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Two Cytotoxic Stereoisomers of Malyngamide C, 8-*Epi*-Malyngamide C and 8-*O*-Acetyl-8-*epi*-Malyngamide C, from the Marine Cyanobacterium *Lyngbya majuscula*

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Abstract

Two new epimers of malyngamide C, 8-*O*-acetyl-8-*epi*-malyngamide C (**1**) and 8-*epi*-malyngamide C (**3**) have been isolated along with known compounds 6-*O*-acetylmalyngamide F (**5**), H (**6**), J (**7**) K (**8**), and characterized from a Grenada field collection of the marine cyanobacterium *Lyngbya majuscula*. The planar structures of these compounds were deduced by 1D- and 2D-NMR and mass spectral data interpretation. The absolute configurations were determined by a combination of CD-spectroscopy, chemical degradation and the variable temperature Mosher's method. Compounds **1**–**5**, **7** and **8** displayed moderate cytotoxicity to NCI-H460 human lung tumor and neuro-2a cancer cell lines, with IC_{50} values ranging between 0.5 and 20 μg/mL.

Keywords

Lyngbya majuscula; Malyngamide; variable temperature Mosher ester method

1. Introduction

Malyngamides are a class of secondary metabolites frequently encountered during chemical investigations of filamentous cyanobacteria, particularly *Lyngbya majuscula* or sea hares of the genus *Bursatella* and *Stylocheilus* (Gerwick et al., 2001; Tan, 2007). In the latter case, however, it has been shown that molluscs incorporate these compounds as a consequence of their diet of cyanobacteria (Pennings and Paul, 1993). Thus, malyngamides are considered metabolites of cyanobacterial origin. To date, more than 30 members belonging to this compound class have been isolated. Structurally, malyngamides consist of a methoxylated fatty acid tail, known as 'lyngbic acid', and a presumed amino acid derived head which are joined

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through an amide linkage. Whereas for the lipid part of malyngamides only five variations are known, namely lyngbic acid and its 12-, 16- and 20-carbon analog (Gerwick et al., 2001) and one 7*R*-epimer (Suntornchashwej et al., 2007), the amine portion of the molecule shows a remarkable structural diversity in carbon skeletons and bears a multitude of functional groups. These include unsaturations, covalently-attached chlorine atoms, and various oxygen-containing functional groups such as ketones, alcohols, epoxides and lactones. In addition to the variations in planar structure, differences in the absolute configuration have also been observed (Kan et al., 2000; Milligan et al., 2000; Suntornchashwej et al., 2007).

In the present study, a collection of *L. majuscula* from Grenada was chemically analyzed in order to identify the compounds responsible for the cytotoxicity observed in the crude extract. Subsequent bioassay-guided fractionation led to the isolation of two new stereoisomers of malyngamide C (**1**, **3**), along with a suite of malyngamides previously reported, including 6-*O*-acetylmalyngamide F (**4**) and malyngamides H (**5**), J (**6**), and K (**7**). This paper describes the isolation, structure elucidation, and biological activity of the two new malyngamides **1** and **3**.

2. Results and discussion

Compound **1** showed an $[M+H]^+$ peak at *m/z* 498.2607, consistent with the molecular formula $C_{26}H_{41}ClNO_6$ by HRESITOFMS. The ^1H NMR data exhibited several resonances typical of a malyngamide-type metabolite. For example, a methoxy singlet signal (δ 3.31) and its corresponding α-methoxy methine multiplet (δ 3.14), two olefinic signals (δ 5.46), three methylenes between δ 2.17 and 2.31, a methylene envelope (δ 1.26 – 1.42) and a terminal methyl (δ 0.87) were indicative of the amide of a lyngbic acid moiety in **1**. In addition, the ^1H and ^{13}C NMR spectra contained resonances consistent with the presence of an exomethylene functionality possessing a vinyl chloride (δ_H 6.39/δ_C 122.7) and an exchangeable amide signal (δ 6.02), revealing further typical structural features of this compound class. Based on its mass and overall NMR features, isolate **1** was initially dereplicated as malyngamide C acetate (Ainslie et al., 1985; Wright and Coll, 1990) using the MarinLit database Blunt and Munro, 2005). However, when the spectral data were carefully compared with those reported in the literature, deviations in the specific rotation in sign and amount as well as in the ^{13}C NMR spectra between **1** and malyngamide C acetate became apparent (Table 1). Analysis of the 2D-NMR data confirmed that **1** had the same planar structure as malyngamide C acetate, and hence, the two molecules must differ in configuration of either the stereogenic centers C-4, C-8, C-9 and C-7′ and/or the geometry of the Δ^2 and $\Delta^{4'}$ double bonds.

Base hydrolysis of **1** yielded the acid portion, which was identical in all respects to that previously reported for 7(*S*)-methoxytetradec-4-(*E*)-enoic acid (**2**), thus establishing the 7′*S*-configuration for **1**. The *Z*-configuration of the Δ^2-double bond was evident from the observation of ROE correlations from H-1_α and H-3 and NOE correlations from NH to H-3. The geometry of the $\Delta^{4'}$-double bond was inferred from ^1H decoupling experiments. The olefinic proton signals of C-4′ and C-5′ were overlapped in $CDCl_3$ (Table 1), but resolved to a distinct pair of multiplets in C_6D_6. Decoupling of the adjacent methylene protons H_2-3′ and H_2-6′ allowed the measurement of the $^3J_{H4'\text{-}H5'}$ as 15.4 Hz, confirming an *E*-geometry for $\Delta^{4'}$ in **1**.

These results focused our efforts on elucidation of the configuration of the cyclohexyl amino moiety of **1**. Moreover, this was the part of the molecule where the majority of ^{13}C NMR chemical shift differences were observed between **1** and malyngamide C acetate (Table 1). Because the empirical reversed octant rule can be applied to determine the absolute configuration of an epoxy-ketone ring system (Djerassi et al., 1965;Kan et al., 1998), the configuration of carbons C-4 and C-9 was clarified on the basis of a circular dichroism (CD)

spectrum. The CD spectrum of **1** showed a negative Cotton effect by the C-5 carbonyl group at 308 nm, indicating that the absolute configuration of the epoxide was 4*S* and 9*S*, as seen in malyngamide C acetate. This result was also supported by comparison of the CD spectra of **1** with an authentic sample of malyngamide C acetate, obtained from a *Lyngbya* species, collected in Madagascar (See Supplementary Data).

In order to investigate the absolute configuration at C-8, **1** was deacetylated and the hydrolysis product was subsequently analyzed by single derivatization with methoxyphenylacetic acid (MPA) followed by variable temperature ^{1}H NMR spectroscopy analysis (Latypov et al., 1998). Complete deacetylation of **1** without ring opening of the epoxide function was accomplished by use of porcine liver esterase. Calculation of the shift differences $\Delta\delta^{T1,\,T2}$ gave negative values for H_2-6 and H_2-7, and a positive value for H-9 (Figure 2), hence revealing the 8-*R* absolute configuration (Seco et al., 2001). Compound **1** was thus proven to be the C-8 epimer of the known compound malyngamide C acetate, and for which we propose the trivial name 8-*O*-acetyl-8-*epi*-malyngamide C.

Compound **3** was analyzed for $C_{24}H_{38}ClNO_5$ by HRMS. Inspection of the ^{1}H and ^{13}C NMR data of **3** showed these data to be extremely similar to those of 8-*O*-acetyl-8-*epi*-malyngamide C (**1**), the major differences being the missing proton and carbon NMR resonances accounting for the acetyl function (Table 2). Indeed, compound **3** was identical in all respects to the hydrolysis product of 8-*O*-acetyl-8-*epi*-malyngamide C (**1**). Thus, compound **3** was shown to be the C-8 epimer of malyngamide C (**4**) and given the name 8-*epi*-malyngamide C. Late in the preparation of this manuscript, we became aware that Kwan et al. (2010) also recently isolated and defined the structure of compound **3**. In this latter work, the absolute configuration was determined using a selective Mitsunobu inversion of C-8. The reported structural data for **3** are in good agreement with our findings and hence, corroborate the assignment of the absolute configuration. The identities of the known compounds **4**–**7** were readily established by direct comparison of HR-MS, ^{1}H NMR and ^{13}C NMR data with published data (Gerwick et al., 1987;Orjala et al., 1995;Wu et al., 1997).

All malyngamide compounds obtained during this study were tested for cytotoxicity and for modulatory activity in the mammalian Voltage-Gated Sodium Channel. Cytotoxicity (VGSC) was evaluated by testing the metabolites towards NCI-460 human lung tumor, neuro-2a mouse neuroblastoma and HCT-116 cells and in the disk-diffusion assay (Table 3). All compounds except malyngamide H (**5**) exhibited moderate activity with IC_{50} values ranging from 0.5 to 20 μg/mL. Of these, only metabolite **3** showed selectivity toward solid-tumor cell lines. 8-*O*-acetyl-8-*epi*-malyngamide C (**1**) and its free alcohol (**3**) were proven to be less active than their respective epimers malyngamide C acetate and malyngamide C, indicating the importance of the absolute configuration at this position for the interaction of malyngamides with their corresponding target structure. In the sodium channel assay (Table 4) compounds **1** and **5** were shown to have moderate blocking activity and malyngamide C and C acetate moderate activating properties.

3. Conclusions

In conclusion, the present work reports on two new natural products (**1**, **3**) in the malyngamide structure class which are C-8 epimers of the reported compounds malyngamide C and malyngamide C acetate. Biosynthetically, the NH, C-1 and C-2 carbons of these malyngamide C derivatives are likely incorporated from glycine, C-3 from an HMGCoA synthase-like reaction as determined for jamaicamide A (Edwards et al., 2004), and this is followed by three rounds of acetate extension. This is interesting in that the C-8 carbon is predicted to derive from C-2 of acetate, and thus, the C-8 hydroxyl functionality is likely introduced as a post-assembly P450 oxidation. That both stereoisomers at C-8 in the malyngamide C structure class

are produced in different populations is intriguing, and appears to be differentiated by the ocean of origin. Literature reports of the 8-*S* isomer (= malyngamide C) all derive from Pacific collections [Fanning Island by Ainslie et al. (1985) and Palmyra Atoll by Taniguchi et al. (2010)] whereas the 8-*R* isomer has now been reported from two Caribbean collections [Grenada in this work and Florida in the recent publication by Kwan et al. (2010)]. Thus, cytochrome P450 enzymes with opposite chiral preferences may have evolved in these different populations of *L. majuscula*. It is additionally intriguing that this modest structural change in the malyngamide C class (e.g. C-8 epimerization) results in an approximately 5-fold reduction in biological activity, and therefore identifies this as an important stereogenic site to be considered in any future medicinal chemistry efforts.

4. Experimental

4.1. General experimental procedures

Optical rotation measurements were recorded on a Jasco P-1010 polarimeter. CD spectra were measured using a model J-720 Jasco spectropolarimeter. UV and FT-IR spectra were obtained employing Hewlett Packard 8452A and Nicolet 510 instruments, respectively. All NMR spectra were recorded on Bruker Avance DRX300, DPX400 and DRX600 spectrometers. Spectra were referenced to residual solvent signal with resonances at $\delta_{H/C}$ 7.26/77.1 ($CDCl_3$) and δ_H 7.15/128.0 (C_6D_6). Low-resolution ESI-MS spectra were obtained on a Thermo Finnigan LCQ Advantage mass spectrometer. High-resolution ESI-TOF and CI mass spectra were recorded on Waters Micromass LCT Classic and JEOL MSRoute mass spectrometers, respectively. HPLC was carried out using a Waters system consisting of a Rheodyne 7725i injector, two 515 pumps, a pump control module and a 996 photodiode array detector. TLC grade (10–40 μm) Si gel was used for vacuum chromatography. All solvents were purchased as HPLC grade.

4.2. Collection

The marine cyanobacterium *Lyngbya majuscula* (voucher specimen available from WHG as collection no. GBI-26Jul95-07) was collected from shallow waters (1–3 m) in True Blue Bay, Grenada, on July 26, 1995 and stored in 2-propanol at −20 °C until workup. Taxonomy was assigned by microscopic comparison with the description given by Desikachary.[7]

4.3. Extraction and isolation

A total of 19.1 g (dry wt.) of the cyanobacterium *L. majuscula* was extracted five times with CH_2Cl_2-MeOH (2:1, v/v) to produce 662 mg of cytotoxic crude organic extract (solid tumor selective at 15 μg/disk: $_{U251}\Delta_{CEM}$ = 400 units). The extract was fractionated by vacuum liquid chromatography (VLC) over silica gel using a stepwise gradient of hexanes-EtOAc and EtOAc-MeOH to give nine fractions. Fractions 5, 6 and 7, eluted with 60% and 80% EtOAc in hexanes and 100% EtOAc, respectively, showed cytotoxic effects (solid tumor selective at 15 μg/disk: $_{C38}\Delta_{L1210}$ = 300 units) and were further investigated. Fraction 5 was further separated by RP-HPLC (column: YMC ODS AQ-323, 250 × 10.0 mm, 5 μm; MeOH-H_2O (85:15), 2 mL/min) giving two distinct fractions. A second chromatography of the first fraction by RP-HPLC on a Phenomenex Synergi Fusion-RP 80 column (250 × 10.0 mm, 4 μm; MeOH-H_2O (79:21), 2 mL/min) yielded 24.3 mg of 8-*O*-acetyl-8-*epi*-malyngamide C (**1**). Further separation of VLC fraction 6 by RP-HPLC (column: YMC ODS AQ-323, 250 × 10.0 mm, 5 μm; MeOH-H_2O (85:15), 2 mL/min) resulted in additional quantities of compound **1** (9.4 mg) and an inseparable mixture of malyngamide H (**6**) and K (**8**) (1.9 mg). Reversed-phase HPLC (column: YMC ODS AQ-323, 250 × 10.0 mm, 5 μm; MeOH-H_2O (85:15), 2 mL/min) of VLC fraction 7 yielded compounds **3** (0.9 mg), 6-*O*-acetyl malyngamide F (**5**) (1.7 mg) and additional quantities of the inseparable mixture of malyngamide H (**6**) and K (**8**) (1.0 mg). ^{1}H NMR profiling of all VLC fractions indicated fraction 8 (eluted with 25% MeOH in EtOAc)

also contained a malyngamide type metabolite. Therefore, VLC fraction 8 was first subjected to C_{18} HPLC (YMC ODS AQ-323, 250 × 10.0 mm, 5 μm; MeOH-H_2O (90:10), 2 mL/min), and those materials eluting at 12 to 14 min were then further purified with RP-HPLC (Phenomenex Synergi Fusion-RP 80, 250 × 10.0 mm, 4 μm; MeOH-H_2O (80:20), 2 mL/min) to give malyngamide J (**7**).

4.4. Basic hydrolysis of 8-O-acetyl-8-epi-malyngamide C (1)

Compound **1** (6.9 mg) was dissolved in 2 mL solution of 10% KOH in EtOH-H_2O (4:1) and the stirred mixture refluxed for 15 h. The hydrolysate was concentrated *in vacuo* and partitioned between H_2O and CH_2Cl_2. The H_2O layer was isolated, acidified, and extracted with CH_2Cl_2 to yield 1.6 mg (45.1%) of lyngbic acid (**2**).

4.5. Enzymatic hydrolysis of 8-O-acetyl-8-epi-malyngamide C (1)

Compound **1** (4 mg, 8.0 μmol) was dissolved in 0.2 mL MeOH and the resulting solution was added to 6 mL of 0.3 M K_2HPO_4 buffer (pH 8.0) containing 100 units porcine liver esterase (Sigma-Aldrich). The solution was stirred for 12 h at room temperature and then repeatedly extracted with ether (3 × 10 mL). The extracts were dried over $MgSO_4$ and the solvent removed *in vacuo* to yield 8-*epi*-malyngamide C (**3**, 3.6 mg, 98.3 % yield).

4.6. Preparation of the (R)-MPA-Cl

Oxalyl chloride (51.9 μL, 0.6 mmol) was added to a mixture of *R*-MPA (10 mg, 0.06 mmol) and DMF (0.47 μL, 0.006 mmol) in hexanes at room temperature. After 48 h, the solvent was evaporated to dryness at reduced pressure to afford 0.06 mmol of *R*-MPA-Cl (11.1 mg, 100%).

4.7. Preparation of the R-MPA ester derivative of 8-epi-malyngamide C (3)

To a solution of 3.6 mg of 8-*epi*-malyngamide C (**3**) in 800 μL CH_2Cl_2 were added 3 mg of DMAP and 7.3 mg of *R*-MPA-Cl. The reaction mixture was reacted for 24 h and then partitioned between EtOAc and 0.1 M $NaHCO_3$, and the EtOAc layers then washed with 0.1 M HCl. The EtOAc layer was evaporated and then separated by RP-HPLC (column: Phenomenex Synergi Fusion-RP 80, 250 × 10.0 mm, 4 μm; MeOH-H_2O (80:20), 2 mL/min) to yield the *R*-MPA ester of **3** (1.8 mg, 37.7 % yield).

4.8. Variable temperature NMR experiments

^1H NMR and ^1H-^1H-COSY spectra (300 MHz) of the R-MPA ester of **3** dissolved in CS_2/CD_2Cl_2 (4:1) were recorded at T1 = 25° (298 K), T1a = −50° (223 K) and T2 = −70° (203 K). Chemical shifts (ppm) were internally referenced to the TMS signal (0 ppm). For low-temperature NMR spectroscopy, the probe temperature was cooled by a constant stream of liquid nitrogen, controlled by a standard unit (See Supplemental data) calibrated with a methanol reference. The sample was allowed to equilibrate for 15 min at each temperature before recording the spectra. $\Delta\delta^{T1T2}$ values ($\delta^{T1} - \delta^{T2}$) of all protons surrounding the chiral secondary alcohol were calculated and applied to the corresponding model for the assignment of the absolute configuration of secondary alcohols by single derivatization, established by the Riguera group (Latypov et al. 1998; Seco et al., 2001).

4.9. Cancer cell line assays (HCT-116, NCI-460, neuro-2a)

Human colon carcinoma cells HCT-116 (Brattain et al., 1981) were grown in 5 mL culture medium (RPMI-1640 + 15% fetal bovine serum containing 1% penicillin-streptomycin, and 1% glutamine) (Moore and Woods, 1977) at 37°C and 5% CO_2 from a starting cell density of 5×10^4 cells/T25 flask. On day 3, duplicate wells containing HCT-116 cells were exposed to various concentrations of the malyngamide analogs. Flasks were incubated for 120 h (5 d) at

5% CO_2 and 37°C, and the cells harvested with trypsin, washed once with Hanks' balanced salt solution (HBSS), re-suspended in HBSS, and counted using a hemocytometer. The results were normalized to an untreated control. The IC_{50} value was determined using Prism 4.0 software (GraphPad, San Diego, CA). Cytotoxicity to NCI-H460 lung tumor cells and neuro-2a cells were run in quadruplicate using the method of Alley et al. (1988) with cell viability being determined by MTT reduction (Manger et al., 1995). Cells were seeded in 96-well plates at 5000 and 8000 cells/well in 180 μL for H460 and neuro-2a cells, respectively. Twenty-four hours later, the test malyngamide was dissolved in DMSO and diluted into medium, and then added at 20 μL/well producing concentrations between 15 and 0.5 μg. DMSO was less than 1% final concentration. After 48 h, the medium was removed and cell viability determined.

4.10. Disk Diffusion Soft Agar Colony Formation Assay

An *in vitro* cell-based assay using murine L1210 (leukemia), C38 (colon), and CFU-GM (normal) cells and human HCT116 (colon), H125 (lung), and leukemia (CEM) cells, assessed general and differential cytotoxicity of pure compounds (Valeriote et al., 2002). Samples were dissolved in 250 μL of DMSO, and duplicate 15 μL aliquots were applied to cellulose disks in agar plates containing cells. After a period of incubation, a zone of cell colony inhibition (z) was measured from the edge of each disk to the edge of colony growth, and expressed as zone units (zu), where 200 zu = 6 mm. General cytotoxic activity for a given sample was defined as an antiproliferation zone of 300 zu or greater. The differential cytotoxicity of a pure compound was expressed by observing a zone differential of 250 units or greater between any solid tumor cell (murine colon C38, human colon HCT-116, human lung H125) and either leukemia cells (murine L1210 or human CEM) or normal cells (CFU-GM).

4.11. Voltage-Gated Sodium Channel Modulation Assays

The malyngamides were evaluated for their capacity to either activate or block sodium channels using the following modifications to the cell-based bioassay of Manger et. al (1995). Twenty-four h prior to chemical testing, cells were seeded in 96-well plates at 8×10^4 cells/well in a volume of 200 μL. Test chemicals, tested in quadruplicate, were dissolved in DMSO were serially diluted with medium and added at 10 μL/well resulting in concentrations of 10, 3 and 1.0 and 0.3 μg/mL. DMSO was less than 1% final concentration. Plates to evaluate sodium channel activating activity received 20 μL/well of either a mixture of 3 mM ouabain and 0.3 mM veratridine (Sigma Chemical Co.) in 5 mM HCl, or 5 mM HCl alone in addition to the test chemical. Plates were incubated for 18 h and results compared to similarly treated solvent controls with 10 μL medium added in lieu of the test chemical. The sodium channel activator brevetoxin PbTx-1 (Calbiochem) was added at 10 ng/well in 10 μL of medium and used as the positive control. Sodium channel blocking activity was assessed in a similar manner except that the ouabain and veratridine stock solution was 5.0 and 0.5 mM, respectively, and the sodium channel blocker saxitoxin (Calbiochem) was used as the positive control. Plates were incubated for approximately 22 h.

4.12. 8-O-acetyl-8-epi-malyngamide C (1)

Clear oil; $[\alpha]_{26D}$ +6 (*c* 0.35, EtOH); UV (EtOH) λ_{max} 216 nm (log ε 3.99), 278 nm (log ε 3.64); IR (KBr) ν_{max} 3305, 2925, 2854, 1726, 1718, 1652, 1541, 1435, 1373, 1233, 1093, 1043 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESITOFMS *m/z* 498.2607 $[M+H]^+$ (calcd. for $C_{26}H_{41}{}^{35}ClNO_6$, 498.2622).

4.13. Lyngbic acid (2)

Brownish oil; $[\alpha]_{26D}$ −6 (*c* 0.5, $CHCl_3$) [lit. −5 (c 0.22, $CHCl_3$) Kan et al., 2000; −10° (c 0.5, $CHCl_3$), Cardellina et al., 1978); HRCIMS *m/z* 257.2110 $[M+H]^+$ (calcd. for $C_{15}H_{29}O_3$, 257.2117); ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (3H, *t*, *J*=6.9 Hz, H-14′), 1.27 m (10H, *m*,

H-9′, H-10′, H-11′, H-12′ and H-13′), 1.42 (2H, m, H-8′), 2.19 (2H, *m*, H-6′), 2.36 (2H, *m*, H-2′), 2.42 (2H, *d*, *J*=5.9 Hz, H-3′), 3.15 (1H, *t*, *J*=5.8 Hz, H-7′), 3.32 (3H, s, H-15′), 5.49 (2H, *m*, H-4′and H-5′).

4.14. 8-Epi-malyngamide C (3)

Clear oil; $[\alpha]_{26D}$ −8 (*c* 0.5, EtOH); UV (EtOH) λ_{max} 208 nm (log ε 3.59), 282 nm (log ε 1.46); IR (KBr) ν_{max} 3322, 2927, 2855, 1714, 1651, 1537, 1432, 1265, 1092, 1071, 971, 915 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRCIMS *m/z* 456.2506 $[M+H]^+$ (calcd for $C_{24}H_{39}{}^{35}ClNO_5$, 456.2517).

4.15. R-MPA ester of 8-epi-malyngamide C

Yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$, See Supplemental data): δ 0.88 (3H, *t*, *J*=6.9 Hz, H-14′), 1.26 m (10H, *m*, H-9′, H-10′, H-11′, H-12′ and H-13′), 1.42 (2H, br t, H-8′), 1.93–2.50 (10H, *m*, H-6, H-7, H-2′, H-3′, H-6′,), 3.13 (1H, *t*, *J*=5.4 Hz, H-7′), 3.31 (3H, s, H-15′), 3.40 (3H, s, MPA-OCH_3), 3.47 (1H, d, *J*=2.6 Hz, H-9), 3.77 (1H, dd, *J*=15.4, 4.7, H-1a), 3.93 (1H, dd, *J*=15.4, 5.7, H-1b), 4.80 (1H, s, MPA-CH), 5.46 (2H, *m*, H-4′and H-5′), 5.54 br s (1H, br s, H-8), 5.96 (1H, br t, NH), 6.30 (1H, br s, H-3), 7.32–7.44 (5H, m, aromatic protons of MPA); LRESIMS *m/z* $[M+Na]^+$ 626.3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:

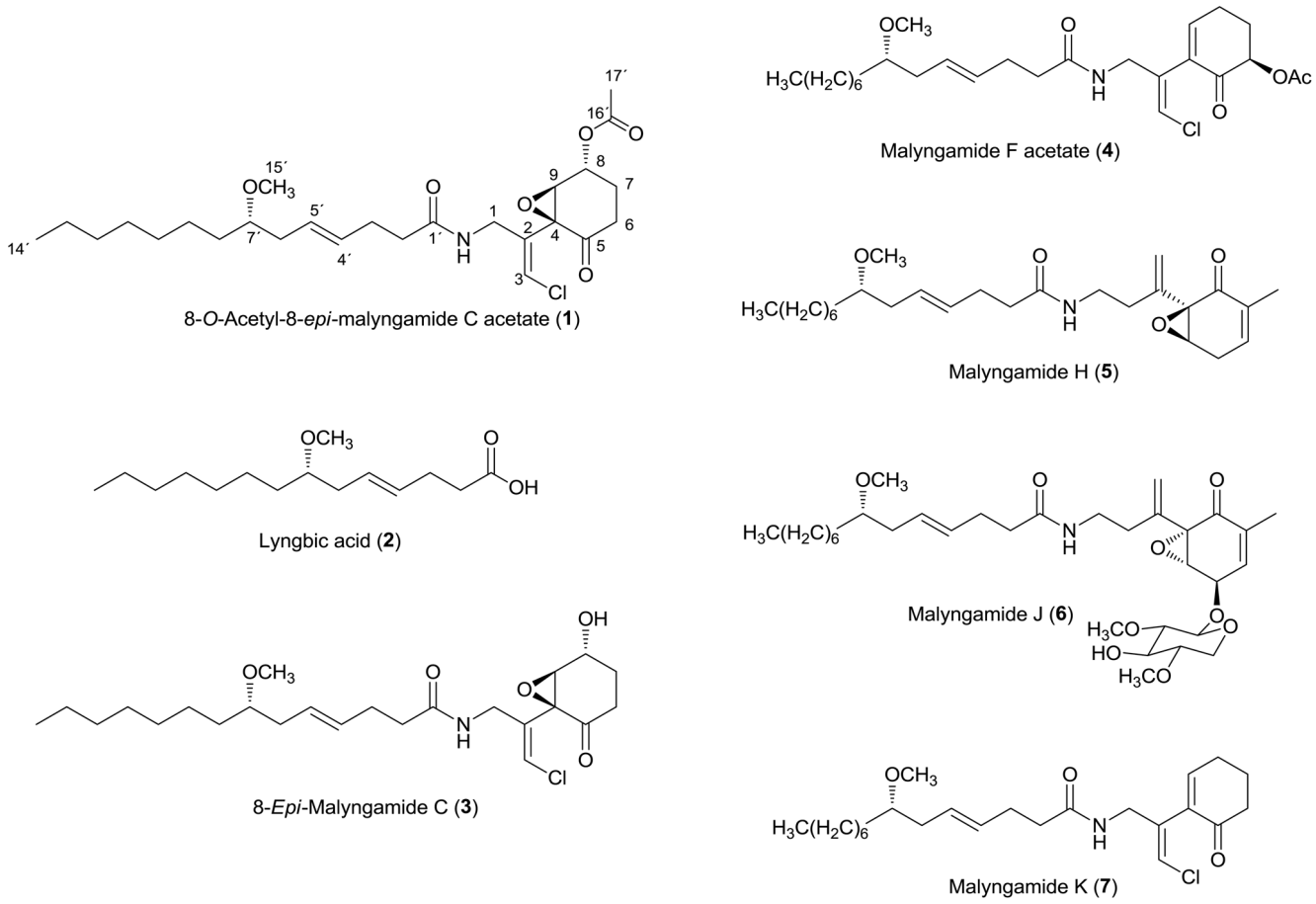

Figure 1.
Structures of lyngbic acid and all malyngamide analogs obtained during this study.

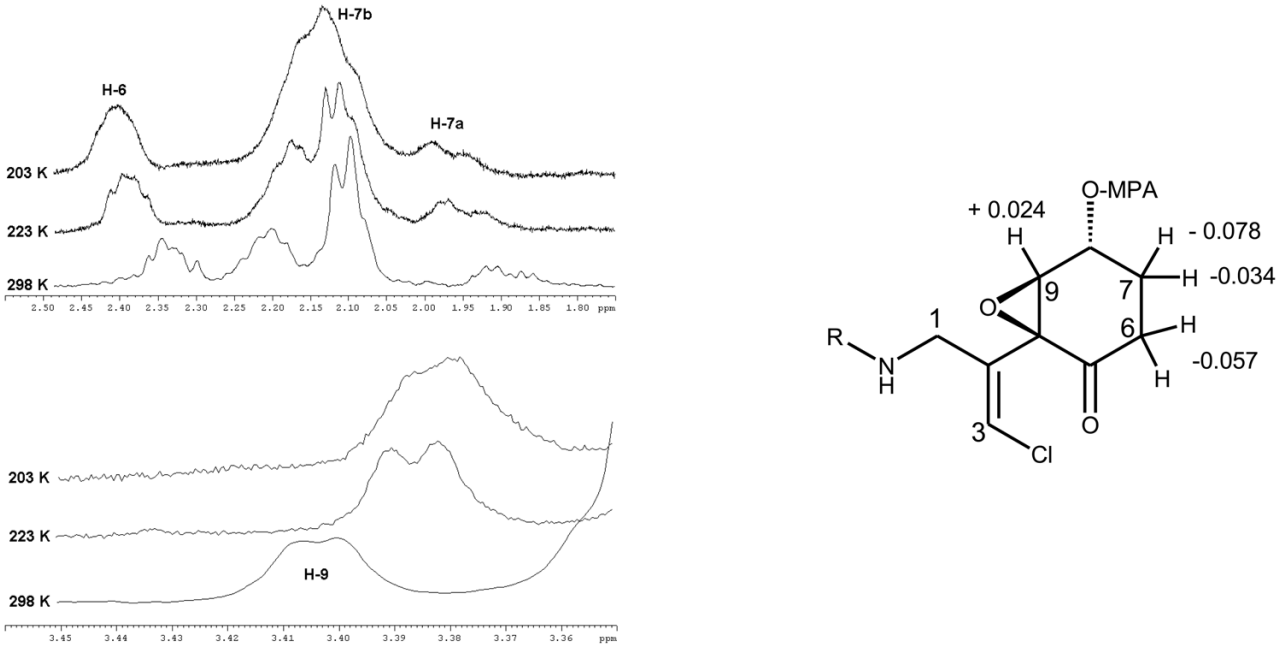

Figure 2.

Results of the variable temperature NMR study on the *R*-methoxyphenylacetate (MPA) derivative of 8-*epi*-malyngamide C. Significant shifts in the ^{1}H NMR spectrum of the protons of interest are shown on the left panel, while the corresponding $\Delta\delta^{T1T2}$ values (ppm) are detailed on the right (R = acyl portion of lyngbic acid).

Table 1

^{1}H and ^{13}C NMR data for 8-*O*-acetyl-8-*epi*-malyngamide C (**1**) and malyngamide C acetate in $CDCl_3$.

	8-*O*-acetyl-8-*epi*-malyngamide C (1)[a]		**malyngamide C acetate[a,b]**		
position	**δ_H**	**δ_C**	**δ_H**	**δ_C**	**$\Delta(\delta_C)$**
1	3.82 *ddd* (14.8, 4.8, 0.7)	40.6 CH_2	3.85 *dd* (4.8, 14.7)	40.3 CH_2	**0.3**
	3.99 *ddd* (14.7, 6.0, 1.2)		3.97 *dd* (4.8, 14.7)		
2		132.8 qC		133.1 qC	**0.3**
3	6.39 *s*	122.7 CH	6.39 *s*	122.8 CH	**0.1**
4		60.7 qC		61.5 qC	**0.8**
5		201.7 qC		200.9 qC	**0.8**
6	2.49 *m*	32.2 CH_2	2.35 *m*	35.0 CH_2	**2.8**
			2.60 *dt* (4.0, 17.8)		
7	1.95 *m*	22.4 CH_2	2.01 *m*	21.5 CH_2	**0.9**
	2.17 *m*				
8	5.49 *ddd* (3.7, 2.9)	66.5 CH	5.44 *m*	68.4 CH	**1.9**
9	3.67 *d* (2.9)	61.6 CH	3.64 *s*	62.6 CH	**1.0**
1′		172.2 qC		172.4 qC	0.2
2′	2.23 *m*	36.3 CH_2	2.17 *m*	36.3 CH_2	0.0
3′	2.31 *m*	28.4 CH_2	2.29 *m*	28.4 CH_2	0.0
4′	5.46 *m*	130.7 CH	5.46 *m*	130.7 CH	0.0
5′	5.46 *m*	127.6 CH	5.46 *m*	127.6 CH	0.0
6′	2.17 *m*	36.4 CH_2	2.25 *m*	36.3 CH_2	0.1
7′	3.14 *m*	80.7 CH	3.12 *m*	80.7 CH	0.0
8′	1.42 *m*	33.3 CH_2	1.42 *m*	33.3 CH_2	0.0
9′	1.26 *m*	31.8 CH_2	1.25 *m*	31.8 CH_2	0.0
10′	1.26 *m*	25.3 CH_2	1.25 *m*	25.3 CH_2	0.0
11′	1.26 *m*	29.3 CH_2	1.25 *m*	29.3 CH_2	0.0
12′	1.26 *m*	29.7 CH_2	1.25 *m*	29.7 CH_2	0.0
13′	1.26 *m*	22.6 CH_2	1.25 *m*	22.6 CH_2	0.0
14′	0.87 *t* (6.7)	14.1 CH_3	0.86 *m*	14.1 CH_3	0.0

	8-*O*-acetyl-8-*epi*-malyngamide C (1)[a]		malyngamide C acetate[a,b]		
position	δ_H	δ_C	δ_H	δ_C	$\Delta(\delta_C)$
15′	3.31 *s*	56.5 CH_3	3.30 *s*	56.4 CH_3	0.1
16′		170.2 qC		170.4 qC	0.2
17′	2.10 *s*	20.8 CH_3	2.14 *s*	20.9 CH_3	0.1
NH	6.02 *dd* (4.8, 4.8)		6.10 *br t* (4.8)		

[a]The coupling constants (*J*) are in parentheses and reported in Hz; chemical shifts are given in ppm.

[b]NMR values taken from Wright and Coll, 1990.

Table 2

^{1}H and ^{13}C NMR data for 8-*epi*-malyngamide C (**3**) and malyngamide C in $CDCl_3$.

	8-*epi*-malyngamide C (3)[a]		malyngamide C[a,b]		
position	δ_H	δ_C	δ_H	δ_C	$\Delta(\delta_C)$
1	3.84 *m*	41.6 CH_2	3.81 *ddd* (14.1, 6.2, 1.3)	40.2 CH_2	**1.4**
	3.99 *m*		4.03 *ddd* (14.1, 4.7, 0.8)		
2		132.9 qC		133.3 qC	**0.4**
3	6.42 *s*	123.6 CH	6.38 *dd* (1.3, 0.8)	122.3 CH	**0.7**
4		61.0 qC		61.8 qC	**0.8**
5		202.6 qC		202.0 qC	**0.6**
6	2.48 *m*	31.8 CH_2	2.24 *ddd* (16.9, 12.5, 6.1)	35.4 CH_2	**3.6**
			2.57 *ddd* (16.9, 4.4, 3.9)		
7	1.89 *m*	26.1 CH_2	1.93 dddd (13.1, 12.5, 9.5, 4.4)	24.8 CH_2	**1.3**
	2.11 *m*		2.01 *dddd* (13.1, 6.1, 5.5, 3.9, 1.1)		
8	4.48 *s*	64.1 CH	4.39 *ddd* (9.5, 5.5, 1.1)	66.2 CH	**2.1**
9	3.59 *d* (2.6)	64.3 CH	3.61 *d* (1.1)	65.3 CH	**1.0**
1′		173.0 qC		172.6 qC	0.4
2′	2.29 *m*	36.3 CH_2	2.29 *m*	36.3 CH_2	0.0
3′	2.30 *m*	28.3 CH_2	2.30 *m*	28.4 CH_2	0.1
4′	5.46 *m*	130.7 CH	5.46 *m*	130.5 CH	0.2
5′	5.46 *m*	127.8 CH	5.46 *m*	127.5 CH	0.3
6′	2.18 *m*	36.2 CH_2	2.20 *m*	36.3 CH_2	0.1
7′	3.15 *m*	80.7 CH	3.15 *m*	80.7 CH	0.0
8′	1.42 *m*	33.3 CH_2	1.42 *m*	33.3 CH_2	0.0
9′	1.27 *m*	31.8 CH_2	1.25 *m*	31.8 CH_2	0.0
10′	1.27 *m*	25.3 CH_2	1.25 *m*	25.3 CH_2	0.0
11′	1.27 *m*	29.3 CH_2	1.25 *m*	29.2 CH_2	0.1
12′	1.27 *m*	29.7 CH_2	1.25 *m*	29.7 CH_2	0.0
13′	1.27 *m*	22.7 CH_2	1.25 *m*	22.6 CH_2	0.1
14′	0.88 *t* (6.7)	14.1 CH_3	0.86 *t*	14.1 CH_3	0.0

	8-*epi*-malyngamide C (3)[a]		malyngamide C[a,b]		
position	δ_H	δ_C	δ_H	δ_C	$\Delta(\delta_C)$
15′	3.32 *s*	56.4 CH_3	3.31 *s*	56.4 CH_3	0.0
NH	6.07 *br s*		6.13 *dd* (6.2, 4.7)		

[a]The coupling constants (*J*) are in parentheses and reported in Hz; chemical shifts are given in ppm.

[b]NMR values taken from Ainslie et al., 1985.

Table 3

Cytotoxic activities of malyngamide analogs towards selected cancer cell lines in conventional cell line assays and in the disk diffusion assay.

compound	cell line assay LC_{50}[μg/mL][a,b]			disk diffusion assay [zu][a,c]
	NCI-H460[d]	Neuro-2a[d]	HCT-116[e]	$_{C38}\Delta_{L1210}$[e]
8-*O*-acetyl-8-*epi*-malyngamide C (**1**)	4.2	5.3	n.d.	< 250
malyngamide C acetate	0.98	0.91	0.8	< 250
8-*epi*-malyngamide C (**3**)	4.5	10.9	n.d.	500
malyngamide C	1.4	3.1	0.2	< 250
6-*O*-acetylmalyngamide F (**4**)	7.6	3.1	-	250
malyngamide H (**5**)[f]	-	-	-	-
malyngamide J (**6**)	10.8	4.0	-	< 250
malyngamide K (**7**)[f]	1.1	0.49	-	< 250

[a] dash = not active

[b] n.d.= not determined

[c] zu = zone units

[d] Four replicates at each test concentration were averaged to construct dose-response curves; LC_{50} values were determined graphically.

[e] A minimum of two measurements were made at each concentration tested

[f] Since in this study, malyngamide H (**5**) and K (**7**) were obtained as an inseparable mixture, for testing, pure reference compounds were taken from an in-house compound library

Table 4

Mammalian Voltage-Gated Sodium Channel (VGSC) modulatory activity of malyngamide analogs.

	Activation [%][a,b]			Blocking [%][a,b,c]			
compound/conc [μg/mL]	**3**	**1**	**0.3**	**10**	**3**	**1**	**0.3**
8-*O*-acetyl-8-*epi*-malyngamide C (**1**)	-	-	-	n.d.	71±16	10±15	-
malyngamide C acetate	toxic	45	-	n.d.	-	-	-
malyngamide C	67±2	19±8	-	n.d.	-	-	-
6-*O*-acetylmalyngamide F (**4**)	19±1	-	-	n.d.	28	15	-
malyngamide H (**5**)[d]	-	-	-	82	69	33	9
malyngamide J (**6**)	-	-	-	n.d.	44±5	19±4	-
malyngamide K (**7**)[d]	-	-	-	n.d.	-	-	-

[a] Values are averages of 4 replicates

[b] dash = not active

[c] n.d. = not determined

[d] Since in this study, malyngamide H (**5**) and K (**7**) were obtained as an inseparable mixture, for testing, pure reference compounds were taken from an in-house compound library