Tetrahedron Letters, Vol. 33. No. 50, pp. 7663-7666.1992
Printed in Great Britain

0040-4039/92 $5.00 + .OO
Pergamon Press Ltd

MARINONE AND DEBROMOMARINONE: ANTIBIOTIC SESQUITERPENOID NAPHTHOQUINONES OF A NEW STRUCTURE CLASS FROM A MARINE BACTERIUM

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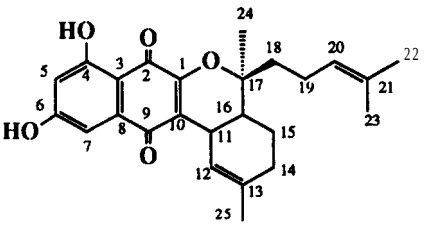
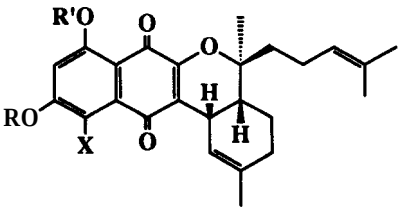
Summary - Marinone (1) and its debromo analog debromomarinone **(2),** antibiotic sesquiterpenoid naphthoquinones of a new structure class, have been isolated from the organic extract of the liquid culture of a marine actinomycete. isolate CNB-632. The structures of the new compounds were assigned on the basis of comprehensive spectroscopic analyses.

Although the production of antibiotic compounds by terrestrial bacteria has been the focus of intense investigation for over 50 years, analogous investigations of marine bacteria have been severely limited.1 Due to problems in the isolation and identification of marine bacteria and fungi, the immensity of this resource has yet to be demonstrated. In preliminary chemical studies of several classes of marine bacteria, a variety of interesting new molecules have recently been discovered.[1,2] In this paper, we report the isolation and structural elucidation of marinone **(1),** a sequiterpenoid naphthoquinone and its debromo analogue, debromomarinone **(2),** new antibacterials of a novel class, isolated from the culture broth of an unidentified marine actinomycete. The new molecules are among a rare group of bacterial metabolites which are produced via mixed biosynthesis involving both acetate and terpene pathways.

The microorganism, culture # CNB-632,[3] was grown in 10L culture and the whole broth was repetitively extracted with ethyl acetate. Concentration of the extract under vacuum yielded 1.73 g of crude material, which was





1 : R = R' = H, X = Br

2 : R = R' = X = H

3 : R = Me, R' = X = H

fractionated by flash chromatography on silica using a gradient of ethyl acetate in isooctane. Fractions eluted with 30% ethyl acetate / isooctane were combined and further purified by normal phase HPLC and reversed-phase preparative TLC (70% MeOH/H_2O) to obtain 14 and 2,5 each as red, non-crystalline solids.

The mass spectrum of 1 showed a doublet of equal intensity at *m/z* 486 and 488, which by HR mass analysis clearly indicated a molecular formula $C_{25}H_{27}BrO_5$ containing bromine. Marinone (1) and 2 possessed very similar ^1H NMR, IR and UV spectra. In the mass spectrum of 2, a parent ion was observed at *m/z* 408 which implied that 2 was the debromo analog of 1. This was confirmed when the molecular formula analysed for $C_{25}H_{28}O_5$ by HREIMS. The proton NMR spectra of 1 and 2 were simple and relatively uninformative. However, analysis of the ^1H NMR COSY spectrum of 1 led to the assignment of several structural fragments. A resonance at δ 5.08 (bt, J = 7 Hz) coupled to a signal at δ 2.0 and to two methyl resonances at δ 1.49 and 1.66. in combination with mass spectral loss of an *m/z* = 83 fragment to give a strong peak at *m/z* 325. indicated a C_6H_{11} terpenoid side-chain in the molecule. Proton NMR COSY correlations involving three methine protons at δ 1.88, 3.39 and 6.03 identified a fragment, $-CH_2CHCHCH{=}CCH_3-$, assigned to C15, C16, Cl 1, and C12. These two fragments were linked through a quaternary carbon (C17) using long range proton-carbon correlations acquired through HMBC experiments. A two bond correlation from a methyl resonance (C24) at δ 1.49 to Cl7 at δ 83.5, and a three bond correlation to Cl8 at δ 37.5 linked C24 and Cl8 through C17. Another three bond correlation from the same methyl protons to Cl6 linked

Table. NMR **Data for Debromomarinone (2)***

C#	Carbon NMR Data (ppm)		Proton NMR Data (δ)	HMBC Data
1				
2	155.1	(C)		H11
	180.9	(C)		
3	105.6	(C)		H5, H7
				H5
4	107.9	(CH)	6.10 (d, 2.5)	H7
6	167.2	(C)a		H5
7	115.6	(CH)	6.83 (d, 2.5)	H5
8	135.7	(C)		
9	186.4	(C)		H7
11	123.5	(C)		H11, H16
12	32:0	(CH)	3.39 (bt, 5.0)	H12, H16
13	121.7	(CH)	6.03 (bd, 5.0)	H11, H14, H25
14	130.7	(C)		H11, H14. H25
		(CH2)	2.00 (m)	H12, H25
15	21.4	(CH2)	1.31 (dt, 6.0. 12.0, 12.0)	H11
			2.00 (m)	
17	39.1	(CH)	1.88 (ddd, 3.0, 6.0, 12.0)	H11, H12, H14, H24
18	37.5	(CH2)		H24
			1.63 (m)	H19, H24
19	23.2	(CH2)	2.00 (m)	H20
20	124.8	(CH)	5.08 (bt, 7.0)	H19, H22, H23
21	132.9	(C)		H19, H22, H23
23	25.8	(CH3)	1.60 (bs)	H20
24	22.7 17.6	(CH3)	1.55 1.49 (bs)	H20
		(CH3)	(s)	
25	23.8	(CH3)	1.66 (bs)	H12

* Spectra were acquired in CD_3OD with reference to internal TMS at 0.00 ppm for ^1H (500 MHz) and CD_3OD at 49.0 ppm for ^{13}C (50 MHz). Assignments are based on the results of XHCORR (50 MHz) and HMBC (500 MHz) experiments. a- assignments may bc interchanged.

Cl6 and C17. Long range correlations from the Cl4 methylene protons to C12, Cl3 and Cl6 allowed the terpenoid portion of the molecule to be fully defined as in **1** and 2.

The remaining aromatic portion of the molecule accounted for 9 degrees of unsaturation, and was characterized by two ^1H NMR resonances at δ 6.83 and 6.10 ppm and ^{13}C NMR resonances for eight sp^2 quaternary carbons, including two quinone carbonyl resonances at δ 186.4 and 180.9. This information, combined with the UV-Vis absorption characteristics of these compounds,[4, 5] (absorbances between 480-510 nm) and the IR absorption at 1638 cm-l of **1** suggested a hydroxynaphthoquinone moiety.[6, 7] In the ^1H NMR spectrum of 2, the 2.5 Hz coupling between the two aromatic protons at δ 6.10 and 6.83 indicated that they were *meta* oriented, thus allowing their assignment at C5 and C7, respectively. The presence of two phenolic OH groups was confirmed by the ^1H NMR characteristics of **3**, the monomethyl ether derivative produced by the treatment of 2 with diazomethane.[8] Because of intense hydrogen bonding with the C9 carbonyl, the hydroxyl at C7 was not methylated under the reaction conditions. The hydroxyl proton appeared at δ 12.0 ppm in the ^1H NMR spectrum of 3, a shift common in this hydrogen bonded arrangement.* The protons of the methyl ether showed strong nOe when either of the aromatic protons were irradiated, allowing the methyl ether to be established at C6.

The naphthoquinone component of debromomarinone was linked to the terpenoid moiety using HMBC correlations. This included a 3-bond correlation from Hl 1 to Cl and two and three bond correlations from H11 and H16 to C10, respectively. To satisfy the molecular formula, Cl had to be linked to Cl7 via a chromanol ether linkage. Finally, the substitution pattern on the aromatic ring was successfully established using HMBC results in combination with ^{13}C NMR data. In naphthoquinone chromenols with peri-substituted hydroxyl groups, the carbonyl adjacent to the chromenol oxygen-bearing carbon is known to experience a significant upfield shift.9 On this basis, the carbonyl resonances at δ 180.9 and 186.4 were assigned to C2 and C9, respectively. A strong 3-bond correlation from H7 to C9 in an HMBC experiment with 2 enabled H7 to be assigned, thus providing the regiochemistry of the aromatic ring. The ^1H NMR spectra of **1** and 2 were almost superimposable except for the aromatic proton signal assigned at C7 in 2. Since this signal was absent in marinone (**1**), the bromine substituent was placed at this position.

The relative configurations at the three chiral centers in **1** and 2 were determined using ^1H NMR nOe results. A strong nOe in 2 from Hl 1 to H16, and vice versa, indicated a *cis* configuration of the Cl l-Cl6 ring junction. The C17-C18 bond was assigned as pseudoaxial (β) on the basis of an nOe between Hl **1** and one of the protons at C18. Curiously, both marinone and debromomarinone displayed no optical rotation at the sodium D line, indicating that they may be racemic.

Marinone and its debromo derivative are the first examples of compounds possessing this novel carbon skeleton. Two related compounds, naphterpin,[10] and naphthogeranine A,[11] both isolated recently from the fermentation broths of terrestrial actinomycetes, possess similar chromenol C and D rings, constructed in these cases from monoterpenoid substituents. Marinone and debromomarinone are formally the sesquiterpenoid analogs of these latter compounds. Both metabolites show significant in vitro antibacterial activity mainly against gram-positive bacteria. For example, marinone(**1**) shows MIC = 1 μg/ml against *Bacillus subtilis*, and debromomarinone shows MIC values of l-2 μg/ml against *Staphylococcus aureus, S. epidermis, S. pneumoniae, S. pyogenes* and S. *epidermis.*

Acknowledgements

This research is a result of financial support from the National Institutes of Health, National Cancer Institute, under grants CA44848 and CA50750. We thank Dr. Robert E. Kessler, Bristol-Myers Squibb Pharmaceutical Institute, for providing comprehensive antibacterial biotesting data, and Mr. Ryan Dwight for collecting and processing the sediment sample.

References

1. a) Austin, B. *J. Appl. Bact.*, **1989, 67, 461.**, b) Fenical, W., and Jensen, P. R.. In: ***Advances in Marine*** *Biotechnology, Volume I., 0.* K. Zaborsky and D. A. Attaway, **Eds.,** Plenum Publishing Co., in **press,** 1992.

2. a) Gustafson, K., Roman, M., Fenical, W., ***J. Am. Chem. Soc.*, 1989, *111*, 7519.**, b) Tapiolas, D. M.. Roman, M., Fenical, W., Stout, T. J., Clardy, J.,***J. Am. Chem. Soc.***, 1991,123, **4682.**, c) Gil-Tumes, M. S., Hay, M. E., Fenical, **W.,** Science. **1989.246.116..** d) Pathirana, C., Jensen, P. R., Dwight, R., Fenical. W..***Tetrahedron Lett.*1991, *32*(21),** 2323.. e) Stout, T. J.. **Clardy,** J., Pathiia, **I.** C., and Fenical, W.. ***Tetrahedron Lett.*, 1991, 47 (22). 351 1., f)** Pathirana, C., Dwight, R.. Jensen, P. R.. Fenical, W., Delgado, A,**Brinen, L.** S., and **Clardy, J.,** *Tetrahedron* ***Lett.*, 1991**, *32* **(48), 7001., g) Gil-Tumes,** M. S., and Fenical. W., ***Biol. Bull.*, 1992, *182*, 105**.

3. The bacterium, isolate CNB-632. was obtained by serial dilution and plating techniques, using media containing rifampicin (5 **μg/ml),** from a sediment sample collected at the **Torrey** Pines Estuary, La Jolla, CA in 1989. The isolate was cultured in a marine medium (Al) consisting of 1% starch, 0.4% yeast extract, 0.2% **peptone, 75% seawater, 25%, deionized** water, buffered with 10 ml 1 .O M Tris buffer and shaken at 230 RPM for 7 days at **23°C.** The whole culture was extracted with **EtOAc** to leave an extract **from** which compounds **1 (2-5 mg/L)** and 2 (7-10 **mg/L)** were isolated. Isolate CNB-632 is an actinomycete which shows brown vegetative **mycelia** and lacks aerial spores and hyphae (Al medium).

4. For Marinone **(1):** UV-Vis **(MeOH)** 508 (e **4100),** 406 **(ε 2290),** 300 **(ε** 10500). 235 **(ε 17400)** nm; IR (film) 3417 (br), **2919, 1638, 1585, 1545, 1452, 1393, 1323, 1244** cm-l; LREIMS ***m/z*** 488 **(20),** 486 (13). 408 (34). 325 **(65),** 323 **(86),** 28 (100) **;** HREIMS ***m/z*** 486.0996. calcd. for $C_{25}H_{27}BrO_5$ 486.1039; ^1H NMR (200 MHz. CD_3OD) **δ** 6.10 (s, **1H),** 5.95 (d, J = 5 Hz. **1H),** 5.03 (bt, J **=** 7 Hz. **1H),** 3.40 **(br, 1H),** 2.0 (m. **5H),** 1.66 (bs, **3H),** 1.60 **(bs, 3H),** 1.5 **(m, 2H),** 1.55 (bs, **3H),** 1.45 **(s,** 3H).

5. For Debromomarinone (2): UV-Vis **(MeOH)** 488 (E 2280). 388 **(ε 2600),** 299 (E **9200),** 269 **(ε 10400),** 226 (e 16600) **nm; IR** (film) **3414(br), 2924, 1631, 1589, 1544, 1469, 1380, 1228** cm-l; LRBIMS ***m/z*** **408 .(M^+, 60),** 325 **(100),** 297 (30); LRCIMS ***m/z*** 409 **(M^++** H); HREIMS ***m/z*** 408.1916, calcd. for $C_{25}H_{28}O_5$ 408.1924, ^1H and ^{13}C NMR: see table.

6. Britton, G., ***The*** *Biochemistry of Natural Pigments,* Cambridge University Press, Cambridge, 1983, p. 77.

7. Stack, M. E., Mazzola, E. P., Eppley, R. M., *Tetrahedron* ***Lett.*, 1979, *52*, 4989**.

8. For 3 : **IR** (film) **2926, 1638, 1591, 1446, 1388, 1312, 1224, 1160 cm^{-1}; ^1H** NMR (500 MHz, CDC13) **δ** 12.0 (s, **1H), 7.15** (d, J **= 2.5 Hz, 1H), 6.55** (d, **J = 2.5** Hz, **1H), 6.07** (d, J-5.5, **1H), 5.02 (bt, J = 5.5 Hz, 1H), 3.46 (bt,** J = 5 Hz, **1H),** 2.0 **(m, 5H),** 1.88 (ddd, J = **3, 6.5,** 12 Hz, **1H),** 1.68 (bs. **3H),** 1.65 **(m, 2H),** 1.62 **(bs, 3H),** 1.56 **(bs, 3H),** 1.54 (s, **3H),** 1.34 (m, **1H).**

9. McDonald, I. A., Simpson, T. J.. Sierakowski, A. F., ***Aust. J. Chem.*, 1977, *30*, 1727.**

10. **Shin-Ya,** K., Imai, S., Furihata, K., Hayakawa, Y., Kato, Y., Van Duyne, G. D., Clardy. J. ***J. Antibiotics,* 1990,** 43,444.

11. Wessels, P., Gohrt, A., Zeeck, A. *J. Antibiotics,* **1991, *44*, 1013.**

(Received in USA 20 August 1992)