Lobatamides A and B, Novel Cytotoxic Macrolides from the Tunicate *Aplidium lobatum*†

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Over the last 25 years, tunicates have proven to be a consistent source of interesting and novel bioactive natural products.[2] Indeed, the first biologically active tunicate metabolites were isolated from the genus *Aplidium*.[3] Here, we report the isolation[4] of two novel, cytotoxic macrolides, lobatamides A (**1**) and B (**2**), from the tunicate *Aplidium lobatum*.

The molecular formula of $C_{27}H_{32}N_2O_8$ was provided for lobatamide A (**1**)[5] by HRFABMS (noba, *m/z* 513.2257, MH^+, calcd 513.2237). The presence of three exchangeable protons was indicated by a CIMS deuterium-exchange experiment using ND_3 as the ionizing agent.[6] The ^{13}C NMR spectrum of **1** contained signals for all 27 carbons, including two ester carbonyls (δ 171.9, 170.0); an amide carbonyl (δ 164.2); 15 sp^2 carbons, 14 of which were accounted for by a phenyl ring and four olefins; three oxygenated methine carbons (δ 73.8, 73.3, 73.0); three methylenes (δ 33.1, 35.6, 38.9); and three methyl groups [δ 62.7 (OCH_3), 20.2, 19.6].

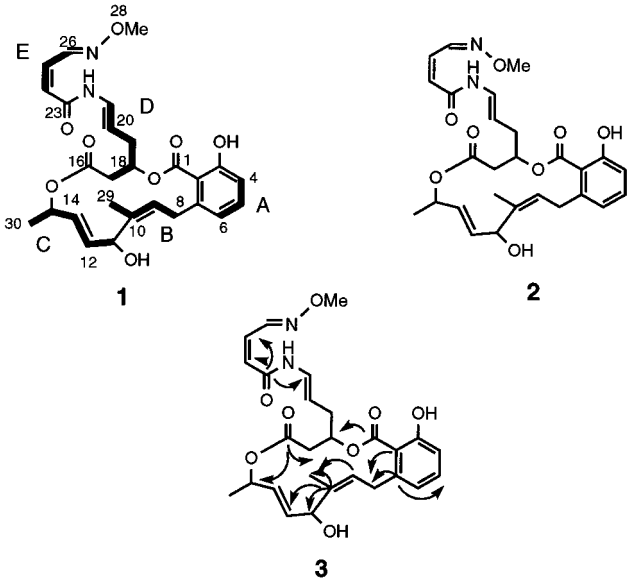

Figure 1. Structures of lobatamides A (**1**) and B (**2**). The bold lines on **1** indicate discrete spin systems identified by NMR experiments. The letter adjacent to each system is used to identify an individual spin system discussed in the text. The observed HMBC correlations that provided links between spin systems are detailed for **1** in **3**.

A series of NMR experiments, including COSY, difference NOE, HMQC, and HMBC, was used to construct the five partial structures depicted in Figure 1 by the emboldened bonds. Four of the five spin systems identified (fragments A−D) were typical structures commonly encountered in structure elucidation. For example, fragment A consisted of three adjacent aromatic protons (δ_C 114.4, δ_H 6.68, d, J = 7.8 Hz; δ_C 131.8, δ_H 7.14, t, J = 7.8 Hz; δ_C 120.8, δ_H 6.63, d, J = 7.8 Hz). Fragment E consisted of three olefinic protons (δ_C 126.1, δ_H 6.04; δ_C 135.6, δ_H 6.45; and, δ_C 148.7, δ_H 8.95). Two of the protons in fragment E (δ_H 6.04 and δ_H 6.45, H24 and H25, respectively) were assigned to a *Z* olefin, based on the $J_{24,25}$ of 11.7 Hz and the observed NOE between the protons. Both the chemical shift of the carbon at 148.7 ppm and the downfield shift of its proton to 8.95 ppm suggested that this carbon was connected to the remaining nitrogen atom in an imine-type bond. In addition, this proton was not correlated to any carbons except the neighboring olefin, already identified as part of fragment E. The carbon chemical shift of δ148.7 is consistent with the presence of an oxime,[7] and such a functional group would account for the remaining nitrogen and oxygen atoms required by the molecular formula. The value of the H25−H26 coupling constant, 11 Hz, plus the NOE correlations observed between these two protons suggested that the olefin and oxime were in an *s-cis* relationship. Together, the five partial structures account for all of the structural elements of **1** except for the methoxyl group. The methoxyl functionality was, in the end, placed on the oxime at N27 based on its carbon chemical shift (δ_C 62.7), which is consistent with other oxime methyl ethers[7] and a weak NOE observed between the methoxyl signal at 3.91 ppm and the proton at 8.95 ppm (H26). The observed NOE and the chemical shifts of the

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† This paper is dedicated to the memory of Luigi Minale (deceased 11 May 97), a leader in marine natural products research and a valued colleague and collaborator.

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(4) These compounds were isolated from *Aplidium lobatum* (Q66C-2612) collected by P. Murphy and colleagues under contract to the National Cancer Institute. The organism was collected off the southwestern coast of Australia due west of Hillary Boat Harbor at −6 m. The taxonomy was provided by Patricia Kott of the Queensland Museum. Vouchers are maintained both at the Australian Institute of Marine Science and the Smithsonian Institution.

(5) **1**: $[\alpha]_D$ −7.9 (c 0.24, MeOH); UV (MeOH) λ_{max} 273 (log ϵ 4.22) nm; IR (film) ν_{max} 3590−3128 (br), 2974, 2933, 1739, 1656, 1523, 1461, 1451, 1374, 1266, 1215, 1169, 1113, 1042 cm^{-1}; ^{13}C NMR (MeOH-d_4) δ 171.9 (s, C16), 170.0 (s, C1), 164.2 (s, C23), 156.7 (s, C3), 148.7 (d, C26), 141.2 (s, C7), 139.4 (s, C10), 135.6 (d, C25), 135.0 (d, C12), 132.7 (d, C13), 131.8 (d, C5), 126.9 (d, C21), 126.1 (d, C24), 125.6 (d, C9), 122.3 (s, C2), 120.8 (d, C6), 114.4 (d, C4), 109.9 (d, C20), 73.8 (d, C14), 73.3 (d, C11), 73.0 (d, C18), 62.7 (q, C28), 38.9 (t, C17), 35.6 (t, C19), 33.1 (t, C8), 20.2 (q, C30), 19.6 (q, C29); ^1H NMR (MeOH-d_4) δ 8.95 (d, J = 11.2 Hz, H26), 7.14 (t, J = 7.8 Hz, H5), 6.82 (d, J = 14.2 Hz, H21), 6.68 (d, J = 7.8 Hz, H4), 6.63 (d, J = 7.8 Hz, H6), 6.45 (dd, J = 11.7, 11.2 Hz, H25), 6.04 (d, J = 11.7 Hz, H24), 5.66 (dd, J = 15.1, 8.8 Hz, H12), 5.58 (m, H18), 5.50 (dd, J = 15.1, 8.3 Hz, H13), 5.34 (dt, J = 14.2, 7.8 Hz, H20), 5.23 (dq, J = 8.3, 6.3 Hz, H14), 5.17 (m, H9), 4.78 (d, J = 8.8 Hz, H11), 3.91 (s, H28), 3.21 (dd, J = 17.1, 8.3 Hz, H8a), 2.93 (br d, J = 17.1 Hz, H8b), 2.67 (d, J = 16.6 Hz, H17a), 2.59 (dd, J = 16.6, 10.7 Hz, H17b), 2.48 (dd, J = 7.3, 6.3 Hz, H19), 1.79 (s, H29), 1.35 (d, J = 6.3 Hz, H30); HRFABMS (noba) MH^+ *m/z* 513.2257 for $C_{27}H_{33}N_2O_8$ Δ +2.0 mmu; FABMS (magic bullet) *m/z* 535 ($M+Na^+$, 8), 513 (MH^+, 16), 495 (15), 460 (20), 289 (65), 239 (22), 176 (30), 154 (100), 138 (100), 107 (83).

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oxime carbon and proton supported *E* (*anti*) geometry for the oxime double bond.[7,8] The presence of an oxime methyl ether was also supported by MS data (vide infra).

The substitution pattern around the phenyl ring of fragment A was determined on the basis of HMBC experiments optimized for 8.3 and 5.5 Hz couplings. The aromatic proton at δ 7.14 (H5), which was coupled to two *ortho* protons, was correlated to both a phenol carbon at δ 156.7 and a second, quaternary carbon at δ 141.2, suggesting that both were *meta* to this proton. The carbon at δ 141.2 was further correlated to the methylene protons at C8, thus identifying it as C7 and requiring attachment of that methylene to this carbon. Correlations to H6 (δ 6.63) were observed from δ 114.4 (C4), 122.3 (C2), and the C8 methylene (δ 33.1), identifiying the macrolide ring attachment site as C7, *ortho* to C6. This left C2 (δ 122.3) as the only point of attachment for the ester carbonyl at δ 170.0 (C1). HMBC experiments provided the final data leading to the gross structure of lobatamide A (**1**). Observed correlations connecting the individual spin systems are summarized in Figure 1.

Additional, critical support for the proposed structure of lobatamide A was provided by mass spectrometry.[9] Compound **1** fragmented to give several structurally significant ions (see Figure 1, Supporting Information), the sequence of which was determined by linked-scan analyses of the fragment ions. In the positive ion mode, elimination of a water molecule from the pseudomolecular (MH^+) ion dominated the upper mass range to give a peak at *m*/*z* 495 (**II**). Although more than one structure can be drawn for this ion, **II** is based on subsequent fragmentation and deuterium-exchange data. Cleavage of the second ester bond yielded a dominant ion at *m*/*z* 239 (**III**, C16-C28) characteristic of the upper half of the molecule. This further fragmented to yield ions at *m*/*z* 128 (**IV**, C16-C22) and *m*/*z* 112 (**V**, C23-C28). This latter fragment (**V**, *m*/*z* 112) strongly supported location of the methoxyl on an oxime. Initial fragmentation at the other ester bond yielded fragment ions at *m*/*z* 275 (**VI**, C1-O15 and C30) and then *m*/*z* 257 (**VII**, C1-C14 and C30). In the negative ion mode, fragmentation was dominated by cleavage at both ester bonds to yield ions at *m*/*z* 291 (**VIII**, O-C1-O15 and C30), *m*/*z* 273 (**IX**, O-C1-C14 and C30), and *m*/*z* 219 (**X**, O-C1-C11 and C29), further characterizing the macrocyclic ring. Upon acetylation, compound **1** yielded a diacetate, *m*/*z* 596, which, on fragmentation in positive ion mode, gave further support to structure **1** (fragment ions comparable to **II**, **VI**, and **VII** showed two acetylations, while analogous structures **III**, **IV**, and **V** showed no change).

Lobatamide B (**2**)[10] was isomeric with **1**. Indeed, the only significant difference in the ^{1}H NMR spectrum of **2** was the downfield shift of H25 to 7.04 ppm (vs 6.45 ppm in **1**) and the upfield shift of H26 to 8.36 ppm (vs 8.95 ppm in **1**). The upfield shift of this proton is consistent with *Z* (*syn*) geometry about the oxime bond.[11] These shifts in the proton spectrum were accompanied by similar changes in the corresponding carbon signals, with C25 appearing at δ 127.5, upfield from 135.6 ppm in **1**, and C26 moving upfield to 144.4 ppm (vs δ 148.7 in **1**). These differences indicated that the geometry of the oxime methyl ether was changed in **2** to *Z*. No NOE was observed between the oxime OMe and H26, as was seen with **1**. The mass spectra of **2** showed the same fragmentations as those seen in **1**, further supporting that only the geometry of the oxime had changed in lobatamide B.

The structures of the lobatamides are completely unrelated to any of the numerous compounds isolated from other collections of *Aplidium*. There is one report of the isolation, from an *Aplidium* sp., of compounds having the same molecular formulas and similar spectroanalytical data as lobatamides A and B; however, the structures described were entirely different from the lobatamides. In particular, we found no evidence for the presence of an *ortho*-nitrite functionality as described by Murray *et al.*[12]

Supporting Information Available: Spectral data (^{1}H and ^{13}C NMR spectra and mass spectral fragmentation pattern) are available for lobatamides A and B (6 pages).

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(9) FABMS were run on a JEOL SX102 spectrometer using a using a 10 kV xenon gun to desorb the samples from a magic bullet matrix (5:1 DTT−DTE). Spectra were run in both positive and negative ionization conditions. Fragmentation analyses were performed by B/E-linked scans of the parent and fragmentation ions. Where necessary, a helium collision gas was used in the first field free region to enhance this fragmentation. Exchange spectra were performed under chemical ionization (CI) conditions on a Finnigan 4500 spectrometer using the direct exposure probe. Exchanges were determined by comparing spectra obtained using ND_3 as the reagent gas with those from NH_3. HRFABMS data for structurally significant fragments are as follows: **II**, 495.2115 for $C_{27}H_{31}N_2O_7$, calcd 495.2131, three exchangeable protons; **III**, 239.1021 for $C_{11}H_{15}N_2O_4$, calcd 239.1032, two exchangeable protons; **V**, 112.0402 for $C_5H_6NO_2$, calcd 112.0399; **VI**, seen in CI only, three exchangeable protons; **VII**, 257.1129 for $C_{16}H_{17}O_3$, calcd 257.1137, two exchangeable protons; **VIII**, 291.1231 for $C_{16}H_{19}O_5$, calcd 291.1231; **IX**, 273.1118 for $C_{16}H_{17}O_4$, calcd 273.1127; **X**, 219.0650 for $C_{12}H_{11}O_4$, calcd 219.0657.

(10) **2**: $[\alpha]_D$ −15.0 (c 0.03, MeOH); UV (MeOH) λ_{max} 288 (log ϵ 4.55); IR (film) ν_{max} 3580−3118 (br), 3056, 2974, 2933, 1733, 1651, 1605, 1584, 1528, 1467, 1451, 1267, 1221, 1175, 1113, 1046 cm^{-1}; ^{13}C NMR (MeOH-d_4) δ 171.9 (s, C16), 170.0 (s, C1), 164.0 (s, C23), 156.7 (s, C3), 144.4 (d, C26), 141.2 (s, C16), 139.4 (s, C10), 135.0 (d, C12), 132.7 (d, C13), 131.8 (d, C5), 127.9 (d, C24), 127.5 (d, C25), 126.8 (d, C21), 125.6 (d, C9), 122.3 (s, C2), 120.9 (d, C6), 114.4 (d, C4), 110.2 (d, C20), 73.8 (d, C14), 73.3 (d, C11), 73.0 (d, C18), 62.6 (q, C28), 38.9 (t, C17), 35.6 (t, C19), 33.1 (t, C8), 20.2 (q, C30), 19.6 (q, C29); ^{1}H NMR (MeOH-d_4) δ 8.36 (d, J = 9.8 Hz, H26), 7.14 (t, J = 7.8 Hz, H5), 7.04 (dd, J = 11.7, 9.8 Hz, H25), 6.82 (d, J = 14.2 Hz, H21), 6.68 (d, J = 7.8 Hz, H4), 6.63 (d, J = 7.8 Hz, H6), 6.05 (d, J = 11.7 Hz, H24), 5.66 (dd, J = 15.1, 8.8 Hz, H12), 5.58 (m, H18), 5.50 (dd, J = 15.1, 8.3 Hz, H13), 5.34 (m, H20), 5.23 (dq, J = 8.3, 6.8 Hz, H14), 5.17 (m, H9), 4.78 (d, J = 8.8 Hz, H11), 3.91 (s, H28), 3.21 (dd, J = 18.1, 8.8 Hz, H8a), 2.93 (br d, J = 18.1 Hz, H8b), 2.67 (dd, J = 16.6, 2.4 Hz, H17a), 2.59 (dd, J = 16.6, 10.7 Hz, H17b), 2.48 (br t, J = 6.8 Hz, H19), 1.79 (d, J = 6.8 Hz, H29), 1.35 (s, H30); HRFABMS (magic bullet) MH^+ *m*/*z* 513.2244 for $C_{27}H_{33}N_2O_8$ Δ +0.7 mmu; FABMS (glyc) *m*/*z* 513 (MH^+, 10), 495 (10), 461 (8), 279 (55), 239 (23), 177 (25), 153 (100), 135 (100).

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