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NOTE

Neomacquarimicin: a new macquarimicin analog from marine-derived actinomycete

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During our screening program for a secondary metabolite with a polycyclic ring system from a bacterial species using LC-MS/UV-based chemical analysis, the production of a novel β-hydroxyl-δ-lactone compound, neomacquarimicin (**1**), with a molecular ion at *m/z* 317.1756 and an absorption maximum at 279 nm, was observed in the culture extract of *Micromonospora* sp. NPS2077, which was isolated from an unidentified marine sponge collected at Uranouchi Bay, Kochi, Japan. Herein, we report the fermentation, isolation, structural elucidation, and biological properties of **1**.

The marine sponge was rinsed three times with sterile artificial seawater to remove the bacteria attached to the surface. The sample was then homogenized in a blender. The homogenized sample was resuspended in sterile seawater and was kept at 55 °C for 5 min in a separate glass container for pretreatment.[1] Isolation of the actinomycetes from the homogenized samples was initially carried out by using serial dilution and spread plate technique on chitin agar plate, which consisted of 0.2% colloidal chitin (Sigma-Aldrich, St Louis, MO, USA), 0.15‰ KH_2PO_4 (Wako Pure Chemical Industries, Osaka, Japan), 0.21‰ K_2HPO_4 (Wako), 0.25‰ $MgSO_4$ $7H_2O$ (Wako), 1.8% artificial seawater (Nihon Pharmaceutical, Tokyo, Japan) and 1.8% agar (Becton, Dickinson and Company, Sparks, MD, USA). The plates were incubated at 28 °C for 7 days.

The 16S rDNA sequence of the NPS2077 strain (1387 base pairs) was deposited in the DDBJ Genbank (AB856417). This strain showed 100% 16S rDNA sequence identity with *Micromonospora* sp. A1-15.[2] The strain was cultured on a rotary shaker (180 r.p.m.) at 28 °C for 3 days in 14 ml glass tube containing 3 ml of the seed medium. The seed medium consisted of 3% soytone (BD), 1.8% artificial seawater (Nihon Pharmaceutical). Strain NPS2077 was cultured in 500 ml baffled shake flasks containing 100 ml of the production medium (modified KG), which consisted of 0.8% glucose (Wako), 0.8% maltose monohydrate (Wako), 0.8% soluble starch (Wako), 1.5% soytone (BD), 0.2% yeast extract (BD), 1.8% artificial seawater (Nihon Pharmaceutical) and 1 ml seed culture. After 10 days of fermentation with shaking at 220 r.p.m. at 28.5 °C, the whole culture broth (900 ml) was extracted with an equal amount of EtOAc. Two MS and UV-guided chromatographic purification steps, reverse-phase chromatography ($3.5 \times 7\,cm^2$, Wako gel C-50, Wako) with aqueous methanol solution (25–100% aq. MeOH in 25% stepwise increments), and partitioned TLC chromatography (0.5 mm, silica gel 60 F_{254}; Merck, Darmstadt, Germany) with an EtOAc/methanol (19/1) solution were performed to afford a pure sample of neomacquarimicin (**1**, 4.8 mg) with an R_f value in EtOAc:MeOH (19:1) of 0.79.

The molecular ion of *m/z* 317.1756 for neomacquarimicin was determined by HR-ESI-time-of-flight positive-ion MS. The ^{13}C NMR and HSQC spectra of neomacquarimicin indicated 19 carbon atoms with 23 attached protons and suggested the presence of functional groups, including a ketone (δ_C 219.1, qC), an ester (δ_C 175.5, qC) and an olefin (δ_C 130.6, CH; δ_C 123.3, CH). Thus, the MW could represent a molecular formula of $C_{19}H_{24}O_4$ with five rings. The 2D homonuclear and heteronuclear NMR experiments showed connectivities consistent with a structure possessing portions similar to macquarimicin C (**2**)[3] but with desorption of the methyl ketone at C-9 and reduction of the ketone at C-19, as shown in Figure 1-I. The 1D (^1H and ^{13}C) NMR spectral data and 2D NMR correlations for **1** are summarized in Table 1.

Key HMBC correlations were observed for the H-1 methine proton (δ_H 4.65) with the ^{13}C NMR signals at δ_C 175.5 (C-3), 23.4 (C-16) and 68.9 (C-19) and for the H-6 methine proton (δ_H 2.43) with the ^{13}C NMR signals at δ_C 28.4 (C-5), 38.5 (C-7), 46.2 (C-11), 130.6 (C-13) and 29.9 (C-14).

The relative configuration of neomacquarimicin was determined by NOESY experiments and from the *J* values. The NOE correlations observed in these experiments are summarized in Figure 1-II. The coupling constant of $J_{7,\,8ab}$ (6.8 Hz) and $J_{8ab,\,9}$ (7.3, 6.9 Hz) supported by the NOE correlations among 5-H, 7-H and 11-H confirmed that the AB ring junction was *cis*. The BC ring junction was also proved to be *cis* by the COSY correlation between H-14 and H-15a and the NOE network in H-6/Hb-8 and H-15a/H-13. The relative stereochemistry of the cyclopentane ring (A ring) and the cyclohexane rings (C and D rings) was determined by the NOE

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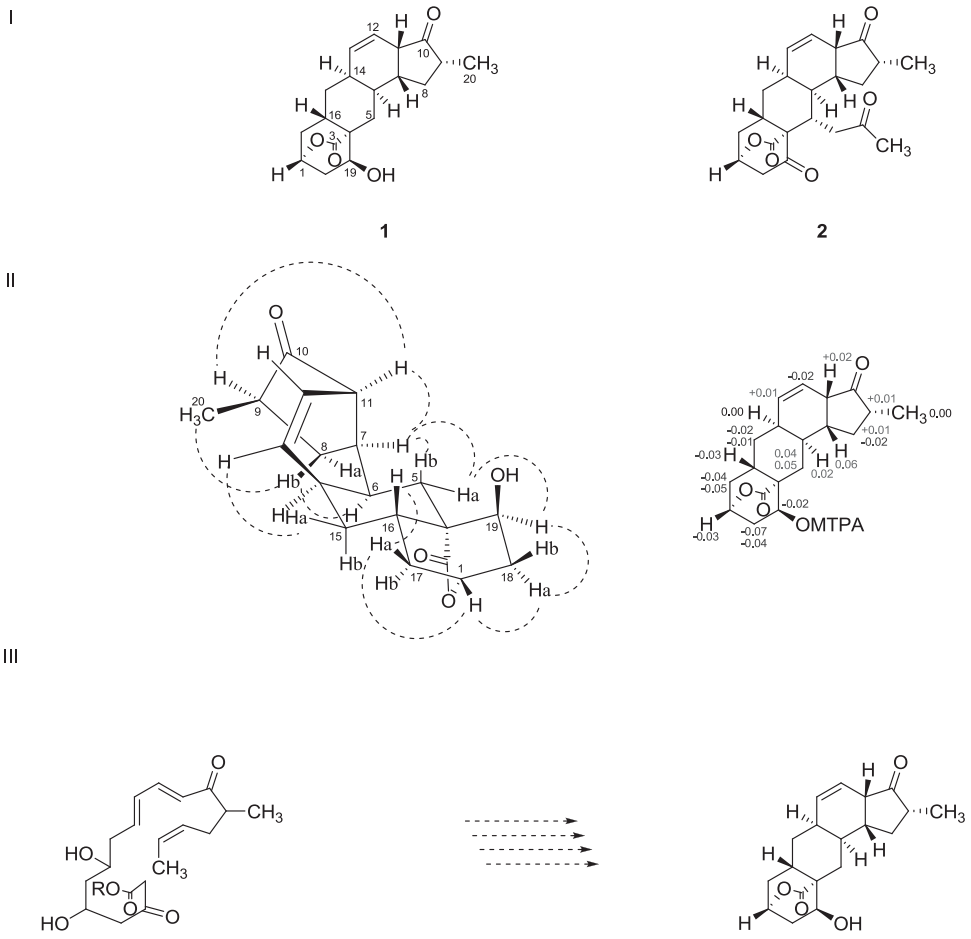

Figure 1 (I) The total structures of neomacquarimicin and macquarimicin C. (II) The relative and absolute configurations of neomacquarimicin and (III) the proposed biosynthesis for the carbocyclic structure of neomacquarimicin. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

correlations observed among H-11/H-9, H-20/H-8b, H-6/H-8b/H-5a, H-5b/H-7 and H-19/H-5a. The relative configuration of neomacquarimicin was determined from these results, as shown in Figure 1-II.

The absolute configuration of **1** was determined using the modified Mosher's method.[4] Neomacquarimicin was esterified to give the corresponding (*R*)- and (*S*)-MTPA esters **1a** and **1b**, respectively. According to the analysis of the Mosher ester, the absolute stereochemistry of **1** was determined to be *S* at C-4, C-6, C-11, C-14, C-16 and C-19, and *R* at C-1, C-7 and C-9 (Figure 1-II). Thus, the relative and absolute configurations of **1** corresponded completely.

Neomacquarimicin is characterized by its β-hydroxy-δ-lactone structure. However, the other moieties of **1** are similar to those in cochleamycin B[5] and macquarimicin C; thus, several of these compounds might have a biosynthetic relationship to **1**. Cochleamycins and macquarimicins, both of which are metabolites of actinomycetes, are carbocyclic polyketides with a β-keto-δ-lactone unit. The biosynthetic origin of cochleamycin has been confirmed by incorporation of ^{13}C-labeled precursors.[6] Starting from the plausible polyketide intermediate shown in Figure 1-III, oxidation followed by the intramolecular Diels–Alder reaction of the triene could form tetrahydroindane. Aldol condensation of tetrahydroindane followed by dehydration could produce the four-ring fused structure in macquarimicin A and cochleamycin A. A reductive transannular cyclization reaction could generate the five-ring fused structure in macquarimicin C and cochleamycin B. Similar to these biosynthetic pathways, a ketone reduction is necessary, in addition to C–C bond formation by PKS, for the construction of the β-hydroxy-δ-lactone of **1**. The stereochemistry of H-9 suggests that the reduction of C-19 occurs during the final step. Attempts to obtain key intermediates are currently underway.

Neomacquarimicin (**1**) was evaluated for inhibitory activity on human acidic sphingomyelinase and rat neutral sphingomyelinase (rnSMase) at concentrations from 0.07 to 700 μM, but no significant responses were noted. In addition, the *in vitro* cytotoxic activity of **1** was evaluated against human umbilical vein endothelial cells, and no cytotoxicity was observed when the cells were exposed to 350 μM **1** for 3 days. The compound **1** was evaluated for activity in the *Bacillus subtilis* ATCC 43223 and *Escherichia coli* ATCC 10536 microbial assays at concentrations that range from 50 to 0.50 mM. Unfortunately, no significant responses were noted at the concentrations tested. A large member of carbocyclic polyketides with a β-keto-δ-lactone unit showed cytotoxicity and antimicrobial activity.[7–9] In addition, macquarimicin A is a weak yet specific inhibitor of rnSMase (IC_{50} value: 145.8 μM).[10] Results on our ongoing structure activity correlation of β-hydroxy-δ-lactone unit will be reported in a forthcoming paper.

Table 1 NMR spectral data for 1 and macquarimicin C in $CDCl_3$

	1					*Macquarimicin C (**2**)*	
C/H n	δ_C, *mult* [a,b]	δ_H, *mult (J in Hz)* [c]	*COSY*	*HMBC*	*NOESY*	δ_C	δ_H
1	74.3, CH	4.65, brs	17b, 18a	3, 16, 19	17a/b, 18a/b	73.1	4.98
3	175.5, qC	—	—	—	—	169.3	—
4	49.0, qC	—	—	—	—	65.9	—
5	28.4, CH_2	1.71, dd (13.2, 4.0)	5b, 6	3, 4, 7, 14, 16, 19	6, 7, 19	28.4	2.66
	—	1.51, dd (13.2, 12.3)	5a, 6	3, 4, 6, 7, 16, 19	7	—	—
6	30.8, CH	2.43, m	5a/b	5, 7, 11, 13, 14	5a, 8b	37.0	2.19
7	38.5, CH	2.32[d]	8a/b, 11	5, 6, 8, 10, 11, 12	5a/b, 11	34.6	2.18
8	33.5, CH_2	2.15, ddd (13.2, 7.3, 6.8)	7, 8b, 9	7, 9, 10, 11	—	34.1	2.06
	—	1.40[d]	7, 8a, 9	7, 9, 20	6, 20	—	1.38
9	44.0, CH	2.25, dquin (7.3, 6.9)	8a/b, 20	8, 10, 20	11	43.6	2.21
10	219.1, qC	—	—	—	—	218.5	—
11	46.2, CH	2.87, brs	7, 12	13	7, 9	46.3	3.30
12	123.3, CH	5.60, d (10.0)	11, 13	11, 14		126.0	5.74
13	130.6, CH	5.48, d (10.0)	12	6, 11	15a	128.7	5.34
14	29.9, CH	2.37[d]	15a			30.3	2.50
15	37.9, CH_2	1.82, brd (13.5)	14, 15b	4, 6, 13, 16	13, 16	37.9	1.90
	—	1.38[d]	15a, 16	4, 13, 14, 16	16	—	1.62
16	23.9, CH	2.35[d]	15b, 17a/b		15a/b, 17a	31.9	2.17
17	33.1, CH_2	2.09, dd (13.7, 10.6)	16, 17b	4, 15, 16	1, 16	33.4	2.19
	—	1.54[d]	1, 16, 17a	1, 18	1		1.85
18	36.8, CH_2	2.50, dd (15.3, 9.0)	1, 18b, 19	1, 17	1, 19	41.9	2.70
	—	1.58, d (15.3)	18a, 19	1, 17, 19	1	—	2.45
19	68.9, CH	3.84, d (9.0)	18a/b, 19-OH	16	5a, 18a	202.8	—
19-OH	—	1.97, s	19	—	—	—	—
20	14.0, CH_3	1.07, d (6.9)	9	8, 9, 10	8b	13.9	1.06
5-CH_2	—	—	—	—	—	48.4	2.81
	—	—	—	—	—		2.41
5-CO	—	—	—	—	—	206.7	
5-CH_3	—	—	—	—	—	29.9	2.13

[a]150MHz.
[b]Assignments by HSQC experiments.
[c]600 MHz.
[d]Multiplicity patterns were unclear due to signal overlapping.

(**1**): colorless oil, $[\alpha]_D^{25}$ +137.08 (*c* 0.30, MeOH); UV (MeCN) λ_{max} (log ε) 279 nm (2.85); IR (KBr) 3432, 2929, 2870, 1732, 1613, 1453, 1377, 1334, 1235, 1174, 1140, 1118, 1101 cm^{-1}; NMR data, see Table 1; HRESITOFMS $[M+H]^+$ *m/z* 317.1756, calcd. for $C_{19}H_{25}O_4$, 317.1753.

Preparation of (*R*)-MTPA ester **1a** and (*S*)-MTPA ester **1b**. A solution of **1** (0.5 mg, 1.6 μmol) in dry CH_2Cl_2 (0.3 ml) was added to (*R*)-MTPA (1.1 mg, 4.7 μmol), DCC (1.0 mg, 4.7 μmol), and DMAP (0.1 mg, 0.8 μmol) at room temperature. After being stirred at room temperature for 16 h, the reaction mixture was concentrated under reduced pressure. Purification by TLC (Hex/EtOAc = 3:2) afforded 0.8 mg of **1a** as a colorless oil. (*S*)-MTPA ester **1b** was obtained by treatment of **1** (0.5 mg, 1.6 μmol) using the method described for Mosher ester **1a**. (*R*)-MTPA ester **1a**: ^1H NMR (600 MHz, $CDCl_3$) δ 7.50-7.46 (2H, m, MTPA-Ph), 7.46-7.41 (3H, m, MTPA-Ph), 5.45 (1H, d, *J* = 10.0 Hz, H-12), 5.36 (1H, d, *J* = 10.0 Hz, H-13), 4.91 (1H, d, *J* = 9.4 Hz, H-19), 4.69 (1H, brs, H-1), 3.50 (3H, s, MTPA-OMe), 2.70 (1H, dd, *J* = 15.8, 9.4 Hz, Ha-18), 2.53 (1H, brs, H-11), 2.39 (1H, m, H-6), 2.33 (1H, brs, H-14), 2.25 (1H, m, H-16), 2.21 (1H, m, H-9), 2.19 (1H, m, H-7), 2.11 (1H, ddd, *J* = 13.2, 7.3, 6.8 Hz, Ha-8), 1.98 (1H, dd, *J* = 14.0, 10.6 Hz, Ha-17), 1.78 (1H, brd, *J* = 13.9 Hz, Ha-15), 1.69 (1 H, dd, *J* = 13.3, 4.0 Hz, Ha-5), 1.61 (1H, m, Hb-17), 1.58 (1H, d, *J* = 15.8 Hz, Hb-18), 1.35 (1H, m, Hb-15), 1.36 (1H, m, Hb-8), 1.28 (1H, dd, *J* = 13.3, 12.3 Hz, Hb-5), 1.06 (3H, d, *J* = 6.9 Hz, H-20); HRESITOFMS $[M-H]^-$ *m/z* 531.1992, calcd. for $C_{29}H_{30}F_3O_6^-$, 531.1994.

(*S*)-MTPA ester **1b**: ^1H NMR (600 MHz, $CDCl_3$) δ 7.49-7.42 (5H, m, MTPA-Ph), 5.43 (1H, dd, *J* = 10.0, 3.1 Hz, H-12), 5.37 (1H, d, *J* = 10.0 Hz, H-13), 4.89 (1H, d, *J* = 9.2 Hz, H-19), 4.66 (1 H, brs, H-1), 3.46 (3 H, s, MTPA-OMe), 2.66 (1 H, dd, *J* = 15.5, 9.2 Hz, Ha-18), 2.55 (1H, brd, *J* = 3.1 Hz, H-11), 2.41 (1H, m, H-6), 2.33 (1H, brs, H-14), 2.25 (1H, m, H-7), 2.24 (1H, m, H-16), 2.22 (1H, m, H-9), 2.12 (1H, ddd, *J* = 13.2, 7.3, 6.8 Hz, Ha-8), 1.94 (1H, dd, *J* = 14.0, 10.6 Hz, Ha-17), 1.77 (1H, brd, *J* = 13.9 Hz, Ha-15), 1.74 (1 H, dd, *J* = 13.3, 4.0 Hz, Ha-5), 1.56 (1 H, m, Hb-17), 1.51 (1H, d, *J* = 15.5 Hz, Hb-18), 1.33 (1H, m, Hb-15), 1.34 (1H, m, Hb-8), 1.32 (1H, dd, *J* = 13.3, 12.3 Hz, Hb-5), 1.06 (3H, d, *J* = 6.9 Hz, H-20); HRESITOFMS $[M-H]^-$ *m/z* 531.1998, calcd. for $C_{29}H_{30}F_3O_6^-$, 531.1994.

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