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PLATISIDINES A-C, *N*-METHYLPYRIDINIUM ALKALOIDS FROM AN OKINAWAN MARINE SPONGE OF *PLAKORTIS* SPECIES

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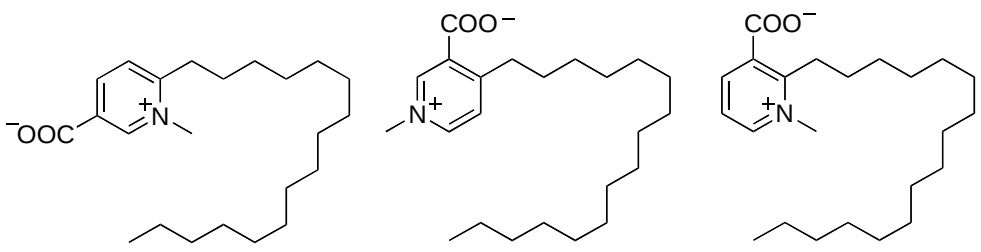
Abstract - Three new *N*-methyl pyridinium alkaloids, platisidines A-C (**1**-**3**), were isolated from an Okinawan marine sponge of the genus *Plakortis*, and the structures were elucidated from spectroscopic data. The structures of platisidines A-C (**1**-**3**) were elucidated to be *N*-methyl nicotinic acid derivatives with a *n*-hexadecanoyl chain. Platisidines A-C (**1**-**3**) showed inhibitory activity against acetylcholinesterase.

INTRODUCTION

Marine sponges of the genus *Plakortis* are known to be a rich source of unique peroxy aliphatic acids and esters.[1] During our search for new metabolites from Okinawan marine sponges, we have isolated some polyketides with unique skeletons from the genus *Plakortis*.[2] Recently, we investigated extracts of an Okinawan marine sponge *Plakortis* sp. (SS-11) and isolated three new *N*-methyl pyridinium alkaloids, platisidines A-C (**1**-**3**). In this paper, we describe the isolation and structure elucidation of **1**-**3**.



1 **2** **3**

RESULTS AND DISCUSSION

The MeOH extracts of the sponge (SS-11) collected off Manzamo, Okinawa, were partitioned between EtOAc and aqueous 3% tartaric acid. Water-soluble materials, which were adjusted to pH 9 with satd. aq. Na_2CO_3, were partitioned with $CHCl_3$. Platisidine A (**1**, 0.000052 %, wet weight) was obtained with four known oxilipins, 43-OMe-manzamenone A,[3-5] 43-OMe-manzamenone B,[3] 5-epi-43-OMe-manzamenone B,[6] and manzamenone F,[3-5] from the EtOAc soluble materials by silica gel and C_{18} column chromatographies. Platisidines B (**2**, 0.000018 %) and C (**3**, 0.000020 %) were isolated from the $CHCl_3$ soluble materials by silica gel and C_{18} column chromatographies followed by silica gel HPLC.

Platisidine A (**1**) showed the pseudomolecular ion peak at *m/z* 362 $[M+H]^+$ in the ESIMS, and the molecular formula, $C_{23}H_{39}NO_2$, was established by HRESIMS (*m/z* 362.30463, $[M+H]^+$, Δ -0.73 mmu). IR absorptions at 3392 (br) and 1646 cm^{-1} indicated the presence of a carboxy group. ^1H and ^{13}C NMR data and the HMQC spectra of **1** disclosed 23 carbon signals due to one carbonyl, two sp^2 quaternary carbons, three sp^2 methines, fifteen sp^3 methylenes, and two methyls. One of the two methyls (δ_C 47.0) was ascribed to one bearing a nitrogen atom. Inspection of the ^1H-^1H COSY spectum of **1** revealed

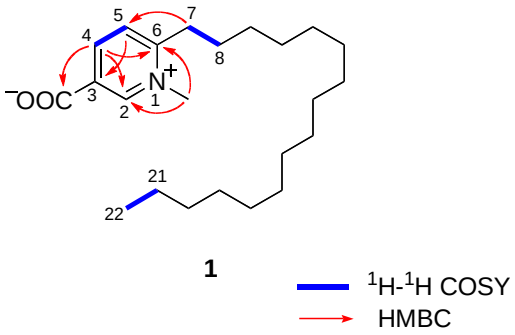


Figure 1. Selected 2D NMR correlations for platisidine A (**1**).

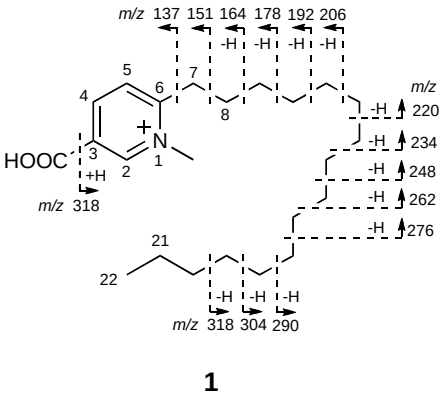


Figure 2. Fragmentation patterns observed in positive ion ESIMS/MS spectrum of platisidine A (**1**) [precursor ion, *m/z* 362 $(M+H)^+$].

connectivities for C-4-C-5, C-7-C-8, and C-21-C-22. HMBC correlations for protons of an *N*-methyl to C-2 and C-6, and H-4 to a carbonyl carbon (δ_C 168.1) as well as those among C-2-C-6 disclosed an *N*-methyl nicotinic acid moiety of **1**. The HMBC correlation for H-7 to C-5 and the molecular formula of **1** suggested that a *n*-hexadecanoyl chain was attached to C-6 of the pyridinium ring of platisidine A (**1**). This was supported by fragmentation patterns observed in the positive ion ESIMS/MS spectrum of platisidine A (**1**) (Figure 2). Thus, the structure of platisidine A (**1**) was assigned as an *N*-methyl nicotinic acid derivative with a *n*-hexadecanoyl chain at C-6.

The molecular formula of platisidines B (**2**) and C (**3**) were established as both $C_{23}H_{39}NO_2$ by HRESIMS. ^{1}H and ^{13}C NMR data of platisidines B (**2**) and C (**3**) indicated that these compounds were analogs of platisidine A (**1**). Inspection of the ^{1}H-^{1}H COSY and the HMBC spectra of **2** and **3** suggested that platisidines B (**2**) and C (**3**) were both *N*-methyl nicotinic acid derivatives with an aliphatic chain (Figure 3). The cross-peaks of H-7/C-3 and H-7/C-5 in the HMBC spectrum of platisidine B (**2**) and the cross-peak of H-7/C-3 in the HMBC spectrum of platisidine C (**3**) revealed that each *N*-methyl pyridinium ring for platisidines B (**2**) and C (**3**) was substituted by an alkyl chain at C-4 and C-2,

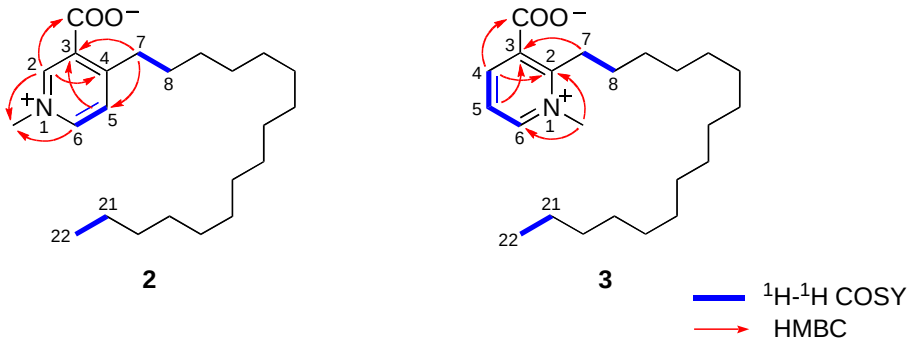


Figure 3. Selected 2D NMR correlations for platisidines B (**2**) and C (**3**).

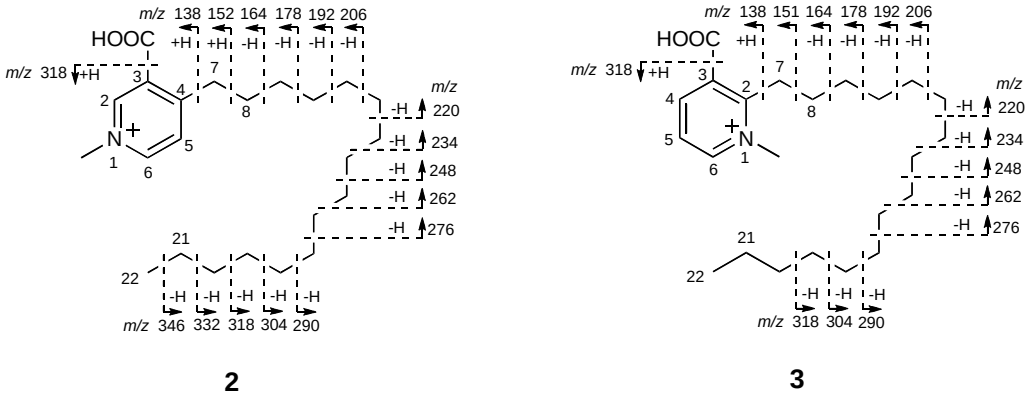


Figure 4. Fragmentation patterns observed in positive ion ESIMS/MS spectrum of platisidines B (**2**) and C (**3**). [precursor ion, *m/z* 362 $(M+H)^+$].

respectively. Molecular formulas and the fragmentation patterns observed in the positive ion ESIMS/MS spectra of platisidines B (**2**) and C (**3**) (Figure 4) disclosed that platisidines B (**2**) and C (**3**) were *N*-methyl nicotinic acid derivartives with a *n*-hexadecanoyl chain at C-4 and C-2, respectively.

Platisidines A-C (**1**-**3**) are new *N*-methyl nicotinic acid derivatives with a *n*-hexadecanoyl chain at C-6, C-4, and C-2, respectively. Only two 4-alkyl *N*-methyl nicotinic acid derivatives have been isolated from sponges of the genus *Plakortis* so far.[7,8] Platisidines A-C (**1**-**3**) showed inhibitory activity against acetylcholinesterase (IC_{50} 2.8, 2.6, and 2.1 mM, respectively).[9-11]

EXPERIMENTAL

General Experimental Procedures.

IR and UV spectra were recorded on a JASCO FT/IR-230 spectrometer and a Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 NMR spectrometer. The 3.35 and 49.8 ppm resonances of residual CD_3OH in CD_3OD were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. Mass spectrometric analyses were performed on a LTQ Orbitrap XL (Thermo Scientific) in positive ion mode. When using an ESI source, samples were infused at the flow rate of 5 μL/min delivered by a built-in syringe pump and source voltage (4.5 kV), capillary temperature (275 ℃), capillary voltage (40 V), and tube lens voltage (135 V) were applied. MS survey scans were performed in the FT cell recording a window between *m*/*z* 150 and 2,000 in the Orbitrap with a resolution of 60,000 (FWHM) at *m*/*z* 400. MS/MS experiments on a LTQ Orbitrap instrument were performed using collision-induced dissociation (CID) and higher energy collision dissociation (HCD). The most intense ions selected for CID and HCD at normalized collision energy of 35 % and 75 %, respectively.

Sponge Description.

The sponge (SS-11) *Plakortis* sp. (order, Homosclerophorida, family Plakinidae) was collected off Manzamo, Okinawa, and kept frozen until used. The sponge was dark brown throughout in ethanol and flattened. The choanosome was pigmented throughout with more dense pigmentation at the surface. Spicules, which were abundant throughout the sponge, were predominentry diods with a central angulation 65-115×1.5-4.5 μm in dimensions. Ocational triods were present. This specimen was a reproductive female, apparentry with incubating embryos. The voucher specimen was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation.

The sponge (SS-11, 2.25 kg, wet weight) was extracted with MeOH (2L x 4), and the extracts were

partitioned between EtOAc (500 mL x 3) and aqueous 3% tartaric acid (500 mL). Water-soluble materials, which was adjusted to pH 9 with satd. aq. Na_2CO_3, were partitioned with $CHCl_3$. EtOAc soluble materials (8.04 g) were subjected to a silica gel column ($CHCl_3$/MeOH), from which a fraction eluted with MeOH (88.3 mg) was then purified by a C_{18} column chromatography (MeOH/H_2O), followed by a silica gel column chromatography to give platisidine A (**1**, 1.16 mg, 0.000052 %, wet weight). $CHCl_3$ soluble material (0.28 g) was subjected to a C_{18} column (MeOH/H_2O), from which a fraction eluted with 90% MeOH (7.2 mg) was then purified by a silica gel column chromatography ($CHCl_3$/MeOH), followed by silica gel HPLC (hexane/EtOAc) to yield platisidines B (**2**, 0.40 mg, 0.000018 %) and C (**3**, 0.46 mg, 0.000020 %).

Platisidine A (1): colorless amorphous solids; UV (EtOH) λ_{max} 274 nm (ε 6200); IR (film) ν_{max} 3392 (br), 2921, 2851, 1646 cm^{-1}; ^1H NMR (CD_3OD) δ_H 9.17 (1H, d, 1.2 Hz, H-2), 8.82 (1H, dd, 8.1, 1.2 Hz, H-4), 8.00 (1H, d, 8.1 Hz, H-5), 4.37 (3H, s, *N*-Me), 3.16 (2H, t, 7.9 Hz, H_2-7), 1.2-1.8 (28H, brs, H_2-8-H_2-21), 0.94 (3H, t, 7.0 Hz, H_3-22); ^{13}C NMR (CD_3OD) δ_C 168.1 (COO), 161.8 (C-6), 149.4 (C-2), 147.0 (C-4), 137.8 (C-3), 129.4 (C-5), 47.0 (*N*-Me), 34.4 (C-7), 24.0-33.0 (C-8-C-21), 15.2 (C-22); ESIMS (pos.) *m/z* 362 $[M+H]^+$; HRESIMS (pos.) *m/z* 362.30463 $[(M+H)^+$, calcd for $C_{23}H_{40}NO_2$, 362.30536].

Platisidine B (2): colorless amorphous solids; UV (EtOH) λ_{max} 272 nm (ε 3700); IR (film) ν_{max} 3420 (br), 2919, 2848, 1652 cm^{-1}; ^1H NMR (CD_3OD) δ_H 8.85 (1H, s, H-2), 8.62 (1H, d, 6.3 Hz, H-6), 7.89 (1H, d, 6.3 Hz, H-5), 4.35 (3H, s, *N*-Me), 3.21 (2H, t, 7.9 Hz, H_2-7), 1.2-1.8 (28H, brs, H_2-8-H_2-21), 0.94 (3H, t, 6.8 Hz, H_3-23); ^{13}C NMR (CD_3OD) δ_C 170.2 (COO), 162.8 (C-4), 146.0 (C-2), 145.0 (C-6), 142.3 (C-3), 130.6 (C-5), 48.7 (*N*-Me), 35.7 (C-8), 24.0-33.0 (14C, C-8-C-21), 15.2 (C-22); ESIMS (pos.) *m/z* 384 $[M+Na]^+$; HRESIMS (pos.) *m/z* 384.28755 $[(M+Na)^+$, calcd for $C_{23}H_{39}NO_2Na$, 384.28785].

Platisidine C (3): colorless amorphous solids; UV (EtOH) λ_{max} 281 nm (ε 4300); IR (film) ν_{max} 3393 (br), 2921, 2851, 1636 cm^{-1}; ^1H NMR (CD_3OD) δ_H 8.74 (1H, dd, 6.2, 1.0 Hz, H-2), 8.49 (1H, dd, 7.7, 1.0 Hz, H-4), 7.84 (1H, dd, 7.7, 6.0 Hz, H-5), 4.39 (3H, s, *N*-Me), 3.39 (2H, t, 7.7 Hz, H_2-7), 1.2-1.8 (28H, brs, H_2-8-H_2-21), 0.94 (3H, t, 6.9 Hz, H_3-23); ^{13}C NMR (CD_3OD) δ_C 171.5 (COO), 158.6 (C-2), 147.6 (C-6), 145.4 (C-4), 144.4 (C-3), 127.0 (C-5), 47.3 (*N*-Me), 42.1 (C-2), 24.0-33.0 (14C, C-8-C-21), 15.3 (C-22); ESIMS (pos.) *m/z* 384 $[M+Na]^+$; HRESIMS (pos.) *m/z* 384.28742 $[(M+Na)^+$, calcd for $C_{23}H_{39}NO_2Na$, 384.28785].

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REFERENCES AND NOTES

1. J. W. Blunt, B. R. Copp, W. P. Hu, M. H. G. Munro, P. T. Northcote, and M. R. Prinsep, *Nat. Prod. Rep.*, 2009, **26**, 170, and references therein.
2. Y. Ishiguro, T. Kubota, J. Fromont, and J. Kobayashi, *Tetrahedron Lett.*, 2009, **50**, 3202, and references therein.
3. S. Tsukamoto, S. Takeuchi, M. Ishibashi, and J. Kobayashi, *J. Org. Chem.*, 1992, **57**, 5255.
4. S. Al-Busafi, M. G. B. Drew, T. Sanders, and R. C. Whitehead, *Tetrahedron Lett.*, 1998, **39**, 1647.
5. S. Al-Busafi and R. C. Whitehead, *Tetrahedron Lett.*, 2000, **41**, 3467.
6. S. Takeuchi, T. Kikuchi, S. Tsukamoto, M. Ishibashi, and J. Kobayashi, *Tetrahedron*, 1995, **51**, 5979.
7. C. Campagnuolo, C. Fattorusso, E. Fattorusso, A. Ianaro, B. Pisano, and O. Taglialatela-Scafati, *Org. Lett.*, 2003, **5**, 673.
8. E. Fattorusso, A. Romano, F. Scala, and O. Taglialatela-Scafati, *Molecules*, 2008, **13**, 1465.
9. G. L. Ellman, K. D. Courtney, V. Anders, and R. M. Featherstone, *Biochem. Pharmacol.*, 1961, **7**, 88.
10. Acetylcholinesterase from electric eel *Electrophorus electricus* (type V-S, lyophilized powder, Sigma-Aldrich) was used for the assay.
11. Galanthamine[12-14] was used as a standerd inhibitor (IC_{50}, 6.4 μM).
12. N. F. Proskurnina and A. P. Yakovleva, *Zhur. Obschchei Khim.*, 1952, **22**, 1899.
13. S. Uyeo and S. Kobayashi, *Pharm. Bull.*, 1953, **1**, 139.
14. S. Kobayashi, T. Shingu, and S. Uyeo, *Chem. Ind.*, 1956, 177.