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Neomarinone, and new cytotoxic marinone derivatives, produced by a marine filamentous bacterium (actinomycetales)

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Abstract

Neomarinone (**1**), a novel metabolite possessing a new sesquiterpene- and polyketide-derived carbon skeleton, and several derivatives, **3**–**5**, of the marinone class of naphthoquinone antibiotics, were isolated from the fermentation broth of a taxonomically-novel marine actinomycete (strain #CNH-099). The structures of the new compounds were determined by comprehensive NMR and mass spectral analyses. Neomarinone (**1**) and several of the marinone derivatives were shown to be moderately cytotoxic toward human cancer cells in in vitro testing. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: marine bacteria; naphthoquinone; mixed biosynthesis.

Marine microorganisms have been recognized as a new source for the production of bioactive secondary metabolites. The large numbers, and diversity, of marine bacteria suggest that this resource will be of significant importance in the discovery of new drugs.[1] Indeed, over the past few years a significant diversity of antibiotics and antitumor agents has been identifed.[2] In this paper we report the isolation of several cytotoxic metabolites related to marinone, a novel marine actinomycete-derived metabolite reported earlier.[3] The new compounds are produced by a marine bacterium found in a sediment sample taken at −1 m in Batiquitos Lagoon, North of San Diego, CA. The bacterium (strain #CNH-099)[4] was cultured in 26 L scale[5] and the culture was repeatedly extracted with ethyl acetate. Concentration of the extract yielded 2.15 g of crude extract which was fractionated by silica column methods, followed by repeated silica HPLC (% EtOAc in isooctane) to yield neomarinone (**1**, 1 mg/L, 35%), marinone (**2**, 1 mg/L, 30%), isomarinone (**3**, 0.5 mg/L, 30%), hydroxydebromomarinone (**4**, 1 mg/L, 50%), and methoxydebromomarinone (**5**, 1 mg/L, 50%). The structure elucidations of the new marinone derivatives **1** and **3**–**5** were accomplished by analysis of MS and NMR data and comparison with the published data for the previously reported metabolites marinone (**2**) and debromomarinone.[3] Neomarinone (**1**)[6a] analyzed for $C_{26}H_{32}O_5$ by HR-FABMS and ^{13}C NMR methods (Table 1). The UV spectrum of **1** showed

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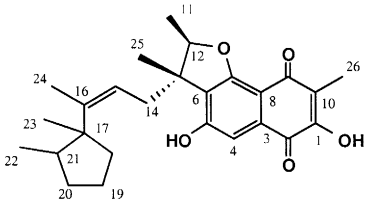
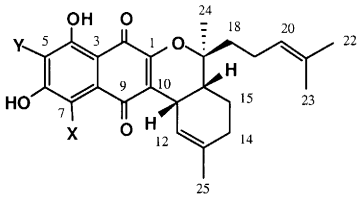
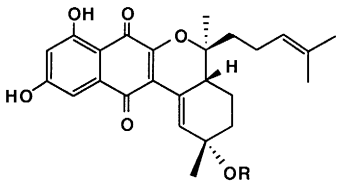
long wavelength absorptions at 398 nm (ε 2700), values similar to the UV characteristics of the previously described naphthoquinone chromophore in marinone. Infrared absorptions at 3299 and 1630 cm^{-1} were characteristic of the quinone and hydroxyl functionalities in this class of molecules. Given that this microorganism produced marinone, it was initially conceived that **1** was a simple modification of the marinone ring system. Analysis of 2D NMR data, however, quickly revealed that neomarinone was composed of an entirely new carbon skeleton. The formula of neomarinone indicated 11 degrees of unsaturation, eight degrees of which were inherent in the bicyclic naphthoquinone moiety. One degree of unsaturation was found in an additional double bond (δ 123.9 and 138.9), hence the remaining two degrees of unsaturation indicated two additional rings to be present.



1



2, X = Br, Y = H
3, X = H, Y = Br



4, R = OH
5, R = OCH_3

The structure assignment of **1** was easily facilitated by the six methyl groups that are present in the molecule, each of which provided informative correlations in the HMBC NMR experiment. The C-26 methyl group protons showed correlations to C-1 and C-9, which taken with correlations from H-4 to C-2, C-3, C-5 and C-6, and correlations from H-12 to C-7, positioned all substituents on the naphthoquinone ring. HMBC correlations also defined a five-membered ether ring that was positioned at C-6 and C-7. The connectivity of the sesquiterpenoid side-chain, and the presence of a methylated cyclopentane ring, were established by HMBC data and also by ^{1}H NMR COSY data. Extensive NMR experiments allowed all protons and carbons to be assigned (Table 1), thus leading to the assignment of **1** without stereochemistry.

Nuclear Overhauser NMR experiments (NOESY) allowed many of the stereochemical features of neomarinone to be assigned. The configuration of the side-chain double bond was assigned as *Z* based on a strong enhancement from H-15 to H-24. A strong correlation between H-11 and H-25 showed that the methyl groups in the dihydrofuran ring are *cis* oriented. Unfortunately, the relative stereochemistry of the two methyl groups (C-22 and C-23) in the cyclopentane ring could not be assigned with confidence from NOESY data because the two signals (δ 0.78 and 0.74) partially overlap in all NMR solvents explored.

Marinone (**2**) and debromomarinone, molecules we previously isolated from a tropical sediment bacterium, were also isolated from this strain. Their spectral data were identical to those from the original samples. The NMR data for marinone is included in Table 1 to facilitate comparisons with the closely related metabolites **3**–**5**.

Isomarinone (**3**), analyzed for $C_{25}H_{28}O_5Br$, a formula isomeric with that of marinone by HRCIMS and ^{13}C NMR methods. Inspection of total spectral data[6b] showed that **3** was an isomeric naphthoquinone possessing the identical side-chain as in **2**. Overall NMR data showed isomarinone to possess bromine at C-7 rather than C-5 as in marinone.

Hydroxydebromomarinone (**4**) analyzed for $C_{25}H_{28}O_6$, a formula indicating the addition of oxygen to the basic marinone composition. Overall spectral data[6c] showed that **4** was modified in the side-chain cyclohexene ring. The NMR data clearly showed that the trisubstituted olefin in **2** was replaced by hydroxylation and double bond migration to the C-11–C-12 position. The stereochemistry at the newly created hydroxyl-bearing carbon could not be assigned from spectral data, but was assigned by analogy to the derivative **5** which was assigned by NOE methods.

Table 1
NMR data for neomarinone (**1**), marinone (**2**) and derivatives (**3**–**5**)

	1		**2**		**3**		**4**		**5**	
C#	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	153.5	10.27 (s)-OH	150.2	-	152.3	-	153.8	-	153.8	-
2	180.3	-	181.9	-	182.0	-	181.7	-	181.4	-
3	131.5	-	108.8	-	107.0	-	107.5	-	107.4	-
4	107.9	7.05 (s)	162.6[a]	12.55 (s)–OH	159.6	12.54 (s)-OH	163.4	11.88 (s)-OH	163.2	11.86 (s)-OH
5	157.6	10.71(s)-OH	106.0	6.64 (s)	101.7	-	106.2	6.45 (d, 2.4)	106.1	6.46 (d, 2.2)
6	127.3	-	162.7[a]	11.99 (bs)-OH	161.8	12.11 (bs)-OH	165.6	11.25 (bs)-OH	165.4	11.29 (bs)-OH
7	159.9	-	104.1	-	107.4	7.09 (s)	108.5	6.94(d, 2.4)	108.4	6.93 (d, 2.2)
8	107.9	-	131.0[b]	-	132.1	-	134.9	-	134.7	-
9	182.7	-	182.4	-	182.4	-	182.5	-	182.1	-
10	120.3	-	124.5	-	123.1	-	125.0	-	127.7	-
11	15.1	1.30 (d, 6.3)	30.4	3.37 (m)	30.3	3.35(m)	116.1	-	115.5	-
12	86.3	4.65 (q, 6.3)	119.7	5.84 (d, 4.4)	120.1	5.95 (d, 5.0)	137.6	7.27(s)	134.2	7.28
13	46.0	-	135.7		135.3		65.6	4.63 (bs)-OH	70.3	-
14	31.0	1.86 (m)	29.3	1.97 (m)	29.1	1.84 (m)	35.7	1.69/1.45 (m)	33.1	1.83/1.40 (m)
15	123.9	5.36 (bs)	19.8	1.89/1.17 (m)	19.7	1.87/1.18 (m	19.4	1.60/1.51 (m)	19.0	1.59/1.45 (m)
16	138.9	-	36.8	1.86 (m)	36.6	1.86 (m)	39.3	2.34(ddd,10.3, 5.4, 2.0)	40.4	2.40 (m)
17	39.6	-	82.0	-	82.4	-	83.0	-	83.2	-
18	30.5	1.31/1.16(m)	36.1	1.53 (m)	35.9	1.54 (m)	38.4	1.77/1.70 (m)	38.2	1.75 (m)
19	25.0	1.92/1.83(m)	22.0	1.94 (m)	22.0	1.94 (m)	20.9	2.18/2.08 (m)	20.9	2.13 (m)
20	26.6	1.37 (m)	123.8	5.04 (bt, 6.5)	123.8	5.04 (bt,6.8)	124.0	5.16 (bt, 6.5)	123.7	5.16 (bt, 7.0)
21	32.7	1.70 (m)	131.1[b]	-	131.1	-	131.4	-	131.2	-
22	15.6	0.78 (d, 6.8)	25.3	1.56 (s)	25.3	1.55 (s)	25.4	1.66 (s)	25.4	1.66 (s)
23	20.9	0.74 (s)	17.3	1.50 (s)	17.3	1.50 (s)	17.5	1.60 (s)	17.4	1.60 (s)
24	18.7	1.52 (s)	21.6	1.41 (s)	21.6	1.43 (s)	18.6	1.09 (s)	18.4	1.09 (s)
25	19.7	1.14 (s)	23.4	1.62 (s)	23.3	1.61 (s)	30.8	1.24 (s)	25.2	1.25 (s)
26	8.5	1.83 (s)							49.4	3.13 (s)

^{13}C spectra were recorded in DMSO-d_6 at 100 MHz (**1** and **4** at 125 MHz). ^{1}H spectra were recorded at 300 MHz (**1** and **4** at 500 MHz). All data are referenced to residual solvent at δ 39.5 and δ 2.49, respectively. [a,b] Assignments may be interchanged. All assignments were made on the basis of COSY, DEPT and HMBC/HMQC data.

Methoxydebromomarinone (**5**) analyzed for $C_{26}H_{30}O_6$ by HR-FABMS and by ^{13}C NMR methods. As in **4**, the overall spectral data for **5**[6d] showed the molecule to be a modified marinone structure. NMR data allowed the modification to be localized in the side-chain cyclohexene ring. Similar to **4**, the structure of **5** was assigned as the C-13 methoxy analog. The relative stereochemistry at C-13 in **5**, with the methyl in the up (β) position, was determined by a strong NOE correlation between H-25 and H-16.

Neomarinone and the new marinone derivatives are all derived from a mixed biosynthetic pathway apparently involving polyketide and terpene pathways. Connection of the sesquiterpenoid side-chain to the naphthoquinone core occurs on the non-quinone side in neomarinone. There are only a few examples where similar metabolites produced by mixed biosynthesis have been observed. Examples are the monoterpene-substituted naphthoquinones, naphterpin[7] and naphthogeranine A.[8] Also, the monoterpene derivatives, furaquinocins A–H, which are similar to neomarinone have been reported.[9,10] The origin of the sesquiterpenoid side-chain in **1** appears complex, possibly being derived from a cation-induced methyl migration as observed in the trichothecenes.[11] Neomarinone (**1**), and the marinones **3**–**5**, displayed

moderate in vitro cytotoxicity, IC_{50}=ca. 8 μg/mL against HCT-116 colon carcinoma. In addition, neomarinone generated a mean IC_{50} value of 10 μM in the NCI's 60 cancer cell line panel.

Acknowledgements

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4. Bacterial strain CNH-099 was isolated from a sediment sample (collected at −1 m, Batiquitos Lagoon) using serial dilution and plating techniques on medium A1 (1.6% agar, 1% starch, 0.4% yeast extract, 0.2% peptone, 100% seawater). As in the original strain producing marinone (CNB-384), strain CNH-099 could not be assigned to any known actinomycete genus by fatty acid methyl ester (FAME) analysis (Microbial ID Inc., Newark, DE).
5. Bacterial strain CNH-099 was cultivated in 26×1 L volumes in Fernbach flasks. The flasks were shaken at 230 rpm at 23°C for 8–10 days.
6. (a) *Spectral data for neomarinone* (**1**): HR-FABMS: 424.2265 $[M]^+$; calcd for $C_{26}H_{32}O_5$: 424.2250 (Δ 3.6ppm) $[\alpha]_D^{25}$ +86° (*c*=0.5, MeOH); IR (film): 3299, 2923, 1659, 1630, 1606, 1569, 1418, 1379, 1347, 1302, 1185, 1052; UV [λ_{max} (ε)]: (MeOH): 398 (2700), 312 (7800), 263 (13900), 259 (sh 13500), 213 nm (19800); (MeOH+KOH): 525 (1100), 358 (6000), 289 (19500), 229 nm (15400). *Spectral data for marinone* (**2**): HR-FABMS: $[M]^+$ *m/z* 486.1033; calcd for $C_{25}H_{27}O_5{}^{79}Br$: 486.1042 (Δ 1.8 ppm), $[\alpha]_D^{25}$ −170° (*c*=0.15, MeOH); IR (film): 3250, 2925, 1639, 1603, 1388, 1274, 1221, 1037 cm^{-1}; UV [λ_{max} (ε)]: (MeOH+HCl): 435 (1400), 395 (1500), 307 (5200), 268 (6900), 202 nm (18100), (MeOH+KOH): 510 (3700), 385 (2300), 335 (5400), 298 (9900), 234 nm (15600); (b) *Spectral data for isomarinone* (**3**): HRCIMS (NH_3): $[M+H]^+$ *m/z* 487.1094; calcd for $C_{25}H_{28}O_5{}^{79}Br$: 487.1120 (Δ 5.4 ppm); $[\alpha]_D^{25}$ −120° (*c*=0.2, MeOH); (film): 3226, 2925, 1634, 1589, 1451, 1378, 1327, 1225, 1045 cm^{-1}; UV [λ_{max} (ε)]: (MeOH+HCl): 390 (1500), 312 (4700), 270 (8100), 265 (8000), 206 nm (17000), (MeOH+KOH): 513 (2000), 385 (2000), 327 (4100), 295 (9500), 230 nm (12200); (c) *Spectral data for hydroxydebromomarinone* (**4**): HR-FABMS: $[M]^+$ *m/z* 424.1870; calcd for $C_{25}H_{28}O_6$: 424.1886 (Δ 3.8 ppm); $[\alpha]_D^{25}$ +280° (*c*=0.2, MeOH); IR (film): 3243, 2925, 1632, 1594, 1561, 1453, 1380, 1326, 1227 cm^{-1}; UV [λ_{max} (ε)]: (MeOH+HCl): 460 (2600), 392 (2800), 328 (7000), 283 (18100), 275 (17000), 228 (16000), 211 nm (18900), (MeOH+KOH): 525 (4700), 358 (8100), 299 (13400), 249 (20700), 238 nm (20300); (d) *Spectral data for methoxydebromomarinone* (**5**): HR-FABMS: $[M]^+$ *m/z* 438.2053; calcd for $C_{26}H_{30}O_6$: 438.2042 (Δ 2.4 ppm); $[\alpha]_D^{25}$ +140° (*c*=0.1, MeOH); IR (film): 3250, 2933, 1631, 1605, 1562, 1454, 1327, 1231 cm^{-1}; UV [λ_{max} (ε)]: (MeOH+HCl): 455 (2100), 385 (2700), 325 (sh 5300), 308 (5900), 283 (12400), 275 (12000), 228 (sh 13200), 208 nm (17800), (MeOH+KOH): 525 (3000), 354 (6000), 298 (10000), 250 nm (15900).
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