Notes

Mololipids, A New Series of Anti-HIV Bromotyramine-Derived Compounds from a Sponge of the Order Verongida†

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A new series of lipids called mololipids have been identified from an Hawaiian sponge of the order Verongida. The structures of these lipids was deduced from spectroscopic data of the lipid mixture combined with GC−MS analysis. The core of this novel series of lipids is a bromotyramine homoserine-derived moiety known as moloka'iamine (**1**) which is found in many Verongid sponge metabolites. Moloka'iamine forms bisamides with a diverse series of fatty acids and the mololipids mixture (**2**) was active against HIV-1 with an EC_{50} of 52.2 μM without cytotoxicity in human lymphocytes (IC_{50} > 100 μM).

Sponges of the order Verongida possess distinct morphological and biochemical characteristics. The animals, frequently light-colored while alive in the ocean, turn progressively darker when exposed to the atmosphere. Their secondary metabolites are bromotyrosine derivatives of widely varying complexity.[1] In an earlier report we showed that a Verongid sponge collected from Moloka'i, Hawaii produced a number of metabolites atypical for this order, including sesquiterpenes and hydrogen cyanide.[2] We now report an unusual new series of anti-HIV bromotyrosine-derived lipids called mololipids (**2**) from this same Verongid sponge.[3]

A yellow sponge, which gradually turned greenish-brown after it was removed from the ocean, was collected at the pier of Kaunakakai Harbor, Moloka'i, in April 1990. Its color change suggested that it was a Verongid sponge. We have subsequently collected the same sponge in waters south of Maui and on several O'ahu beaches. The striking physiological property of hydrogen cyanide emission when broken apart combined with the unusual diversity of secondary metabolites has made this organism an intriguing candidate for further chemical investigation. As a result, a new series of lipids has been isolated from this sponge, which is the subject of this report. *Psammaplysilla purpurea* (Porifera, Demospongiae, Verongida, Aplysinellidae) has previously been described from Hawaiian waters and its description fits this sponge most closely.[4] This species has also been collected in the Red Sea and at Fiji, and its chemistry has been studied.[5,6] Previous chemical studies on *P. purpurea* have not yielded any information on isoprenoid, cyanide, or bromotyramine-derived lipids as secondary metabolites. This sponge, which is the topic of the present investigation, now informally referred to as "the Moloka'i sponge", is most closely comparable to the genera *Psammaplysilla* and *Pseudoceratina* but appears to be a member of an undescribed genus.

Extraction of 2.8 kg of (wet) sponge twice each with ethanol, then methylene chloride, yielded crude extracts which showed activity against a KB cancer cell line. The extracts were combined, reduced in volume, and partitioned, first against hexane, then methylene chloride, and finally butanol. The previously reported compound, moloka'iamine (**1**) was isolated from the butanol and aqueous phases in gram quantities (0.25% wet wt) as a light-brown amorphous powder and could be purified by repeated recrystallization from methanol. Its structure is typical of other metabolites of Verongid sponges. Moloka'iamine has been frequently reported as a substructure of Verongid metabolites.[7] It is the core substructure in the mololipids.

The combined hexane partition fractions yielded a number of compounds by repeated preparative and semi-preparative HPLC on reversed-phase C_{18} columns. The most abundant compound in a series of bioactive sesquiterpenes was identified as puupehenone.[8] Included in this series is the hydrogen cyanide adduct cyanopuupehenol.[9] The most polar compound from the hexane partition was the previously reported 5-bromo-2,3-dihydroxy-6-methoxybenzaldehyde.[2]

The mololipids, a minor lipophilic component (0.005% w/w), were isolated in the form of a mixture as a white wax; they are compounds in which bromotyramine **1** has formed bisamides with long-chain fatty acids. Separation of these compounds into a mixture composed of a single structural class was accomplished by normal-phase HPLC (hexanes−EtOAc 1:1). The compounds ranged in size from 750 to 1100 daltons. Since the mixtures appear to differ only by a few carbons in chain length, repeated normal-phase HPLC failed to separate them. However, character-

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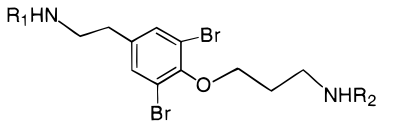
Table 1. Fatty Acid Composition of the Mololipids

t_R (min)	rel t_R[a]	%	MS (m/z)[b] M$^+$	MS (m/z)[b] important ions	identification	ECL[e]
24.068	0.779	0.22	242	227,199,157,143,87[c]	myristic acid	(14:0)
26.302	0.852	2.65	256	225,213,185,143,87[c]	pentadecanoic acid	(15:0)
26.569	0.860	0.85	260	237,225,213,199,143,87[c]	unknown	
29.695	0.962	0.75	270	227,143,87[c]	hexadecanoic acid[d]	(16:0)
29.927	0.969	1.25	270	227,143,87[c]	hexadecanoic acid[d]	(16:0)
30.877	1.000	1.49	270	239,227,143,87[c]	palmitic acid	(16:0)
32.291	1.046	3.93	284	243,199,143,87[c]	heptadecanoic acid[d]	(17:0)
32.940	1.067	0.76	284	241,199,143,87[c]	margaric acid	(17:0)
33.149	1.074	0.53	284	255,241,199,143,87[c]	unknown	(17:0)
36.301	1.176	3.50	296	265,264,222,99[c],87,83	oleic acid	(18:1)
37.160	1.203	2.12	298	255,143,87[c]	stearic acid	(18:0)
38.412	1.244	4.68	312	269,213,143,87[c]	nonadecanoic acid	(19:0)
42.948	1.391	1.31	326	283,255,87[c]	arachidic acid	(20:0)

[a] Retention time relative to methyl palmitate. [b] Mass cutoff set at 80 amu. [c] Base peak. [d] Branched fatty acids without standards. [e] Equivalent chain length (ECL).

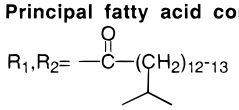
ization as a series could be accomplished through acid hydrolysis followed by GC−MS.

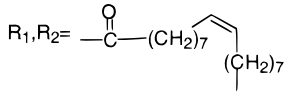


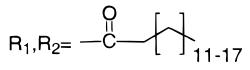
R$_1$=R$_2$=H$_2$Cl

1

Principal fatty acid composition of 2







A study of the fatty acids after hydrolysis of the mololipid mixture (**2**) revealed a homologous series of saturated linear and iso-methyl branched fatty acids ranging from C_{14} to C_{20}. Included is at least one monounsaturated fatty acid of 18 carbon atoms, with the double bond at C-9. There are also mono-, di-, and trimethyl, internally branched, fatty acids containing 15, 17, and 19 carbons. The positions of the methyl substituents varied in the carbon chain. No fatty acids with more than one double bond were detected. The relative percentage of each fatty acid is given in Table 1 and represents the percentage from all compounds eluted by GC, including the unsaponifiable materials. Analysis of HMBC and DEPT experiments combined with ^{13}C NMR chemical shifts supported the presence of *iso*-methyl branched fatty acids. Terminal *gem*-dimethyl protons resonating at 0.85 ppm (carbons at 22.6 ppm) showed HMBC correlations to a methine at 27.9 ppm and a methylene carbon at 39.0 ppm. Internally branched fatty acids showed terminal methyl protons resonating at 0.87 ppm (carbons at 14.0 ppm) with HMBC correlations to methylene carbons at 22.6 and 31.8 ppm. Internally branched methyls resonating at 0.83 ppm (carbons at 19.7 ppm) displayed HMBC correlations to a methine at 32.7 ppm and methylene carbons at 36−37 ppm. The ^{13}C NMR data clearly showed that the internal branching of the most abundant fatty acids did not occur at positions α, β, or γ from the carbonyl carbon or the terminal methyl group. There were no data to support or dismiss the possibility that the individual acids may occur randomly on either nitrogen of the core moloka'iamine nucleus.

On the basis of our experience we concluded that the chemistry of this Verongid sponge is extraordinarily rich and unprecedented. Up to now its only typical Verongid constituent was the bromotyramine fragment **1**, which is present in great abundance, and perhaps the somewhat enigmatic 5-bromo-2,3-dihydroxy-6-methoxybenzaldehyde. The mololipids (**2**) represent additional metabolites which would appear to be more typical of a Verongid sponge. However, they are unique in that they are the first series of bisamides with two fatty acid-derived side chains of the moloka'iamine (**1**) core.

Release of free hydrogen cyanide, when the sponge is broken apart after removal from the ocean, remains an interesting observation. A possible link between hydrogen cyanide and moloka'iamine is the recently characterized cyanoformamide-containing metabolite ceratinamine.[10] The diversity of metabolites produced by this organism and the elucidation of their possible biological functions make this animal an intriguing target for continued chemical and ecological studies. The mololipids were examined in a number of assays including, cytotoxicity, antimicrobial and antiviral. The selective activity against HIV-1 with an EC_{50} of 52.2 μM without cytotoxicity against human peripheral blood mononuclear cells (PBM) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (IC_{50} > 100 μM)[11] suggests that this series of anti-HIV lipids has potential for future studies. Due to the anti-HIV activity of the mololipids combined with the previously reported HSV 2 (10 μg/mL) activity[2] of moloka'iamine (**1**) a high-throughput anti-HIV lead optimization study using moloka'iamine as a scaffold is currently underway in our laboratories.

Experimental Section

General Experimental Procedures. IR and UV spectra were obtained using AATI Mattson Genesis Series FTIR and Perkin-Elmer Lambda 3B UV/vis spectrophotometers. NMR spectra were recorded on Bruker DRX 500 and DRX 400 spectrometers using the solvent peak as the internal standard. HRFABMS spectra were conducted on a Fisons/VG Autospec Q mass spectrometer. Semipreparative HPLC was carried out on Waters 510 model system.

Sponge Material. The sponge was collected from a pier in Kaunakakai Harbor, Moloka'i, in April 1990, and from the south shore of O'ahu island, Hawaii, at a depth of 15 m, in September 1992. The sponge formed a compressible thick encrustation, the surface of which is sparsely conulose. The

texture of the sponge is slightly rubbery, but the sponge is easily torn due to the presence of sand and spicular debris. The sponge is found in association with fouling organisms such as polychaete worms and calcareous algae. The sponge is fleshy and has dense homogeneous tissue, and fibers appear to consist of a very fine pith-like material and are frequently packed with debris. The sponge is comparable in morphology to the genera *Psammaplysilla* and *Pseudoceratina* (Porifera, Demospongiae, Verongida, Aplysinellidae). A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, FL (Catalog No. 003:00852).

Extraction and Isolation. The first collection of 2.8 kg of (wet) sponge, obtained in April 1990, was extracted twice each with ethanol, then methylene chloride, yielding a crude extract that showed activity against a KB cancer cell line. The extracts were combined, concentrated, and partitioned, first against hexane, then methylene chloride, and finally butanol.

The mololipids were isolated from the EtOAc fraction (following CH_2Cl_2) of a silica gel flash column (hexane partition) as a white waxy solid. Further purification was accomplished using Sephadex LH-20 from which the mololipids eluted first. Separation into saturated and unsaturated fatty acids was achieved using silica gel HPLC with hexanes−EtOAc (1:1). Acid hydrolysis with 6 N HCl at 100 °C for 24 h, esterification of the acids to the methyl esters followed by GC−MS provided the composition of the individual fatty acids using an HP 5890A GC with a 5970A quadrupole mass analyzer. GLC conditions: column DB-1 (15 m × 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific Inc., Folsom, CA), temperature program, 1 min at 70 °C and then 3 °C/min to 250 °C.

Mololipids mixture (2): white waxy solid; UV (MeOH) λ_{max} 240 (ϵ 1900), 277 (ϵ 650) 284 (ϵ 620) nm; IR ν_{max} neat (NaCl) 3281 (m), 2922 (s), 2852 (m), 1635 (s), 1552 (m), 1456 (m), 1376 (m), 1259 (m), 1040 (m), 738 (m) cm^{-1}; EI and FAB, *m/z* 750−1,100 amu.

Moloka'iamine (1) substructure: ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 7.33 (2H, s, H-2, 6), 6.07 (m, C-11, NH), 5.61 (m, C-8, NH), 4.05 (2H, t, 6.0 Hz, H-9), 3.55 (2H, q, 6.0 Hz, H-11), 3.44 (2H, q, 6.3 Hz, H-8), 2.73 (2H, t, 6.9 Hz, H-7), 2.05 (2H, m, H-10); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 151.5 (C-4), 138.0 (C-1), 132.9 (C-2, 6), 118.1 (C-3, 5), 71.9 (C-9), 40.3 (C-8), 39.0 (C-11), 34.5 (C-7), 29.3 (C-10).

Fatty acid substructure (mixture): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.37 (m, 2−4H, olefinic), 2.16 (m, α-H), 2.00 (m, allylic H), 1.25 (CH_2 envelope), 0.85 (methyls); ^{13}C NMR (DEPT) ($CDCl_3$, 125 MHz) δ 173.1 (amide), 130.5, 130.2, 128.9 (d, olefin), 36.0−41.0 (CH_2 envelope), 32.7 (CH), 31.8 (CH_2), 29.0−30.0 (CH_2 envelope), 27.9 (CH), 25.5−27.5 (CH_2 envelope), 22.6,19.7,14.0 (CH_3).

Current HIV Studies. The HIV selective activity of the mololipid mixture (**2**) combined with the challenges of isolating a single pure compound for biological evaluation has prompted further investigations. An SAR study utilizing parallel synthesis with acid chlorides available from Aldrich (Combikits) and readily available moloka'iamine is currently underway in our laboratories and will be the subject of a following report.

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Supporting Information Available: ^{1}H, ^{13}C, DEPT, HMQC, and HMBC NMR spectra; EIMS, FAB MS, and GC−MS of standard and constituent fatty acids; and a table of GC-MS data of standard fatty acid methyl esters. This material is available free of charge via the Internet at http://pubs.acs.org.

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