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Malonganenones A–C, novel tetraprenylated alkaloids from the Mozambique gorgonian *Leptogorgia gilchristi*

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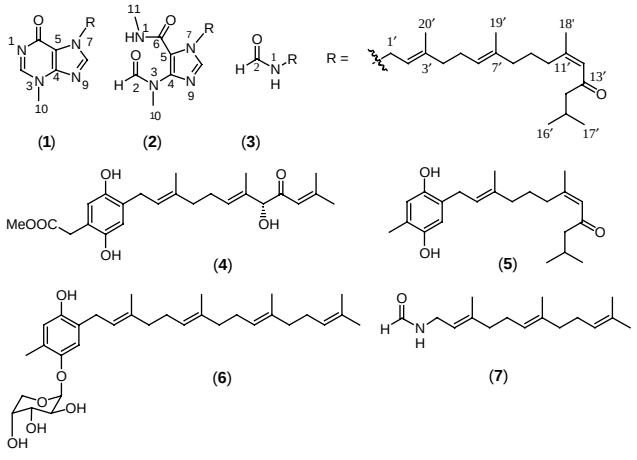
Abstract—Three novel tetraprenylated alkaloids, malonganenones A–C (**1**–**3**), were isolated from the gorgonian *Leptogorgia gilchristi* collected near Ponto Malongane, Mozambique. Compound **1** is the first 3,7-disubstituted hypoxanthine to be discovered from a marine source, while **2** and **3** represent the first formamides to be isolated from gorgonians. The unexpected facile exchange of the formamide proton of **2** with a deuteron originating from the NMR solvent used for spectroscopic analysis hampered initial attempts at the structural elucidation of this compound. The anti-oesophageal cancer activities of **1**–**3** are compared with that of rietone (**4**), a related triprenylated compound previously isolated from a South African soft coral.

1. Introduction

Gorgonians produce a plethora of novel natural products with diverse carbon skeletons and biological activities. There have, however, been very few examples of nitrogenous compounds reported from gorgonian species. Examples include nucleosides,[1] diterpene alkaloids,[2–4] sphingosines,[5] and a β-carboline carboxylic acid.[6] In continuation of our search for novel anti-oesophageal cancer agents from southern African marine invertebrates,[7] we have examined a moderately cytotoxic methanol extract of the gorgonian *Leptogorgia gilchristi* collected off a reef near Ponto Malongane, Mozambique. Exhaustive chromatography of an EtOAc partition fraction of the *L. gilchristi* extract yielded three novel cytotoxic tetraprenylated alkaloids, malonganenones A–C (**1**–**3**). Interestingly, polyprenylated metabolites are relatively common in southern African octocorals. We have previously reported the isolation of rietone (**4**) from the soft coral *Alcyonium fauri*[8] and a cohort of nine closely related triprenylated quinones and hydroquinones, for example, **5** from the arminacean nudibranch *Leminda millecra*, a conspicuous predator of various southern African octocorals including the gorgonian *Leptogorgia palma*.[9] A tetraprenylhydroquinone, nephthoside (**6**), has also been isolated from a southern African soft coral *Nephthea* sp.[10]



2. Results and discussion

Initial attempts to isolate **1**–**3** were unsuccessful. Although several fractions generated from silica flash-chromatography and normal-phase HPLC appeared to contain single compounds from ^{1}H NMR ($CDCl_3$), the corresponding

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1**, **2**, and **3**

Position	**1**[a]				**2**[b]				**3**[b]			
	δ_C	δ_H	mult. J (Hz), Int.	$^{1}J_{C,H}$[c]	δ_C	δ_H	mult. J (Hz), Int.	$^{1}J_{C,H}$[c]	δ_C	δ_H	mult. J (Hz), Int.	$^{1}J_{C,H}$[c]
1	—	—	—	—	—	8.00	br q (4.9), 1H	—	—	8.00	br s, 1H	—
2	149.9	8.25	d (2.3), 1H	—	162.2	8.18	s, 1H	199	160.6	7.97	s, 1H	189
4	149.1	—	—	—	140.1	—	—	—	—	—	—	—
5	115.9	—	—	—	117.0	—	—	—	—	—	—	—
6	164.5	—	—	—	159.9	—	—	—	—	—	—	—
8	143.3	8.08	br s, 1H	213	136.3	7.66	d (2.5), 1H	210	—	—	—	—
10	35.5	3.87	d (2.6), 3H	143	31.2	3.07	s, 3H	140	—	—	—	—
11	—	—	—	—	25.7	2.70	d (4.5), 3H	138	—	—	—	—
1′	45.5	5.07	t (7.2), 2H	142	43.8	4.72	dd (6.5, 3.4), 2H	141	35.0	3.67	dd (10.5, 6.3), 2H	137
2′	119.9	5.50	dddd (7.1, 6.0, 2.3, 1.1), 1H	158	119.3	5.25	t (6.8), 1H	158	120.8	5.12	t (6.3), 1H	155
3′	143.5	—	—	—	140.3	—	—	—	137.5	—	—	—
4′	40.4	2.16	mult., 2H	—	38.9	2.00	mult., 2H	—	38.9	2.02	mult., 2H	—
5′	27.0	2.13	mult., 2H	—	25.7	2.05	mult., 2H	—	25.9	2.04	mult., 2H	—
6′	125.1	5.08	t (7.1), 1H	150	127.6	5.05	t (5.9), 1H	—	120.8	5.08	t (6.2), 1H	151
7′	136.3	—	—	—	134.6	—	—	—	134.5	—	—	—
8′	40.8	1.95	t (7.3), 2H	—	39.1	1.95	mult., 2H	—	39.3	1.95	mult., 2H	—
9′	27.6	1.46	mult., 2H	—	25.8	1.47	mult., 2H	—	25.8	1.45	mult., 2H	—
10′	34.4	2.46	td (8.0, 6.2), 2H	—	32.6	2.42	dd (8.1, 7.6), 2H	—	32.6	2.43	t (7.8), 2H	—
11′	161.2	—	—	—	157.7	—	—	—	157.8	—	—	—
12′	125.0	6.12	br d (1.5), 1H	155	124.1	6.10	s, 1H	152	124.1	6.10	s, 1H	154
13′	202.8	—	—	—	199.6	—	—	—	199.7	—	—	—
14′	54.3	2.26	d (7.1), 2H	124	54.3	2.24	t (7.9), 2H	125	52.6	2.24	d (7.0), 2H	125
15′	26.7	2.06	mult., 1H	—	24.4	2.02	mult., 1H	—	24.5	2.04	mult., 1H	—
16′	22.9	0.90	dt (6.7, 2.1), 3H	125	22.3	0.85	dt (6.0, 2.1), 3H	125	22.3	0.84	d (6.6), 6H	125
17′	22.9	0.90	dt (6.7, 2.1), 3H	125	22.3	0.85	dt (6.0, 2.1)3H	125	22.3	0.84	d (6.6), 6H	125
18′	25.6	1.87	d (1.3), 3H	127	24.8	1.83	d (0.9), 3H	126	24.9	1.83	d (1.0), 3H	126
19′	16.0	1.57	s, 3H	125	15.6	1.55	s, 3H	125	15.6	1.56	s, 3H	125
20′	16.6	1.83	s, 3H	126	15.7	1.70	s, 3H	126	15.9	1.62	s, 3H	126

[a] CD_3OD.
[b] d_6-DMSO.
[c] Detected as one-bond correlations in HMBC experiment.

^{13}C spectra revealed a doubling of resonances, which we initially attributed to possible acid catalysed *E*/*Z* isomerization of an enone functionality (evident from the preliminary NMR data) either during silica chromatography or from NMR analysis performed in $CDCl_3$ solution. Acetylation of this mixture of perceived isomers, in an attempt to improve their chromatographic properties, did not resolve the apparent isomeric mixture. Compounds **1**–**3** were eventually successfully isolated using non silica-based chromatography (polymeric reversed-phase separation followed by normal-phase flash-chromatography and HPLC using diol solid supports) and by avoiding $CDCl_3$ as an NMR solvent.

Malonganenone A (**1**; 29 mg) was isolated as a colorless glass. The molecular formula of **1**, established as $C_{26}H_{38}N_4O_2$ from HRFABMS data ($[M+H]^+$, 439.3730, calcd 439.3730), implied ten degrees of unsaturation. Two carbonyl stretching bands (1681, 1643 cm^{-1}) were the only significant absorbances observed in the IR spectrum. All 38 proton resonances were observed in the ^1H NMR spectrum (CD_3OD) indicating that no exchangeable protons were present. Conversely, only 25 carbon resonances were observed in the ^{13}C NMR spectrum, with a pair of overlapping signals assigned to the terminal methyl groups of the side-chain. Of the 25 carbon resonances observed (Table 1), three could be attributed to a trisubstituted enone functionality (δ_C C=O, 202.8, C=C 161.2, 125.0), four to two trisubstituted olefins (δ_C 143.5, 119.9 and 136.3, 125.1), two to a tetrasubstituted olefin (δ_C 149.1, 115.9), and one each to an imine (δ_C 149.9), a purine carbonyl (δ_C 164.5) and a *N*-methyl (δ_C 35.5), and finally three to vinylic methyls (δ_C 25.6, 16.6, 16.0). These carbon resonances accounted for eight degrees of unsaturation, which therefore required **1** to contain a bicyclic moiety.

The structure of the tetraprenyl side-chain of **1** was readily deduced from standard analysis of COSY and HMBC data. The proton resonances of the terminal isopropyl moiety of the tetraprenyl side-chain were assigned from a COSY cross-peak between the overlapping methyl resonances (H_3-16′ and H_3-17′) and the methine proton (H-15′). A further COSY correlation was observed between H-15′ and the methylene protons H_2-14′ while HMBC correlations from the olefinic proton H-12′, H_2-14′ and H-15′ to the α,β unsaturated carbonyl C-13′ (δ_C 202.8) linked the terminal isopropyl to the trisubstitutued enone moiety. Contiguous methylene groups in the tetraprenyl side-chain were assigned from COSY correlations, which were then connected on the basis of two- and three-bond HMBC correlations from the three vinylic methyls (Fig. 1). The geometries of the three olefins in the tetraprenyl side-chain were established from analysis of both chemical shift and NOE data. The ^{13}C chemical shift of vinyl methyl C-18′ (δ_C 25.6) in **1** was in accordance with the chemical shift of the analogous olefinic methyl in **5** (δ_C 25.2)[9] and suggested that the $\Delta^{11'}$ olefin in **1** also possessed a *Z* geometry. The $\Delta^{11'}$ *Z* assignment was further supported by an NOE correlation between H_3-18′ and H-12′. The ^{13}C chemical shifts of the two other olefinic methyls C-19′ and C-20′ (δ_C 16.6 and 16.0) in the tetraprenyl side-chain were consistent with *E* geometries for the $\Delta^{2'}$ and $\Delta^{6'}$ olefins, respectively.[9]

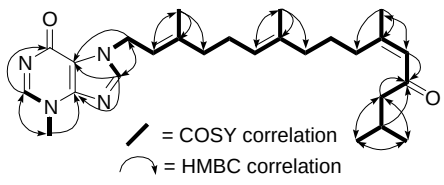


Figure 1. Key COSY and HMBC correlations used to establish the structure of **1**.

The evidence needed to position a bicyclic moiety at C-1′ in the side-chain of **1** was conclusively provided by a long range ^4J COSY correlation observed between the methylene protons H_2-1′ and the deshielded aromatic proton H-8 in addition to reciprocal HMBC correlations between these protons and their corresponding carbon resonances. HMBC data also unequivocally established the structure of the bicyclic ring system ($C_4H_2N_4O$) as a 3,7-disubstituted hypoxanthine (Fig. 1). Interestingly, there has only been one previous report of a dialkylated hypoxanthine marine natural product viz. 1,9-dimethylhypoxanthine isolated from the sponge *Spongosorites* collected off South Australia.[11] Conversely, hypoxanthine containing nucleosides (inosines) are more common, for example, trachycladine B from the Australian sponge *Trachycladus laevisiruifer*,[12] shimofuridins A–G from the tunicate *Aplidium multiplicatum*[13,14] and 3′-*O*-(α-D-glucosyl)inosine from the crustacean *Ligia exotica*.[15] Malonganenone A (**1**) is the first example of a 3,7-disubstituted hypoxanthine natural product from a marine source.

The molecular formula of malonganenone B (**2**; 14 mg), also isolated as a colorless glass, was established with some difficulty as $C_{27}H_{43}N_4O_3$ ($[M+H]^+$ 471.3335, calcd 471.3341) from HRESIMS data where a much larger peak at *m*/*z* 472.3398 dominated the HRESI mass spectrum. We eventually confirmed that this latter peak matched a formula of $C_{27}H_{42}DN_4O_3$, which suggested that one proton in **2** had been exchanged for a deuteron during the acquisition of the NMR data (CD_3OD) prior to mass spectral analysis. Comparison of the 1D and 2D NMR data of **2** (d_6-DMSO) with those of **1** (Table 1) confirmed that both compounds shared the same tetraprenyl side-chain structure, which accounted for four of the nine double bond equivalents required by the molecular formula, and limited the differences between these compounds to a cyclic moiety at C-1′.

HMBC data (Fig. 2) proved crucial in positioning a trisubstituted imidazole ring at C-1′ and provided evidence in support of the *N*-methylamide and *N*-methylformamide

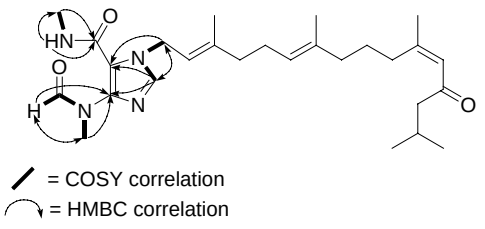


Figure 2. Key COSY and HMBC correlations used to establish the structure of the trisubstituted imidazole ring of **2**.

substituents on this ring. The sequence of HMBC correlations from H_2-1′ to C-8 and C-5 and from H-8 to both C-4 and C-5 together with the ^1H and ^{13}C chemical shifts of H-8 and C-4, C-5 and C-8 were reminiscent of those observed for the imidazole portion of the hypoxanthine ring system in **1** and suggested that both compounds shared an imidazole ring at C-1′.

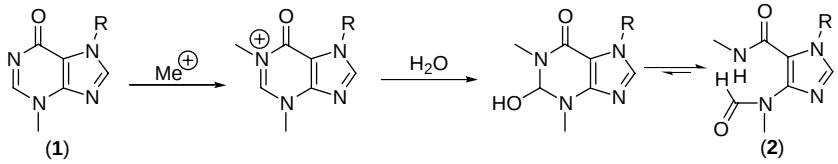
Establishing the structure of the *N*-methylformamide substituent on the imidazole ring proved to be challenging. HMBC correlations from the *N*-methyl protons (H_3-10) to C-4 quickly confirmed the attachment of an *N*-Me substituent at C-4 analogous to **1**. The *N*-methyl proton resonance (H_3-10), however, showed further HMBC correlations to two weak carbonyl resonances (δ_C 161.5 and 162.2) not observed in the ^{13}C NMR spectrum of **2** recorded at 100 MHz. A weak one-bond ^{13}C–^1H correlation was noted from the less shielded of the two resonances (δ_C 162.2) to proton H-2 in the HSQC spectrum. The integration of H-2 initially yielded a very small value when compared to integral values for other resonances observed in the ^1H spectrum of **2** (ca. 10% of H-8). Paradoxically, the H-2 resonance was sufficiently strong to detect a weak HMBC correlation from this proton to C-4 and C-10. A weak but significant $^1J_{C,H}$ coupling constant (199 Hz) for H-2/C-2 was measured in the HMBC spectrum which, although slightly larger, did compare favorably with that of DMF (191 Hz).[16] These NMR data were therefore deemed sufficient for the tentative incorporation of H-2 into a *N*,*N*-disubstituted formamide moiety and the presence of this functionality thus precluded the *N*-Me substituent at C-4 from being included in a second ring as in **1**.

The remaining substituent on the imidazole ring was established as an *N*-methylamide from a COSY correlation between the methyl doublet (H_3-11) and the broad exchangeable proton quartet (H-1) in addition to HMBC correlations from these protons to the amide carbonyl C-6 (δ_C 159.9). Although HMBC data could not place the *N*-methylamide substituent unequivocally at C-5, this was the only possible position for attachment of this substituent given the quaternary character of C-5 and the evidence supporting the positioning of the other two substituents at C-4 and N-7 on the imidazole ring.

The reduced proton integral for H-2, the HMBC evidence for two carbonyl ^{13}C resonances with similar chemical shifts and the presence of a deuterated analogue of **2** inferred from the HRESIMS data suggested that the formamide proton, H-2, had been exchanged for a deuteron while **2** was dissolved in the CD_3OD NMR solvent. This solvent had been used for all the NMR analyses during the isolation protocol and also for the initial structural elucidation studies. To confirm the suspected deuteron exchange with the formamide proton, the deuterated sample of **2** was lyophilized, re-dissolved in MeOH and allowed to stand for several days at room temperature, the MeOH removed under reduced pressure and the sample dried and dissolved in d_6-DMSO for further NMR analysis. As anticipated, the integral value of the H-2 resonance when the ^1H NMR spectrum of **2** was acquired in d_6-DMSO was found to be in accordance with other one proton signals in the ^1H NMR spectrum of this compound. The apparent splitting of the carbonyl resonance was attributed to the deuterium isotope effect in which the deuterated carbon is more shielded than the equivalent protonated carbon.[16,17] In addition, the absence of resonances in the ^{13}C NMR spectrum of **2** for these two forms of the carbonyl could have resulted from the small amount of the protonated formamide carbonyl carbon (δ_C 162.2) present in solution, and the reduction in signal intensity of the deuterated formamide carbonyl carbon (δ_C 161.5) through the combined effect of quadrupolar splitting of the ^{13}C resonance by the deuterium and also the generally longer T_1-relaxation time of ^{13}C–D.[16,17] Deuterium isotopic effects have been used previously in the structural elucidation of marine natural products although generally these have involved long range couplings over two or three-bonds.[18,19]

Although we were unable to establish the mechanistic details of the deuterium exchange of the acidic formamide proton in **2**, such exchange is not unprecedented in *N*,*N*-disubstitutued formamides and Simchen et al.[20,21] have reported this exchange to be particularly prevalent in CD_3OD solutions of the acetal derivatives of these compounds. The structural similarities between **1** and **2** suggest a possible biosynthetic link between these two compounds (Scheme 1).

Malonganenone C (**3**; 4 mg) was isolated as a yellow solid and the molecular formula readily deduced from HRESIMS as $C_{21}H_{36}NO_2$ ($[M+H]^+$ 334.2752, calcd 334.2746). The molecular formula of **3** differed from **1** by the loss of five carbons, three protons and three nitrogens, and thus precluded the presence of either the purine or imidazole rings characteristic of **1** and **2**, respectively. In particular, the molecular formula required only five double-bond equivalents as opposed to the ten required for **1** or nine for **2**. Careful analysis of the COSY and HMBC spectra of **3** again confirmed the presence of the same side-chain as that found in **1** and **2**, which left only one carbon, one oxygen, one nitrogen and two protons to be assigned. A one-bond coupling noted between the remaining carbonyl resonance (δ_C 160.6) and the deshielded proton resonance (δ_H 7.97, H-2) in the HSQC spectrum suggested that the only way to accommodate this aldehyde moiety was as part of a formamide functionality. The presence of the terminal formamide was supported by reciprocal HMBC correlations



Scheme 1. Putative biosynthesis of **2** from **1**.

Table 2. Anti-oesophageal cancer activity of **1**–**4**

Compound	IC_{50} (μM)						
	WHCO1	WHCO5	WHCO6	KYSE70	KYSE180	KYSE520	MCF12
1	17.0	31.6	29.1	35.9	21.7	17.8	20.7
2	25.1	>100.0	50.7	26.9	24.6	18.9	18.7
3	57.7	55.7	58.6	55.0	35.5	>100.0	>100.0
4	49.1	32.2	40.9	>100.0	50.6	84.9	7.3

from the formamide proton (H-2) to C-1′ of the side-chain and from H_2-1′ to the formamide carbonyl carbon (C-2). The chemical shifts of the formamide moiety were in complete agreement with those observed for other previously reported formamides, both natural products,[22–24] and also the synthetic product **7**, which possesses a triprenylated functionality analogous to that found in **1**–**3**.[25] Finally, a one-bond correlation was observed in the HMBC spectrum of **3**, which allowed the $^1J_{C,H}$ value of the formamide to be measured as 189 Hz (Table 1), comparing favorably with the literature value of 190 Hz.[17]

Oesophageal cancer is a prevalent form of cancer amongst the poor rural populations in southern Africa, and its prevalence is attributed to a combination of extraneous factors including alcohol use, the inadvertent ingestion of carcinogenic fungal toxins from contaminated grain, and the continuous exposure of many individuals to excessive wood and cigarette smoke.[26] With the age-standardized incidence rate of oesophageal cancer (16.22 per 100,000) in South Africa greater than those observed in many other parts of the world, we have initiated a program in South Africa to search for potential anti-oesophageal cancer agents from marine organisms.[7,27] Cisplatin and 5-fluoruracil are the common chemotherapeutic agents presently used to treat oesophageal cancer. However, even if either of these agents are administered following an early diagnosis of the disease, they can only effect remission in 20–30% of patients.[27] Given the moderate cytotoxicity of the original *L. gilchristi* extract, malonganenones A–C (**1**–**3**) were evaluated against several oesophageal cancer cells lines (WHCO1, WHCO5, WHCO6, KYSE70, KYSE180, KYSE520). MCF12 cells, derived from a benign breast tumour, were used as a control cell line, and activity was compared to the triprenyl hydroquinone rietone (**4**) (Table 2). All three malonganenones showed moderate cytotoxicity against almost all seven-cell lines with **1** the most and **3** the least active. Interestingly, while **1** exhibited activity against WHCO1 cells comparable with that of cisplatin (IC_{50}=15 μM), **3** appeared to be more selectively active than **1** or **2** against the oesophageal cancer cell lines compared to the control line (MCF12), which may indicate its potential as a chemotherapeutic agent with fewer side effects. Compound **4** showed varying activity against the oesophageal cancer cell lines, but high cytotoxicity against the control cell line. The anti-microbial activity of **1** was also evaluated against the human pathogenic bacteria *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative), and the fungus *Aspergillus niger* at concentrations of 20 and 100 μg/disk in zone inhibition assays (disk diameter=10 mm). No inhibition by **1** was noted against *E. coli* or *A. niger* and only mild activity (17 mm mean inhibition zone) against *S. aureus* was observed at 100 μg/disk.

3. Conclusion

The paucity of purine, rearranged purine and formamide secondary metabolites from marine gorgonians contributes to the significance of our discovery of malonganenones A–C (**1**–**3**) in extracts of *L. gilchristi*. The prenylated side-chains of **1**–**3** form a common link between these compounds and known prenylated hydroquinones and quinones isolated from other southern African octocoral species and a nudibranch *L. millecra* known to prey exclusively on both soft corals and gorgonians.[9] Interestingly, three of the nine prenylated metabolites previously obtained from *L. millecra* share the uncommon *Z* configuration of the α,β unsaturated ketone functionality also found in **1**–**3**.[9] The incorporation of deuterium into **2** from the CD_3OD used as an NMR solvent confounded initial attempts at the structural elucidation of this compound and serves as a caution to those involved in natural product structural elucidation that the unusual functional groups often found in natural products can have unexpected interactions with solvents commonly used for NMR analysis. The moderate cytotoxicity of **1**–**3** towards oesophageal cancer cell lines was established.

4. Experimental

4.1. General

IR spectra were collected using a Perkin-Elmer Spectrum 2000 FT spectrometer with compounds as films (neat) on NaCl plates. UV spectra were recorded using a Varian Cary 500 UV–vis–NIR spectrophotometer. NMR spectra were measured on a Brüker AVANCE 400 MHz spectrometer using standard pulse sequences. Chemical shifts are reported in ppm and are referenced to residual solvent resonances (CD_2HOD δ_H 3.31, δ_C 49.00 or d_5-DMSO δ_H 2.50, δ_C 39.52).[28] HRFABMS data were obtained on a JEOL SX102 FABMS at the Potchefstroom campus of the North West University or using a Waters API Q-TOF Ultima ESI mass spectrometer at Stellenbosch University. Diaion HP-20 polystyrene divinylbenzene beads (supplied by Supelco) and Macherey-Nagel Chroma-Bond OH Diol (0.45 μm) were used for initial chromatographic separations. High-performance liquid chromatography was performed using a Macherey-Nagel Nuecleosil 100-7-OH Diol semi-preparative column (10 mm i.d., 250 mm) on an HP Agilent 1100 series gradient HPLC system equipped with diode array detection.

4.2. Collection and extraction of *L. gilchristi* and isolation of 1–3

Specimens of *L. gilchristi* (Hickson 1904) [Phylum: Cnidaria, Class: Anthozoa, Order: Alcyonacea, Family: Gorgoniidae] were collected by SCUBA at a depth of 20 m from a reef near Ponto Malongane, Mozambique in the autumn of 1995 and frozen immediately after collection. Freeze dried specimens of *L. gilchristi* (148 g) were exhaustively extracted with MeOH, and the MeOH extract concentrated in vacuo and partitioned between H_2O and EtOAc. The two partition fractions were evaporated to dryness under reduced pressure to yield an aqueous fraction (4.29 g) and an organic fraction (3.08 g), respectively. A portion (915 mg) of the organic fraction was re-dissolved in MeOH (50 mL), diluted with H_2O (5 mL) and partitioned with hexane (2×25 mL) to remove fats. Both partition fractions were evaporated to give a MeOH (655 mg) and a hexane (271 mg) fraction. The MeOH fraction was loaded onto an HP-20 column (1.25×10 cm, 50 mL) and eluted with aliquots (150 mL) of increasing concentration of Me_2CO in H_2O (0, 25, 50, 70, 90 and 100% Me_2CO). The 70% $Me_2CO_{(aq)}$ eluent was concentrated in vaccuo (415 mg), re-dissolved in MeOH (ca. 5 mL) and evaporated onto a small portion of diol stationary phase (1 mL). The diol onto which the organic material had been absorbed was transferred as a hexane slurry onto a flash column of diol (1×11 cm, 9 mL), which was eluted with aliquots (100 mL) of (i) hexane, (ii) EtOAc/50% hexane, (iii) EtOAc, (iv) 50% MeOH/50% EtOAc, (v) MeOH and (vi) 50% H_2O/50% MeOH. Elution (iii) (145 mg) from the diol flash column was further chromatographed using diol HPLC under isocratic conditions (2.5% MeOH/97.5% EtOAc) to yield malonganenone A (**1**) (29 mg). Elution (ii) (80 mg) was further chromatographed using diol HPLC with gradient elution from 30% EtOAc/70% hexane to EtOAc to yield malonganenone C (**3**) (4 mg). Finally, one of the HPLC fractions collected from the gradient elution was re-chromatographed on diol HPLC under isocratic conditions (75% EtOAc/25% hexane) to give malonganenone B (**2**) (14 mg).

4.3. Malonganenone A (1)

Colorless glass; UV (MeOH) λ_{max} (ε) 221 (30,900), 252 (28,800); ν_{max} (liquid film) 2956, 2870, 1681, 1643, 1383, 1203, 1012 cm^{-1}; δ_H and δ_C, see Table 1; HRMS (FAB): MH^+, found 439.3073. $C_{26}H_{38}N_4O_2$ requires 439.3073.

4.3.1. Malonganenone B (2). Colorless glass; UV (MeOH) λ_{max} (ε) 238 (14,400); ν_{max} (liquid film) 2956, 2858, 1653, 1642, 1556, 1377, 1212, 1138 cm^{-1}; δ_H and δ_C (d_6-DMSO), see Table 1; δ_H (400 MHz, CD_3OD) δ 8.23 (1H, s, H-2), 7.68 (1H, d, J=2.1 Hz, H-8), 6.14 (1H, dd, J=2.6, 1.4 Hz, H-12′), 5.32 (1H, m, H-2′), 5.12 (1H, dddd, J=6.8, 6.4, 2.5, 1.3 Hz, H-6′), 4.82 (2H, dd, J=7.2, 3.4 Hz, H-1′), 3.20 (3H, s, H-10), 2.85 (3H, s, H-11), 2.50 (2H, dd, J=8.0, 6.2 Hz, H-10′), 2.28 (2H, td, J=9.5, 2.5 Hz, H-14′), 2.15 (2H, m, H-5′), 2.11 (2H, m, H-4′), 2.08 (1H, m, H-15′), 2.04 (2H, m, H-8′), 1.89 (3H, d, J=1.3 Hz, H-18′), 1.77 (3H, s, H-20′), 1.61 (3H, s, H-19′), 1.54 (2H, dd, J=15.4, 8.1 Hz, H-9′), 0.91 (6H, dt, J=6.7, 2.0 Hz, H-16′, H-17′); δ_C (100 MHz, CD_3OD) δ 203.0 (C, C-13′), 163.8 (CH, C-2), 162.4 (C, C-6), 161.3 (s, C-11′), 143.3 (s, C-3′), 141.6 (s, C-4), 138.2 (d, C-8), 136.4 (s, C-7′), 125.1 (d, C-6′), 125.0 (d, C-12′), 119.8 (d, C-2′), 119.5 (s, C-5), 54.3 (t, C-14′), 45.8 (t, C-1′), 40.9 (t, C-8′), 40.5 (t, C-4′), 34.5 (t, C-10′), 32.4 (q, C-10), 27.6 (t, C-9′), 27.3 (t, C-5′), 26.5 (q, C-11), 26.4 (d, C-15′), 25.6 (q, C-18′), 22.9 (q, C-16′), 22.9 (q, C-17′), 16.5 (q, C-20′), 16.0 (q, C-19′); HRMS (ESI): MH^+, found 471.3341. $C_{27}H_{43}N_4O_3$ requires 471.3335, MH^+, found 472.3397. $C_{27}H_{42}DN_4O_3$ requires 472.3398.

4.3.2. Malonganenone C (3). Yellow solid; UV (MeOH) λ_{max} (ε) 230 (8800), 276 (1400); ν_{max} (liquid film) 3321, 2958, 2023, 2863, 1727, 1682, 1616, 1384, 1275, 1126, 1074 cm^{-1}; δ_H and δ_C (d_6-DMSO) see Table 1; δ_H (400 MHz, CD_3OD) δ 8.01 (1H, d, J=2.8 Hz, H-2), 6.15 (1H, s, H-12′), 5.21 (1H, tdt, J=6.9, 2.5, 1.2 Hz, H-2′), 5.13 (1H, tt, J=6.8, 1.2 Hz, H-6′), 3.81 (2H, d, J=6.6 Hz, H-1′), 2.28 (2H, d, J=7.1 Hz, H-14′), 2.51 (2H, td, J=7.8, 6.3 Hz, H-10′), 2.13 (2H, m, H-5′), 2.12 (2H, m, H-4′), 2.07 (1H, m, H-15′), 2.04 (2H, m, H-8′), 1.89 (3H, d, J=1.3 Hz, H-18′), 1.70 (3H, s, H-20′), 1.62 (3H, s, H-19′), 1.54 (2H, m, H-9′), 0.92 (6H, d, J=6.7 Hz, H-16′, H-17′); δ_C (100 MHz, CD_3OD) δ 203.1 (s, C-13′), 163.4 (s, C-2), 161.5 (s, C-11′), 140.7 (s, C-3′), 136.1 (s, C-7′), 125.4 (d, C-6′), 125.0, (d, C-12′), 121.0 (d, C-2′), 54.4 (t, C-14′), 40.9 (t, C-8′), 40.5 (t, C-4′), 36.8 (t, C-1′), 34.5 (t, C-10′), 27.7 (t, C-9′), 27.3 (t, C-5′), 26.5 (d, C-15′), 25.8 (q, C-18′), 22.9 (q, C-16′), 22.9 (q, C-17′), 16.2 (q, C-20′), 15.9, (q, C-19′); HRMS (ESI): MH^+, found 334.2752. $C_{21}H_{36}NO_2$ requires 334.2746.

4.4. Bioassays

Anti-oesophageal cancer bioassays were carried out as described previously.[7]

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