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A NOVEL BROMINATED LIPID FROM AN AUSTRALIAN CYANOBACTERIUM, *LYNGBYA* SP.

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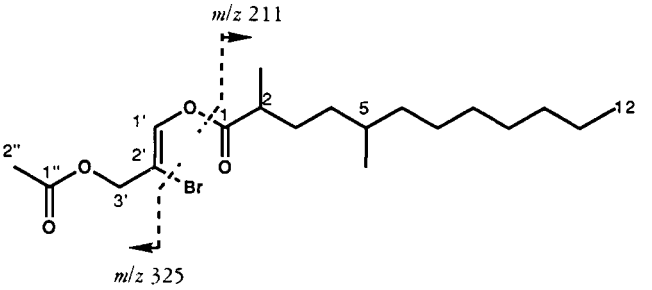
ABSTRACT.—A *Lyngbya* sp. collected from intertidal rock platforms at Point Lonsdale, Victoria, Australia, has been found to contain the novel secondary metabolite [(1′*Z*)-3′-acetoxy-2′-bromo-1′-prop-1′-enyl]-2,5-dimethyldodecanoate [**1**]. The structure of **1** was determined by spectroscopic analysis and degradation.

Cyanobacteria (Cyanophyta), and in particular marine cyanobacteria, have in recent years been shown to be rich sources of unusual secondary metabolites (1). Unlike many other marine organisms that have attracted the attention of natural product chemists, such as red, green, and brown algae or sponges (2), marine cyanobacteria are often difficult to collect in quantity. Indeed in some recent studies (3) sufficient material for chemical investigation was secured only after large-scale culturing in the laboratory. As techniques in structure elucidation have developed, so too the minimum amount of biomass required for a successful chemical investigation has decreased. This report describes the isolation of the novel brominated lipid **1** from a *Lyngbya* sp. found growing on the intertidal rock platform at Point Lonsdale, Victoria. This cyanobacterium occurs sparsely distributed in small patches 1–3 mm thick (2–5 cm^2) on exposed flat surfaces at the extreme seaward edge of the rock platform. Sample collection was only feasible at times of both low tide and calm sea, which for most times of the year is an uncommon event along that portion of coast.

Extraction of a fresh collection of the *Lyngbya* sp. with CH_2Cl_2-EtOH (1:9) followed by concentration under reduced pressure yielded a dark green



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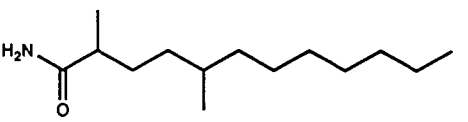

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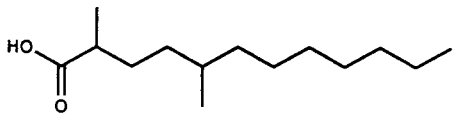

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gum. Preliminary cleanup by solvent partitioning and rapid silica filtration followed by purification on normal phase hplc yielded **1** as a colorless oil. Compound **1** was stable provided it was stored in the dark at <0°; however, if stored at room temperature in the presence of light for any appreciable time (several hours) the sample underwent significant decomposition. The ^{1}H-nmr spectrum of **1** revealed one primary (δ 0.88) and two secondary (δ 0.85 and 1.21) methyls, together with an acetoxy methyl (δ 2.10), and two deshielded methylene protons as an AB_q (δ 4.91 and 4.93, J_{ABq} 13.0 Hz) displaying a small allylic coupling (1.2 Hz) to an olefinic proton (δ 7.58). The ^{13}C-nmr spectrum confirmed the presence of two ester carbonyls [170.4 (s) and 172.1 ppm (s)] and a trisubstituted double bond [137.6 (d) and 106.8 ppm (s)]. Examination of the 15 eV eims of **1** showed a very weak highest mass ion at *m/z* 325, which in keeping with the nmr data ($C_{19}H_{33}$) was attributed to the formula $C_{19}H_{33}O_4$. This was clearly not the molecular ion, which was subsequently identified in the fabms by a pair of equal intensity isotope ion peaks at *m/z* 405 and 407, requiring the formula $C_{19}H_{33}BrO_4$. With the required three double bond equivalents accounted for in two ester functionalities and a trisubstituted double bond, **1** was acyclic.

The deshielded character of the ^{1}H-nmr resonance for the allylic methylene protons, together with the lack of any additional couplings to this resonance [other than the allylic coupling (1.2 Hz) mentioned above], confirmed that the methylene carbon was directly attached to the oxygen of an ester moiety. This was further supported by the deshielded ^{13}C-nmr resonance for this methylene carbon (61.3 ppm). Heteronuclear 2D nmr experiments confirmed a long-range (3-bond) correlation between the allylic methylene protons and the upfield ester carbonyl carbon (172.1 ppm), as well as a short range (2 bond) correlation between this same ester carbonyl carbon and the acetoxy methyl protons (δ 2.10). Thus **1** incorporated an allylic acetate moiety.

As neither the ^{1}H- nor ^{13}C-nmr spectra of **1** revealed deshielded resonances other than those already described, the remaining ester and bromo substitents could only be attached to the molecule via the olefinic carbons. Furthermore, the significantly shielded nature of the quaternary olefinic carbon (106.8 ppm) required, on the basis of tabulated substituent effects (4), that the ester be attached through oxygen to the tertiary olefinic carbon, leaving the bromine substituted at the quaternary olefinic carbon. An nOe enhancement of 4% measured for the olefinic proton on irradiation of the allylic methylene protons was taken to suggest a *Z* rather than *E* geometry about the double bond. It should, however, be noted that in the absence of a corresponding measurement for the *E* isomer this assignment remains tentative (5).

Heteronuclear 2D nmr experiments established a short-range (2-bond) coupling between a deshielded alkyl methine proton (δ 2.51) and the upfield ester carbonyl carbon (170.4 ppm). Furthermore, this alkyl methine proton was shown by homonuclear decoupling experiments to be coupled (J_q = 7.0 Hz) to a deshielded secondary methyl group (δ 1.21), and by the multiplicity of the resonance (tq) to have additional coupling (J_t = 7.0 Hz) to a methylene moiety. The remaining spectroscopic features of **1** were consistent with the second ester unit being derived from a dimethyldodecanoic acid precursor. The existence of a primary methyl terminus (δ 0.88, J_t 7.0 Hz), together with the aforementioned multiplicity of the alkyl methine α to the ester moiety, restricted placement of the remaining secondary methyl to one of carbons C-4 to C-10. Mild hydrolysis of **1** with methanolic NH_3 at room temperature returned the crystalline amide **2**. Exami-

nation of the ^{1}H-nmr spectrum of **2** confirmed the presence of two secondary (δ 0.85 and 1.16) and one primary methyl (δ 0.88), as well as a deshielded alkyl methine proton α to the amide carbonyl (δ 2.18), and two distinct amide proton resonances (δ 5.42 and 5.50). While the eims of **1** was difficult to interpret because of many facile cleavages, the corresponding spectrum of **2** contained numerous diagnostic fragment ions. A 15 eV eims revealed an $[M+1]^+$ ion peak at *m/z* 228 in addition to the very weak $[M]^+$ ion peak at *m/z* 227. Although the mass spectrum at 70 eV revealed a very informative homologous fragmentation pattern (interpreted as sequential loss of 14 amu from the molecular ion), because the origin (parent ions) of individual ions (daughter ions) was not known, an unambiguous placement of the remaining secondary methyl substituent could not be made. Placement of this secondary methyl was eventually achieved by examining the daughter ions from *m/z* 227 in the metastable reaction monitoring mass spectrum of **2** (Figure 1). The absence of a daughter ion at *m/z* 114, together with the presence of one at *m/z* 100, strongly suggested the presence of a C-5 secondary methyl substituent. On careful examination of the ^{1}H-nmr spectrum for **1** it was noted that the magnetically non-equivalent methylene protons at C-3 were coupled as two 16-line multiplets, indicating that in addition to mutual geminal coupling each was further coupled to three other protons (H-2 and H_2-4). This is consistent with the methylation pattern proposed above from mass spectrometric considerations.

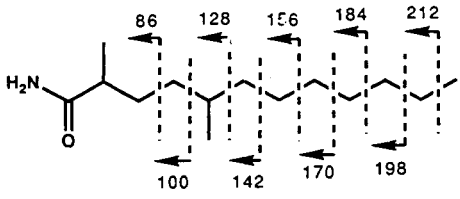

FIGURE 1. Ms fragmentation of [**2**] (metastable reaction monitoring, 70 eV, *m/z* 227→).

Also dominant in the eims of **2** was the expected McLafferty rearrangement ion peak at *m/z* 73. The elemental compositions of the molecular ion (*m/z* 227) and McLafferty rearrangement ion (*m/z* 73) were established by accurate mass measurement. Both the natural product **1** and the degradation product **2** are chiral; however, because of lack of material the relative and absolute stereochemistry about these chiral centers could not be determined.

To the best of our knowledge compound **1** represents the only reported example of this structure class, which can be considered biosynthetically as a diacyldehydro-bromo analogue of glycerol. It is worth noting that 2,5-dimethyldodecanoic acid [**3**] has previously been reported (6) as a metabolite from the Hawaiian marine cyanobacterium *Lyngbya aestuarii*, at which time it was described as possessing plant growth inhibitory properties. The ecological role of **1** in the Victorian *Lyngbya* sp. is unknown.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General experimental procedures were as reported by Barrow and Capon (7).

IDENTIFICATION.—The specimen was identified in the classical sense as a *Lyngbya* sp. in part because it possessed sheaths that extended beyond the trichomes and cell contents that were uniformly granular. The trichomes were 5–6 μm in diameter, with individual cells 4–8 μm in length. The ends of the trichomes were not tapered and had a hemisperical cell at the tip that was not secondarily thickened. In the alternate taxonomic system of Drouet the specimen would have been classified as *Schizothrix mexicana* (Gomont) 1968 (8). A sample has been deposited with the University of Melbourne, Department of Botany, Herbarium collection, accession number MELU A-38634.

COLLECTION, EXTRACTION, AND ISOLATION.—Freshly collected cyanobacteria (dry wt 28 g) were stored in a plastic bag, packed in ice, and transported to the laboratory where they were transferred to a polythene sample bottle and steeped in EtOH-CH_2Cl_2 (9:1). The decanted extract was then partitioned into CH_2Cl_2 solubles (1.15 g) and CH_2Cl_2 insolubles (1.08 g). Tlc [silica, EtOAc-hexane (1:1) visualized with 5%

vanillin in 50% H_2SO_4] and ^{1}H-nmr analysis encouraged further fractionation of the CH_2Cl_2 solubles by rapid silica filtration [20% stepwise gradient from petroleum ether (40–60°) to EtOAc], and then by normal phase hplc (eluent 5% EtOAc/hexane), to yield **1** (16 mg, 0.06%).

[(1′*Z*)-3′-ACETOXY-2′-BROMO-1′-PROP-1′-ENYL]-2,5-DIMETHYLDODECANOATE [**1**].—A pale yellow viscous oil, stable in the dark at < 0° but unstable when exposed to light at room temperature: $[\alpha]_D$ −4.7° (*c* = 0.51, $CHCl_3$); ν max (film) 1759, 1659 cm^{-1}; λ max ($CHCl_3$) 259 nm (ε 4800); ^{1}H nmr ($CDCl_3$) δ 0.85 (d, *J* = 7.0 Hz, 5-C*H*$_3$), 0.88 (t, *J* = 7.0 Hz, H_3-12), 1.21 (d, *J* = 7.0 Hz, 2-C*H*$_3$), 1.2–1.35 (bm, H_2-4, -6, -7, -8, -9, -10, -11), 1.37 (m, H-5), 1.50 and 1.67 (2m, H_2-3), 2.10 (s, H_3-2″), 2.51 (tq, *J* = 7.0, 7.0 Hz, H-2), 4.91 and 4.93 (dABq, *J* = 1.2, 13.0, H_2-3′), 7.58 (t, *J* = 1.2 Hz, H_2-1′); ^{13}C nmr ($CDCl_3$) 14.1 (C-12), 16.6 (2-Me), 19.5 (5-Me), 20.8 (C-2″), 22.7 (C-11″), 27.0, 29.3, and 29.9 (C-7, C-8, and C-9), 30.9 (C-4), 31.9 (C-3), 32.7 (C-5), 34.2 (C-10), 36.8 (C-6), 39.5 (C-2), 61.3 (C-3′), 106.8 (C-2′), 137.6 (C-1′), 170.4 (C-1″), 172.1 (C-1); eims *m/z* (%) (70 eV) 211 (25), 193 (15), 85 (47), 71 (70), 57 (100), (15 eV) 325 (3), 211 (100), 193 (33), 183 (20), 99 (18), 85 (26), 71 (30), 57 (29); fabms (thioglycerol) $[M+H]^+$ 405/407 (5%). Attempts to independently secure the molecular formula for **1** by accurate mass spectral measurements were unsuccessful due to extremely facile cleavages under ei conditions and an inability to undertake hrfab mass measurements before the sample decomposed. The scarcity and instability of the sample precluded combustion analysis.

2,5-DIMETHYLDODECANOIC AMIDE [**2**].—Mild hydrolysis of **1** (5 mg) with NH_4OH-MeOH (1:1) (0.5 ml) at room temperature for 3 h afforded on concentration in vacuo a crude product from which **2** precipitated as a white solid (2 mg, 80%) on addition of 5% EtOAc/hexane: $[\alpha]_D$ +17.1° (*c* = 0.102, $CHCl_3$) (the very small amount of material available makes it difficult to be confident in the absolute value of the $[\alpha]_D$ measurement; it is clearly positive); ^{1}H nmr ($CDCl_3$) δ 0.85 (d, *J* = 7.0 Hz, 5-Me), 0.88 (t, *J* = 7.0 Hz, H_3-12), 1.16 (d, *J* = 7.0 Hz, 2-Me), 1.20–1.40 (bm, H_2-4, -6, -7, -8, -9, -10, -11, H-5), 1.43 and 1.60 (2m, H_2-3), 2.18 (tq, *J* = 7.0, 7.0 Hz, H-2), 5.42 (bs, $W_{1/2}$ = 8.0 Hz, NH), 5.50 (bs, $W_{1/2}$ = 8.0 Hz, NH); eims (15 eV) *m/z* (%), $[M+1]^+$ 228 (2), 227 (<1), 210 (2), 180 (2), 167 (1), 166 (7), 153 (2), 152 (4), 139 (4), 138 (13), 125 (8), 124 (15), 111 (100), 110 (70), 97 (32), 96 (28), 86 (16), 83 (33), 73 (79), 71 (25); eims (70 eV) *m/z* (%) 195 (<1), 194 (5), 181 (<1), 180 (3), 170 (2), 167 (1), 166 (9), 153 (2), 152 (22), 139 (3), 138 (11), 125 (6), 124 (17), 111 (69), 110 (80), 97 (24), 96 (32), 86 (16), 85 (31), 83 (33), 73 (87), 71 (56), 55 (100); hreims *m/z* 227.2250 ($C_{14}H_{29}NO$ requires 227.2249), 73.0527 (C_3H_7NO requires 73.0528).

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