M^+ 400; ν_{max} (CH_2Cl_2) 1768, 1688 cm^{-1};

λ_{max} (MeOH) 228 nm (12,000). Under identical conditions 4 gave rise to a 4:10 mixture of 6 and 7 [M^+ 400, ν_{max} (CH_2Cl_2 1763, 1680 cm^{-1}; λ_{max} (MeOH) 228 nm (13,500)], which were separable by HPLC (Lichrosorb, EtOAc/hexane, 1:1). Irradiation of a benzene solution of 7 at 254 nm for 0.5 h yielded a 1:1 mixture of 6 and 7. ^1H NMR data of 6 and 7 (Table 3) show that the C-25 alcohol had resisted oxidation.

Table 2. Partial ^1H NMR data (360 MHz; $CDCl_3$) of 3 and 4

	H-2	H-4	H_a-5	H_b-5	H-6	H-10	H_2-24	H_2-25
3	6.03 s	5.06 t J=5	2.46 ddd J=16,8,5	2.70 ddd J=16,8,5	5.38 t J=8	5.13 t	4.06 s	4.49 AB
4	6.0 s	5.09 t J=5	2.56 ddd J=16,8,5	2.77 ddd J=16,8,5	5.24 t J=8	5.09 t	4.07 AB	4.46 AB

Table 3. Partial ^1H NMR Data (100 MHz; $CDCl_3$) of 6 and 7

	H-2	H-4	H_a-5	H_b-5	H-6	H-10	H-24	H_2-25
6	6.10 s	5.15 t	2.73 m	3.02 m	6.44 t	5.15 t	9.40 s	4.58 br s
7	6.05 s	5.13 t	2.92 m	3.21 m	6.31 t	5.13 t	10.00 s	4.57 br s

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