Chemistry of Sponges. 19. Novel Bioactive Metabolites from *Hamigera tarangaensis*

Keri D. Wellington,[†] Richard C. Cambie,*[,†] Peter S. Rutledge,[†] and Patricia R. Bergquist[‡]

Department of Chemistry, and School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

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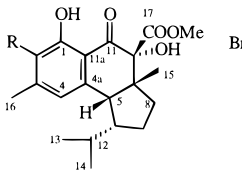
Seven new compounds (**1**−**6** and **10**) with a unique carbon skeleton have been isolated from the sponge *Hamigera tarangaensis*, and the structure of a previously reported metabolite has been revised from **12** to **8**. The structures have been assigned from extensive NMR examination. Compounds **3**−**6** showed moderate in-vitro cytotoxicity against P-388, while **3** showed 100% in-vitro virus inhibition against both the Herpes and Polio viruses, with only slight cytotoxicity.

Recently we reported[1] the isolation of a new brominated phenolic compound **12**, from the poecilosclerid sponge *Hamigera tarangaensis*[2] Bergquist and Fromont (family Anchinoidae, syn. Phorbasidae) collected from the Hen and Chicken Islands, which lie east of Whangarei in New Zealand. Continued examination of the MeOH extract of the freeze-dried sponge has afforded a further seven new compounds: hamigeran A (**1**), debromohamigeran A (**2**), hamigeran B (**3**), 4-bromohamigeran B (**4**), hamigeran C (**5**), hamigeran D (**6**), and debromohamigeran E (**10**). In the course of elucidating the structures of these compounds, it was determined that the structure previously proposed for **12** was incorrect and thus has been revised to hamigeran E (**8**).

The order Poecilosclerida is composed of 14 families, of which the Mycalidae, Microcionidae, Tedaniidae, Latrunculiidae, and Myxillidae have received most attention. From the Mycalidae, compounds such as the mycalamides,[3,4] mycalolides,[5,6] and ptilomycalin A[7] have been isolated, while from the Microcionidae such compounds as the episulfide-containing polyether acanthifolicin,[8] the clathrynamides,[9] and the indole-derived dilemmaones[10] have been isolated. From the Tedaniidae small aromatic alkaloids are the most prevalent metabolites,[11] although the most studied compound is the macrolide tedanolide.[12] Among the compounds isolated from the Latrunculiidae are the discorhabdins[13−18] and the latrunculins,[19,20] while from the Myxillidae members of the halichondrin series of compounds have been found.[21] Yet, apart from our study[1] on *Hamigera tarangaensis*, only the genus *Phorbas* (syn. *Anchinoe*) of the family Anchinoidae has been investigated for secondary metabolites. Among the compounds isolated from the latter genus are the phorboxazoles[22,23] and the anchinopeptolides.[24]

Results and Discussion

The freeze-dried sponge was extracted with MeOH, and a portion of the extract was separated by flash chromatography on Si gel to yield a mixture containing **8** and **10**. This fraction was ethylated and purified further by column chromatography to afford **9** and **11** in 0.93% and 0.11% yields, respectively. The parent phenols **8** and **10** were designated as hamigeran E and debromohamigeran E, respectively. The remaining MeOH extract was subjected to a hexanes−CH_2Cl_2−EtOAc solvent partition sequence, and the hexane-soluble portion was then separated by flash column chromatography and PLC to yield hamigeran A (**1**), debromohamigeran A (**2**), hamigeran B (**3**), 4-bromohamigeran B (**4**), hamigeran C (**5**), and hamigeran D (**6**) in 0.46, 0.32, 0.63, 0.07, 0.09, and 0.09% yields, respectively.



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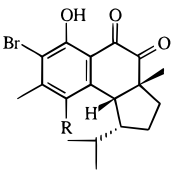

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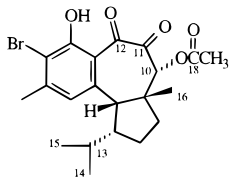

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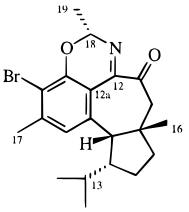

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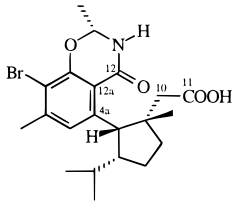

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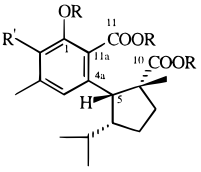
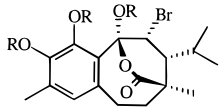


8 R = H, R' = Br
9 R = Et, R' = Br
10 R = H, R' = H
11 R = Et, R' = H



12 R = H
13 R = Et

Hamigeran A (**1**), $[\alpha]_D$ −22.5°, was isolated as yellow needles, mp 207−209 °C, and its molecular formula, $C_{20}H_{25}BrO_5$, was established from high-resolution desorption electron impact mass spectroscopy (HRDEIMS) and ^{13}C NMR data. From this it followed that the compound possessed eight degrees of unsaturation. The IR spectrum

* To whom correspondence should be addressed. Tel.: 64 9 373-7999. Fax: 64 9 373-7422. E-mail: *c.cambie@auckland.ac.nz.*
[†] Department of Chemistry.
[‡] School of Biological Sciences.

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Table 1. ^{1}H, ^{13}C, COSY, and HMBC NMR Data for **1** (in $CDCl_3$)

C	δ_C	δ_H (mult, *J* in Hz)	COSY	HMBC
1	157.6			11.75 (O*H*)
2	110.5			H-4, H-16, 11.75 (O*H*)
3	148.3			H-16
4	122.4	6.79 (s)	H-5, H-16	H-5, H-16
4a	142.8			H-5, H-6
5	50.0	3.50 (d, 5.6)	H-6	H-4, H-8β, H-12, H-15
6	53.4	2.03 (m)	H-5, H-7α, H-7β, H-12	H-8α, H-13, H-14
7	26.9	1.62 (m) (β)	H-6, H-8α, H-8β	H-5
		0.70 (m) (α)	H-6, H-8α, H-8β	
8	34.0	1.72 (m) (α)	H-7α, H-7β	H-5, H-15
		1.55 (m) (β)	H-7α, H-7β	
9	47.2			H-5, H-8α, H-8β, H-15, 4.33 (O*H*)
10	89.3			H-8α, H-8β, H-15, 4.33 (O*H*)
11	198.1			H-4, 4.33 (O*H*)
11a	114.7			H-4, H-5, 11.75 (O*H*)
12	27.3	1.58 (m)	H-6, H-13, H-14	
13	23.4	1.22 (d, 6.5)	H-12	H-14
14	22.0	0.83 (d, 6.5)	H-12	H-13
15	24.3	1.36 (s)		H-5, H-8α, H-8β
16	24.6	2.47 (s)	H-4	H-4
17	169.5			3.61 (OC*H*$_3$), 4.33 (O*H*)
OCH_3	53.1	3.61 (s)		
C-1-O*H*		11.75		
C-10-O*H*		4.33		

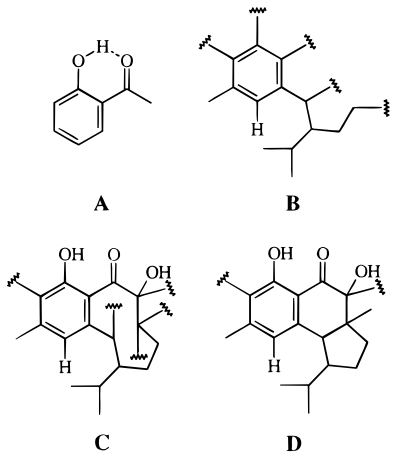


Figure 1. Partial structures of **1**.

contained bands at 3454 (br, hydroxyl), 1742 (ester), and 1640 cm^{-1} (α,β-unsaturated, β-hydroxy ketone).[25] The ^{13}C NMR spectrum (Table 1) contained signals for ketone and ester carbonyl groups at δ 198.1 and 169.5, and six sp^2 carbon signals at δ 157.6, 148.3, 142.8, 122.4 (CH), 114.7, and 110.5, corresponding to a penta-substituted aromatic ring. From this information two degrees of unsaturation are unaccounted for. In the lowfield region of the ^{1}H NMR spectrum (Table 1), a sharp singlet at δ 11.75 indicated the presence of a hydrogen-bonded phenol that provided further evidence to support the positioning of a phenolic group β to the ketone and thereby the partial structure A (see Figure 1). A singlet at δ 6.79 also confirmed the existence of a penta-substituted aromatic ring. Two three-proton singlets at δ 3.61 and 2.47 suggested the presence of a methoxy group and an aryl methyl group, respectively, while signals at δ 0.83 (d, 3H, J = 6.5 Hz) and 1.22 (d, 3H, J = 6.5 Hz) were indicative of an isopropyl group. A COSY experiment provided a spectrum that displayed one distinct 13-spin system involving the signals δ 3.50, 2.03, 1.58, 1.22, 0.83, 1.62, 0.70, 1.55, and 1.72 and corresponding to a fragment CHCH[CH(CH$_3$)$_2$]CH$_2$CH$_2$. Long-range proton−proton coupling was observed between the aromatic proton H-4 (δ 6.79) and the benzylic proton H-5 (δ 3.50), as well as between the aryl methyl H-16 (δ 2.47) and the aromatic proton H-4 (δ 6.79). This information gives rise to partial structure B (Figure 1). An HMQC experiment permitted unequivocal assignment of all proton-bearing carbons (Table 1), as well as proving that the protons responsible for signals at δ 11.75 and 4.33 were those of hydroxyl groups, and thus required the compound to be tricyclic. The HMBC experiment provided information to complete the structure by bridging the quaternary centers.

In the latter spectrum, C-11 (δ 198.1) exhibited coupling to H-4 and the C-10 hydroxyl, while C-10 (δ 89.3) showed coupling to the C-8 methylene protons (δ 1.72 and 1.55) and the H-15 methyl group protons, thus allowing extension of the partial structure to C (Figure 1). The benzylic carbon C-5 (δ 50.0) gave key positional data by showing correlations to H-4 (δ 6.79), the H-15 methyl (δ 1.36), the methylene proton H-8β (δ 1.55), and the isopropyl proton H-12 (δ 1.58), thus defining it as a bridgehead methine and allowing the expansion of the partial structure to D (Figure 1). The ester carbonyl C-17 (δ 169.5) showed coupling to the methoxy protons (δ 3.61) as well as to the C-10 hydroxyl proton (δ 4.33). The only position left for the bromine atom was at C-2, and this placement was consistent with the relatively highfield chemical shift of C-2 (δ 110.5).

The HMBC spectrum also provided information that permitted assignment of the carbon atoms composing the aromatic ring. The signal at δ 142.8 (C-4a) showed coupling to H-5 and H-6, placing it at the ring A−B junction. The signal at δ 114.7 (C-11a) coupled to H-4, H-5, and the phenolic proton, thus placing it at the other ring A−B junction. The signal at δ 110.5 (C-2) showed coupling to the phenolic proton, H-4, and H-16, thereby placing it ortho to C-1. In the NOESY spectrum, the H-15 methyl group (δ 1.36) showed through-space coupling to H-5, H-6, and H-8β (δ 1.55), whereas H-6 showed a NOE correlation to H-7β (δ 1.62). Thus, the relative stereochemistry of the cyclopentane ring at C-5, C-6, and C-9 was assigned as shown in **1**. The alkyl hydroxyl proton showed a NOE correlation to H-8α (δ 1.72), indicating that both were α-oriented and, thus, the methyl ester group was β-oriented. The relative stereochemistry of **1** was confirmed, and the absolute stereochemistry was established by single-crystal X-ray analysis.[26]

Debromohamigeran A (**2**), $[\alpha]_D$ −38.5°, was isolated as a colorless semicrystalline solid, mp 88.5−90 °C, and its

Table 2. ^{1}H, ^{13}C, COSY, and HMBC Data for **3** (in $CDCl_3$)

C	δ_C	δ_H (mult, *J* in Hz)	COSY	HMBC
1	160.8			H-5, H-8α, H-8*β*, H-15
2	111.6			12.64 (O*H*), H-4, H-16
3	150.2			H-4, H-16
4	124.3	6.82	H-5, H-16	H-5, H-16
4a	142.8			H-5
5	56.2	3.39 (d, 9.3)	H-4, H-6	H-4, H-7α, H-7*β*, H-8α, H-12, H-15
6	51.3	2.31 (m)	H-5, H-7α, H-7*β*, H-12	H-7*β*, H-8α, H-12, H-13, H-14
7	26.8	1.81 (m) (α)	H-6, H-7*β*, H-8α, H-8*β*	H-12
		1.69 (m) (*β*)	H-6, H-7α, H-8α, H-8*β*	
8	33.8	2.63 (ddd, 13.0, 7.6, 5.4) (α)	H-7α, H-7*β*, H-8*β*	H-6, H-15
		1.56 (m) (*β*)	H-7α, H-7*β*, H-8α	
9	57.0			H-5, H-6, H-7α, H-7*β*, H-8α, H-15
10	199.1			H-5, H-8α, H-8*β*, H-15
11	184.4			H-4
11a	117.3			12.64 (O*H*), H-4, H-5
12	28.1	1.21 (m)	H-6, H-13, H-14	H-5, H-7α, H-7*β*
13	19.7	0.53 (d, 6.5)	H-12	H-6, H-14
14	23.3	0.45 (d, 6.5)	H-12	H-6, H-13
15	24.3	1.29 (s)		H-5, H-8*β*
16	24.4	2.51 (s)	H-4	H-4
1-*OH*		12.64 (s)		

molecular formula, $C_{20}H_{26}O_5$, was established from HR-DEIMS and ^{13}C NMR data. The IR spectrum contained bands at 1742 (ester) and 1639 cm^{-1} (α,*β*-unsaturated, *β*-hydroxy ketone). The ^{13}C NMR spectrum was very similar to that of **1**, but it contained two aromatic methine signals (*δ* 121.5 and 115.1) rather than one. In the ^{1}H NMR spectrum, the only significant difference from that of **1** was the presence of two aromatic proton singlets (*δ* 6.66 and 6.64) instead of one. From this information, debromohamigeran A was assigned structure **2**, which was confirmed from COSY and HMBC correlations.

Hamigeran B (**3**), $[\alpha]_D$ −151.1°, was isolated as yellow plates, mp 163−165 °C, and its molecular formula, $C_{18}H_{21}BrO_3$, was established from HRDEIMS and ^{13}C NMR data. The IR spectrum contained bands at 1725 (ketone) and 1634 cm^{-1} (α,*β*-unsaturated, *β*-hydroxy ketone). The ^{1}H NMR spectrum (Table 2) contained signals identifying it as a member of the same series as **1** and **2**, that is, a phenolic proton signal at *δ* 12.64, an aryl proton singlet at *δ* 6.82, an aryl methyl singlet at *δ* 2.51, a benzylic proton doublet at *δ* 3.39 (H-5, *J* 9.3 Hz), a three-proton tertiary methyl group singlet (*δ* 1.29), and two methyl doublets of an isopropyl group at *δ* 0.53 (*J* = 6.5) and 0.45 (*J* = 6.5). The ^{13}C NMR spectrum (Table 2) was very similar to that of **1**, but it contained two lowfield quaternary carbon signals at *δ* 199.1 and 184.4, and it lacked an alkyl quaternary carbon signal corresponding to C-10. Instead, the latter was replaced by a second ketone carbonyl signal (*δ* 199.1) in addition to that of C-11. From these data it was deduced that **3** was an α-diketone. An HMBC spectrum showed correlations between the C-10 carbonyl at *δ* 199.1 and four aliphatic signals, H-5, H-8α, H-8*β*, and H-15, which indicated that this carbonyl was adjacent to the cyclopentane ring. The NOESY spectrum of **3** provided evidence that permitted assignment of the relative stereochemistry. Thus, H-5 showed NOE correlations to H-6, H-7*β* (*δ* 1.81), H-8*β* (*δ* 1.56), and H-15 (Figure 2), which indicated that H-5, H-6, and H-15 were all *β*-oriented. From the skeletal similarity between **1** and **3** and the same sign of rotation, it is probable that they also possess the same absolute stereochemistry.

4-Bromohamigeran B (**4**), $[\alpha]_D$ −81.2°, was isolated as a yellow semicrystalline solid, mp 144−148 °C, and its molecular formula, $C_{18}H_{20}Br_2O_3$, was established from HREIMS and ^{13}C NMR data. The molecular ion cluster in the mass spectrum had the expected 1:3:1 height ratio expected for a compound containing two bromine atoms. The IR spectrum contained bands at 1723 (ketone) and 1637 cm^{-1} (α,*β*-unsaturated, *β*-hydroxy ketone). The ^{13}C NMR spectrum was very similar to that of **3** except there was no aromatic methine signal, and the only significant difference in the ^{1}H NMR spectrum from that of **3** was the absence of an aromatic proton signal. From this information the structure of 2,4-dibromohamigeran B was assigned as **4**. COSY and HMBC spectra correlations were in accord with the structure **4**.

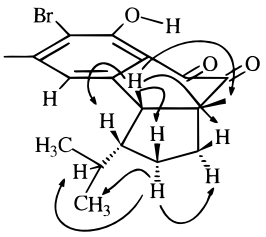


Figure 2. NOE correlations for hamigeran B (**3**).

Hamigeran C (**5**), $[\alpha]_D$ −136.0°, was isolated as fine yellow needles, mp 156−158 °C, and its molecular formula, $C_{21}H_{25}BrO_5$, was established from HRDEIMS and ^{13}C NMR data. The IR spectrum contained bands at 1749 (ester), 1737 (ketone), 1626 (α,*β*-unsaturated, *β*-hydroxy ketone), and 1230 cm^{-1} (ester). The ^{1}H NMR spectrum (Table 3) contained signals indicative of a carbon skeleton similar to that of **1**−**4**, that is, a phenolic proton signal at *δ* 12.04, an aryl proton singlet at *δ* 6.80, an aryl methyl singlet at *δ* 2.49, a benzylic proton doublet at *δ* 3.49 (H-5, *J* 11.2), a three-proton tertiary methyl group singlet at *δ* 1.34, and two methyl doublets indicative of an isopropyl group at *δ* 0.68 (*J* = 6.5) and 0.27 (*J* = 6.5). In addition, an acetate methyl group singlet appeared at *δ* 2.16 and a one-proton singlet at *δ* 5.08, indicating an oxymethine proton. The ^{13}C NMR spectrum (Table 3) exhibited signals confirming the presence of an acetate group (*δ* 170.0) and of an oxymethine carbon (*δ* 81.6). Two lowfield quaternary carbons at *δ* 191.2 and 187.4 were indicative of the presence of an α-diketone, as in **3** and **4**. In the HMBC spectrum, the oxymethine carbon (C-10) showed coupling to the protons at H-5, H-6, and H-8*β* and to the methyl protons H-16 (*δ* 1.34), thus placing it adjacent to the cyclopentane ring. The only location for an α-diketone was to include it in a seven-

Table 3. ^{1}H, ^{13}C, COSY, and HMBC Data for **5** (in $CDCl_3$)

C	δ_C	δ_H (mult, *J* in Hz)	COSY	HMBC
1	160.4			12.04 (O*H*)
2	113.6			12.04 (O*H*), H-4, H-17
3	148.8			H-4, H-17
4	128.2	6.80 (s)	H-17	H-5, H-17
4a	139.7			H-5, H-6
5	59.8	3.49 (d, 11.2)	H-6	H-4, H-7*β*, H-8α, H-13, H-16
6	53.2	2.02 (m)	H-5, H-7α, H-7*β*, H-13	H-8α, H-14, H-15
7	31.6	1.05 (dddd, 13.8, 13.2, 13.2, 6.6) (α)	H-6, H-7*β*, H-8α, H-8*β*	H-5, H-6
		1.74 (dddd, 13.2, 7.0, 6.6, 0.8) (*β*)	H-6, H-7α, H-8α, H-8*β*	
8	34.8	2.30 (ddd, 13.8, 6.3, 0.8) (α)	H-7α, H-7*β*H-8*β*,	H-8*β*, H-10, H-16
		1.41 (ddd, 13.2, 13.2, 6.3) (*β*)	H-7α, H-7*β*, H-8α	
9	47.6			H-5, H-7*β*, H-8α, H-8*β*, H-10, H-16
10	81.6	5.08 (s)	H-8*β* (weak)	H-5, H-6, H-8α, H-16
11	191.2			H-10
12	187.4			H-4
12a	118.3			12.04 (O*H*), H-4, H-5
13	29.6	0.85 (m)	H-6, H-14, H15	H-5, H-7α
14	22.1	0.68 (d, 6.5)	H-13	H-6, H-15
15	22.3	0.27 (d, 6.5)	H-13	H-6, H-14
16	28.9	1.34 (s)		H-5, H-8*β*, H-10
17	24.0	2.49 (s)	H-4	H-4
18	170.0			H-10, H-19
19	20.4	2.16 (s)		
1-O*H*		12.04 (s)		

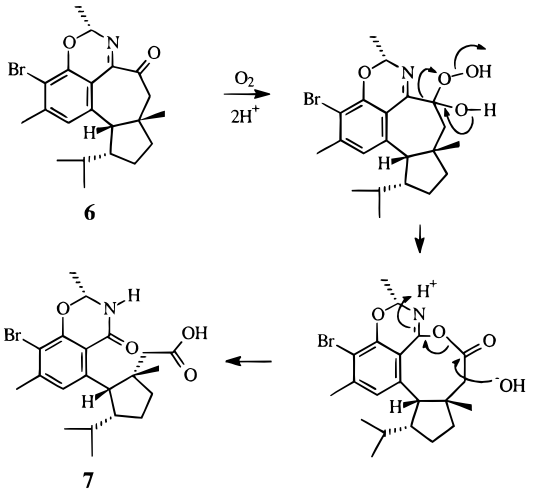
Table 4. ^{1}H, ^{13}C, COSY, and HMBC Data for **6** (in $CDCl_3$)

C	δ_C	δ_H (mult, *J* in Hz)	COSY	HMBC
1	155.3			H-4, H-17, H-18, H-19
2	110.5			H-4, H-17
3	144.3			H-4, H-17
4	129.1	6.82 (s)	H-5, H-17	H-5, H-17
4a	138.2			H-5, H-19
5	59.3	3.27 (d, 10.8)	H-4, H-6	H-4, H-7*β*, H-8α, H-10a, H-10b, H-13, H-16
6	53.9	1.92 (m, 13.1, 10.8, 6.5)	H-5, H-7α, H-7*β*, H-13	H-8α, H-13, H-14, H-15
7	31.9	1.15 (dddd, 13.1, 13.1, 13.1, 6.1) (α)	H-6, H-7*β*, H-8α, H-8*β*	
		1.72 (ddd, 13.1, 6.5, 6.5) (*β*)	H-6, H-7α, H-8*β*	
8	39.9	2.03 (dd, 13.1, 6.1) (α)	H-7α, H-8*β*	H10a, H10b, H-16,
		1.59 (dddd, 13.1, 13.1, 6.5, 0.6) (*β*)	H-7α, H7*β*, H-8α, H-10a	
9	46.9			H-5, H-7*β*, H-8α, H-8*β*, H-10a, H-10b, H-16
10	54.9	2.64 (dd, 11.6, 0.6) (a)	H-8*β*, H-10b	H-5, H-8α, H-8*β*, H-16
		2.47 (d, 11.6) (b)	H-10a	
11	196.5			H-10a, H-10b, H-16, H-18
12	160.8			H-4, H-10a, H-10b, H-18, H-19
12a	117.9			H-4, H-5, H-17
13	29.6	0.86 (m)	H-6, H-14, H-15	H-5, H-7α
14	22.1	0.68 (d, 6.5)	H-13	H-15
15	22.4	0.38 (d, 6.5)	H-13	H-6, H-14
16	32.4	1.28 (s)		H-5, H-10a, H-10b, H-8*β*
17	23.3	2.45 (s)	H-4	H-4
18	86.6	5.11 (q, 6.1)	H-19	
19	21.7	1.96 (d, 6.1)	H-18	

seven-membered ring. The C-11 carbonyl carbon signal at δ 191.2 only showed coupling to H-10. As for **1**−**4**, the relative stereochemistry of **5** was assigned from a consideration of the NOESY spectrum. In addition to the usual NOE correlations for H-5, H-6, and H-16, which permitted assignment of their relative configurations about the cyclopentane ring, the benzylic proton H-5 showed NOE correlations to the H-16 methyl protons (δ 1.34) and to the H-10 methine (δ 5.08), indicating that the acetate group was oriented α to the plane of the ring. Again, from skeletal similarities, the absolute stereochemistry of **5** is assigned to be the same as **1**−**4**.

Hamigeran D (**6**), was isolated as a pale yellow, amorphous solid, $[\alpha]_D$ −47.1°, and its molecular formula, $C_{21}H_{26}BrNO_2$, was established from HRDEIMS and ^{13}C NMR data. The IR spectrum contained bands at 1715 (ketone), 1589 (imine),[27] and 1184 cm^{-1} (ether), while the ^{1}H NMR spectrum (Table 4) showed signals similar to those of the cyclopentane moiety of **1**−**5**, that is, an alkyl methyl singlet at δ 1.28, a benzylic proton doublet at δ 3.27 (H-5, J = 10.8), and two isopropyl methyl doublets at δ 0.68 (J = 6.5) and 0.38 (J = 6.5). Immediately obvious was the absence of a phenolic proton signal at lowfield as in the spectra of **1**−**5**, and no signal for a proton attached to nitrogen was present. A new one-proton quartet at δ 5.11 and a methyl doublet at δ 1.96 suggested the possibility of an aromatic ether linkage at C-1. In the ^{13}C NMR spectrum (Table 4) there was only one ketone carbon signal (δ 196.5), but a carbon signal suggesting the presence of an imine (C-12) appeared at δ 160.8, and a methylene group signal at δ 54.9 replaced the C-10 quaternary carbon signals in **1**−**4** and the C-10 methine carbon signal in **5**. In the HMBC spectrum the imine carbon C-12 showed a weak ^4J coupling to the aromatic proton, thus allowing its placement adjacent to C-12a. The C-12 signal also showed coupling to the C-10 methylene protons as did C-5, C-8, and C-16, thereby permitting placement of the methylene carbon adjacent to the cyclopentane ring. The C-1 signal showed coupling to

Scheme 1. Proposed Mechanism of Decomposition of **6**



H-18 (δ 5.11) as did C-12, showing that C-18 was bound to both oxygen and nitrogen atoms, and the relatively deshielded nature of the chemical shift of C-18 (δ 86.6) supported this fact. Both the COSY and ^{1}H NMR spectra showed that H-18 was coupled to the H-19 methyl protons. The ketone carbon (C-11) showed ^4J coupling to H-18 as well as to H-16, and it also showed ^2J coupling to the methylene protons C-10, thereby placing it adjacent to the imine carbon and the methylene carbons. The C-19 methyl group was determined to be oriented α to the ring from the lowest energy configuration of both C-18 epimers, using the computer software PCMODEL, which utilizes the MMX forcefield. From this information, hamigeran D was assigned the structure **6**.

During an attempt to carry out a NOESY experiment on **6**, crystals of a different compound formed in an NMR tube when **6** was dissolved in a hexanes−$CDCl_3$ mixture. A ^{1}H NMR spectrum of this new compound, **7**, showed a set of signals that included an N−H signal at δ 9.23 and a doublet of a quartet (H-18) at δ 5.17. From this information the decomposition product was assigned the structure **7**, and this was confirmed from an X-ray[26] structure showing that **6** had decomposed into the amide **7**. The proposed mechanism of decomposition (Scheme 1) strongly supports the structure of **6**. The X-ray structure of **7** also provided the absolute stereochemistry of **6**, assuming there was no inversion at any of the stereocenters during decomposition.

The triethyl derivative (**9**) of hamigeran E (**8**), was isolated as a colorless oil, $[\alpha]_D$ + 32.3°, and its ^{1}H and ^{13}C NMR spectra were identical with those published[1] previously for compound **13**. However, in view of the structures assigned to **1**−**6** and the confirmation of that of **1** by X-ray crystallography, it was apparent that structure **13** was incorrect and required reexamination.

The molecular formula of **9**, $C_{24}H_{35}BrO_5$, was reconfirmed from HREIMS and ^{13}C NMR data, while a broad band at 1725 cm^{-1} in the IR spectrum previously assigned to a δ-lactone was reassigned to that of an ester, and this was supported by further ester bands at 1265 and 1211 cm^{-1}. The low-field region of the ^{13}C NMR spectrum (Table 5) contained two ester carbonyl signals at δ 175.4 and 167.3 (previously assigned to δ-lactone and oxygenated aryl carbons), five aromatic quaternary carbon signals (δ 153.0, 139.5, 137.8, 129.9, 117.7), and one aromatic methine carbon signal (δ 126.7). The ^{1}H NMR spectrum contained signals indicative of a cyclopentyl unit, that is, a doublet at δ 3.11 (H-5, J 7.1), a three-proton tertiary methyl group singlet at δ 1.40 (H-15), and two isopropyl methyl doublets at δ 0.74 (J = 6.5) and 0.83 (J = 6.5). In the HMBC spectrum, the ester carbonyl resonating at δ 175.4 (C-10) showed coupling to the ethoxy methylene protons at δ 3.79 and 3.59. In **13** this would be a ^5J coupling compared to a ^3J coupling in **9**. The aromatic methine carbon (C-4) showed coupling to H-5, again a ^5J coupling versus a ^3J coupling. C-5 showed coupling to H-15 (^4J vs ^3J), while C-7 showed coupling to H-5 (^4J vs ^3J), and C-8 showed coupling to H-15 (^4J vs ^3J). The alkyl methyl (H-15) showed ^3J coupling to H-5, H-8α, and H-8β, all of which correspond to ^4J couplings in **13**. The relative stereochemistry of **9** was determined by NOESY correlations. The signal for H-15 showed NOE correlations with H-5, H-7β (δ 2.06), and H-8β (δ 1.58), while H-6 also showed an NOE correlation with H-8β. The signal for H-7α (δ 1.78) showed NOE correlations with H-8α (δ 2.71) and H-12 (δ 1.29). From the evidence outlined above, structure **12** should be changed to that of **8**. The compound could arise by oxidative cleavage between C-10 and C-11 of hamigeran B (**3**).

The triethyl derivative (**11**) of debromohamigeran E (**10**) was isolated as a colorless oil, $[\alpha]_D$ +38.6°, and its molecular formula, $C_{24}H_{36}O_5$, was assigned from HRDEIMS and ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum was very similar to that of **9**, but it contained two aromatic methine signals (δ 121.9 and 110.9) rather than one. In the ^{1}H NMR spectrum the significant difference from that of **9** was the presence of an additional aromatic proton signal (δ 6.53). From this information, **11** was assigned as debromohamigeran E. COSY and HMBC spectra were consistent with this structure.

Evaluation of compounds **1**−**7**, and **9** for biological activity showed hamigeran D (**6**) to have the strongest in-vitro antitumor activity against P-388, with an IC_{50} of 8 μM. Hamigeran B (**3**) and 4-bromohamigeran B (**4**) both showed similar activities, with IC_{50}s of 13.5 and 13.9 μM, respectively, while hamigeran C (**5**) had an IC_{50} of 16.0 μM. Hamigeran A (**1**), triethylhamigeran E (**9**), and the amide **7** all had low cytotoxicities, with IC_{50}s of 31.6, 54.2, and 74.2 μM, respectively. None of the compounds tested showed any activity against the Gram-negative bacterium *Escherichia coli* or the yeast *Candida albicans*, and compounds **1** and **9** exhibited no antimicrobial activity at all. Against the Gram-positive bacterium *Bacillus subtilis*, however, compounds **5**−**7** all showed a 3-mm inhibition zone outside the disk at assay loadings of 96, 150, and 156 μg, respectively. Compounds **3** and **4** both showed slight inhibition, while **1** and **9** showed no inhibition of bacterial growth. Compounds **3**, **5**, and **6** showed slight activity against *Trichophyton mentagrophytes*, although the rest were inactive. The most pronounced biological activity was observed in the antiviral assays. Hamigeran B (**3**) showed 100% virus inhibition against both the Herpes and Polio viruses, with only slight cytotoxicity throughout the whole well at a concentration of 132 μg per disk. None of the other compounds showed any antiviral activity.

Experimental Section

General Experimental Procedures. MS were determined on a Varian VG 70-SE mass spectrometer. ^{1}H and ^{13}C NMR spectra at highfield were recorded on a DRX-400 MHz NMR spectrometer in $CDCl_3$. All 1D and 2D spectra (COSY, gHMQC, gHSQC, gHMBC, NOESY) were recorded on the DRX-400 spectrometer using UXNMR software. IR spectra were recorded on a Perkin−Elmer 1600 FT−IR spectrometer, and optical rotations were measured with a Perkin−Elmer 141 polarimeter on CH_2Cl_2 solutions. Si gel (type 60, Merck) was used for column chromatography, and aluminum-backed plates coated with Si gel F_{254} (Merck) were used for TLC. PLC plates

Table 5. ^{1}H, ^{13}C, COSY, and HMBC Data for **9** (in $CDCl_3$)

C	δ_C	δ_H (mult, *J* in Hz)	COSY	HMBC
1	153.0			H-4, H-16, 4.02 (OCH_2)
2	117.7			H-4, H-16
3	139.5			H-16
4	126.7	6.75 (s)	H-16	H-4, H-5, H-16
4a	137.8			H-5, H-6
5	54.5	3.11 (d, 7.1)	H-6	H-5, H-15
6	51.7	2.12 (m)	H-5, H-7α, H-7*β*, H-12	H-13, H-14
7	29.8	2.06 (m) (*β*)	H-6, H-7α, H-8α, H-8*β*	H-5
		1.78 (m) (α)	H-6, H-7*β*, H-8α, H-8*β*	
8	34.0	2.71 (m) (α)	H-7α, H-7*β*, H-8*β*	H-5, H-15
		1.58 (m) (*β*)	H-7α, H-7*β*, H-8α	
9	56.2			H-5, H-8α, H-15
10	175.4			H-8α, H-15, 3.59, 3.79 (OCH_2)
11	167.3			H-4, 4.39 (OCH_2)
11a	129.9			H-4, H-5
12	29.3	1.29 (m)	H-6, H-13, H-14	H-7α
13	22.2	0.83 (d, 6.5)	H-12	H-14
14	21.8	0.74 (d, 6.5)	H-12	H-13
15	28.0	1.40 (s)		H-5, H-8α, H-8*β*
16	23.8	2.39 (s)	H-4	H-4
C_1−O$CH_2$$CH_3$	70.4	4.03, 4.02 (dq)	1.36	
C_{10}−O$CH_2$$CH_3$	60.1	3.79, 3.59 (dq)	0.87	
C_{11}−O$CH_2$$CH_3$	61.2	4.39, 4.37 (dq)	1.39	
C_1−OC$H_2$$CH_3$	15.3	1.36 (t, 7.0)	4.03, 4.02 (CH_2)	
C_{10}−OC$H_2$$CH_3$	13.6	0.87 (t, 7.2)	3.79, 3.59 (CH_2)	
C_{11}−OC$H_2$$CH_3$	14.2	1.39 (t, 7.0)	4.39, 4.37 (CH_2)	

(1 mm) were prepared using Si gel 60 $PF_{254+366}$ on 25 × 25 cm glass plates. All solvents were distilled prior to use.

Biological Material. A single specimen of *H. tarangaensis* was collected at the Hen and Chicken Islands, New Zealand, from a depth of 30 m by hand using scuba in December 1997, and stored in ice for two weeks before being freeze-dried. A voucher specimen (P. O. R 103) has been deposited in the Museum of New Zealand, Wellington. Taxonomic identification was provided by one of us (P.R.B.).

Extraction and Isolation. The freeze-dried sponge (6.76 g) was extracted at room temperature with MeOH (2 × 100 mL). A portion (0.15 g) of the combined MeOH extracts (0.95 g) was separated by flash column chromatography on silica to yield a mixture of **8** and **10**. The remaining MeOH extract was subjected to a hexanes−CH_2Cl_2−EtOAc solvent partition sequence. The hexanes extract (0.54 g) was then separated by flash column chromatography on silica using a hexanes−EtOAc solvent gradient of increasing polarity to give 10 fractions. Compounds **1**−**6** were finally isolated by PLC using EtOAc−hexanes mixtures as the mobile phase.

Hamigeran A (1): 26 mg; 0.46%; mp 207−209 °C; $[\alpha]^{25}_D$ −22.5° (*c* 0.50, CH_2Cl_2); IR (film) 3454, 1742, 1640, 1608, 1232, 1173 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; DEIMS *m*/*z* 426 (55), 424 (55), 367 (90), 365 (100), 283 (45), 281 (48), 255 (30), 253 (33); HRDEIMS *m*/*z* 426.0871, 424.0890 (calcd for $C_{20}H_{25}BrO_5$, 426.0865, 424.0885).

Debromohamigeran A (2): 18 mg; 0.32%; mp 88.5−90 °C; $[\alpha]^{25}_D$ −38.5° (*c* 0.11, CH_2Cl_2); IR (film) 1742, 1639, 1596 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 10.97 (1H, s, O*H*), 6.66 (1H, s, H-4), 6.64 (1H, s, H-2), 4.40 (1H, s, O*H*), 3.60 (3H, s, OCH_3), 3.53 (1H, d, *J* = 5.7 Hz, H-5), 2.35 (3H, s, H-16), 2.02 (1H, m, H-6), 1.73 (1H, m, H-8α), 1.62 (1H, m, H-7*β*), 1.58 (1H, m, H-12), 1.55 (1H, m, H-8*β*), 1.37 (3H, s, H-15), 1.21 (3H, d, *J* = 6.5 Hz, H-13), 0.82 (3H, d, *J* = 6.5 Hz, H-14), 0.70 (1H, m, H-7α); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 197.7 (s, C-11), 169.2 (s, C-17), 161.2 (s, C-1), 149.1 (s, C-3), 144.1 (s, C-4a), 121.5 (d, C-4), 115.1 (d, C-2), 113.7 (s, C-11a), 83.7 (s, C-10), 53.4 (d, C-6), 52.9 (q, OCH_3), 50.2 (d, C-5), 47.2 (s, C-9), 34.1 (t, C-8), 27.3 (d, C-12), 26.9 (t, C-7), 24.4 (q, C-15), 23.4 (q, C-13), 22.7 (q, C-16), 22.0 (q, C-14); DEIMS *m*/*z* 346 (38), 287 (100), 203 (45), 175 (30); HRDEIMS *m*/*z* 346.1776 (calcd for $C_{20}H_{26}O_5$, 346.1780).

Hamigeran B (3): 36 mg; 0.63%; mp 163−165 °C; $[\alpha]^{25}_D$ −151.1° (*c* 0.15, CH_2Cl_2); IR (film) 1725, 1634, 1606, 1282 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; DEIMS *m*/*z* 366 (40), 364 (40), 338 (62), 336 (62), 295 (77), 293 (75), 256 (80), 254 (100); HRDEIMS *m*/*z* 366.0634, 364.0668 (calcd for $C_{18}H_{21}BrO_3$, 366.0654, 364.0674).

4-Bromohamigeran B (4): 3.7 mg; 0.07%; mp 144−148 °C; $[\alpha]^{25}_D$ −81.2° (*c* 0.37, CH_2Cl_2); IR (film) 1723, 1637 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 12.94 (1H, s, O*H*), 3.71 (1H, d, *J* = 10.2 Hz, H-5), 2.79 (3H, s, H-16), 2.76 (1H, m, H-6), 2.68 (1H, m, H-8α), 1.84 (1H, m, H-7α), 1.67 (1H, m, H-7*β*), 1.52 (1H, m, H-8*β*), 1.28 (3H, s, H-15), 1.21 (1H, m, H-12), 0.52 (3H, d, *J* = 6.6 Hz, H-13), 0.46 (3H, d, *J* = 6.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 198.0 (s, C-10), 185.0 (s, C-11), 160.1 (s, C-1), 149.8 (s, C-3), 142.1 (s, C-4a), 119.3 (s, C-4), 117.6 (s, C-11a), 113.3 (s, C-2), 57.8 (d, C-5), 57.7 (s, C-9), 47.4 (d, C-6), 33.7 (t, C-8), 28.5 (d, C-12), 26.6 (q, C-16), 25.3 (t, C-7), 23.5 (q, C-15), 22.9 (q, C-13), 19.0 (q, C-14); EIMS *m*/*z* 446 (33), 444 (66), 442 (33), 364 (24), 362 (48), 360 (26), 334 (95), 309 (87), 307 (87), 41 (100); HREIMS *m*/*z* 445.9732, 443.9745, 441.9771 (calcd for $C_{18}H_{20}Br_2O_3$, 445.9738, 443.9759, 441.9780).

Hamigeran C (5): 5 mg; 0.09%; mp 156−158 °C; $[\alpha]^{25}_D$ −136.0° (*c* 0.29, CH_2Cl_2); IR (film) 3421, 1749, 1737, 1626, 1230 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; DEIMS *m*/*z* 438 (4), 436 (4), 396 (23), 394 (23), 368 (38), 272 (17), 270 (17), 43 (100); HRDEIMS *m*/*z* 438.0868, 436.0879 (calcd for $C_{21}H_{25}BrO_5$, 438.0865, 436.0885).

Hamigeran D (6): 5 mg; 0.09%; $[\alpha]^{25}_D$ −47.1° (*c* 0.21 CH_2Cl_2); IR (film) 1715 (br), 1589, 1184 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; DEIMS *m*/*z* 405 (100), 403 (100), 390 (51), 388 (51), 362 (56), 360 (56); HRDEIMS *m*/*z* 405.1136, 403.1151 (calcd for $C_{21}H_{26}BrNO_2$, 405.1126, 403.1147).

Hamigeran D decomposition product:[7] 0.8 mg; mp 244.5−246 °C; $[\alpha]^{25}_D$ −1.98° (*c* 0.31, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 9.23 (1H, br s, N*H*), 6.65 (1H, s, H-4), 5.17 (1H, dq, *J* = 5.7, 1.7 Hz, H-18), 5.06 (1H, d, *J* = 7.2 Hz), 2.43 (3H, s, H-17), 1.69 (3H, d, *J* = 5.7 Hz, H-19), 1.25 (3H, s, H-16), 0.92 (3H, d, *J* = 6.4 Hz, H-14), 0.81 (3H, d, *J* = 6.4 Hz, H-15).

Ethylation of 8 and 10: A crude mixture containing **8** and **10** (0.15 g) was dissolved in dry acetone (50 mL) and heated under reflux overnight with EtI (1 mL) and anhydrous K_2CO_3 (0.6 g). Workup and purification by silica column chromatography (5% EtOAc−hexanes) gave the triethyl derivatives **9** and **11** as colorless oils.

Triethylhamigeran E (9): 12 mg; $[\alpha]^{25}_D$ +32.3° (*c* 1.00, CH_2Cl_2); IR (film)1725, 1265, 1211, 1094 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 5; EIMS *m*/*z* 484 (14), 482 (14), 438 (22), 436 (22), 395 (100), 393 (100), 365 (51), 363 (40); HREIMS *m*/*z* 484.1630, 482.1661 (calcd for $C_{24}H_{35}BrO_5$, 484.1647, 482.1668).

Triethyldebromohamigeran E (11): 1.4 mg; $[\alpha]^{25}_{D}$ +38.6° (*c* 0.05, CH_2Cl_2); IR (film) 1727, 1281, 1116 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.53 (1H, s, H-2), 6.49 (1H, s, H-4), 4.37 (1H, dq, $C_{11}-OCH_2CH_3$), 4.34 (1H, dq, $C_{11}-OCH_2CH_3$), 3.98 (1H, dq, $C_1-OCH_2CH_3$), 3.97 (1H, dq, $C_1-OCH_2CH_3$), 3.75 (1H, dq, $C_{10}-OCH_2CH_3$), 3.62 (1H, dq, $C_{10}-OCH_2CH_3$), 3.14 (1H, d, J = 7.2 Hz, H-5), 2.73 (1H, m, H-8α), 2.29 (3H, s, H-16), 2.10 (1H, m, H-6), 2.05 (1H, m, H-7β), 1.81 (1H, m, H-7α), 1.58 (1H, obscured, H-8β), 1.40 (3H, s, H-15), 1.36 (3H, t, $C_{11}-OCH_2CH_3$), 1.33 (3H, t, $C_1-OCH_2CH_3$), 1.32 (1H, m, H-12), 0.85 (3H, t, $C_{10}-OCH_2CH_3$), 0.81 (3H, d, J = 6.6 Hz, H-13), 0.77 (3H, d, J = 6.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.8 (s, C-10), 168.6 (s, C-11), 155.6 (s, C-1), 139.1 (s, C-4a), 138.9 (s, C-3), 123.6 (s, C-11a), 121.9 (d, C-4), 110.9 (d, C-2), 64.2 (t, $C_1-OCH_2CH_3$), 60.6 (t, $C_{11}-OCH_2CH_3$), 60.0 (t, $C_{10}-OCH_2CH_3$), 56.3 (s, C-9), 54.7 (d, C-5), 51.8 (d, C-6), 34.1 (t, C-8), 29.8 (t, C-7), 29.2 (d, C-12), 28.3 (q, C-15), 22.2 (q, C-16), 22.1 (q, C-13), 22.1 (q, C-14), 14.7 (q, $C_1-OCH_2CH_3$), 14.3 (q, $C_{11}-OCH_2CH_3$), 13.5 (q, $C_{10}-OCH_2CH_3$); DEIMS *m/z* 404 (11), 315 (100), 285 (33), 241 (22); HRDEIMS *m/z* 404.2549 (calcd for $C_{24}H_{36}O_5$, 404.2563).

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