ABSTRACT

Two new polyketides, namely lucentides A (1) and B (2), together with 19-hydroxyprotylonolide (3) were isolated from *Nocardiopsis lucentensis* DSM 44048. Their structures were elucidated by analysis of their high-resolution mass spectrometry (HR-MS) and 1D, 2D nuclear magnetic resonance (NMR) spectroscopic data. The antibacterial activities of compounds 1–3 were evaluated.

1. Introduction

The *Nocardiopsis* is an actinobacteria genus consisting of former members of the genus *Actinomadura* that Meyer reclassified in 1976 (Meyer 1976). Although *Nocardiopsis* does not represent a major group within actinomycetes, a ubiquitous distribution of *Nocardiopsis* strains has been reported in various environments, such as saline soil (Al-Zarban et al. 2002), marine sediment (Sabry et al. 2004), salterns (Chun et al. 2000; Hamedi et al. 2011) and marine sponges (Schneemann et al. 2010). Moreover, many bioactive secondary metabolites have been isolated from this genus, for example nocapyrones A–D (Schneemann et al. 2010), nocapyrones H–J (Kim et al. 2013), lucentamycins A–D (Cho et al. 2007), pyranone derivatives (Lin et al. 2010), nocardiopsins A and B (Wu et al. 2013), griseusin D (Li et al. 2007), nocazins A–C, nocratrones A and B (Kim et al. 2014), dopisamine (Takahashi et al. 1986), nocazoline A (Fu et al. 2011) and quinoline alkaloid (Tian et al. 2014).
In a continuation of our search for bioactive small molecules from halophilic actinomycetes, *Nocardiopsis lucentensis* DSM 44048, which is purchased from the DSM Microbial Type Culture Collection (Yassin et al. 1993), was selected on the basis of cytotoxicity screenings against human tumour cell lines. It is a moderately halophilic actinomycete which shows optimal growth characteristics at 37 °C in pH 7.6 medium containing 10% (w/v) NaCl. Our previously results suggested that new benzoxazole derivatives from DSM 44048 were responsible for cytotoxicity against HepG2 and HeLa (Sun et al. 2015) cell lines. Further investigation of extracts revealed the presence of additional new metabolites and now reported two new polyketides, lucentides A (1) and B (2), as well as the known 19-hydroxyprotylonolide (3) (Figure 1) (Tian et al. 2014).

2. Results and discussion

Compound 1 was obtained as a colourless oil. The molecular formula was determined to be C_{12}H_{22}O_{4} on the basis of the positive-ion-mode HRESIMS data (m/z 231.1592 [M + H]^+). The ^1H- and ^13C-NMR spectra along with HMQC experiments revealed the signals of four Me, two CH₂, four CH groups and two quaternary C-atoms, including an ester C=O group (δ_C 181.1 (C-1)). The C12 (from C-1 to C-9) unit was determined to be 5-(1,3-dihydroxy-2-methylpentyl)-3,5-dimethylfuran-2(3H)-one by unambiguous nuclear magnetic resonance (NMR) assignments with the aid of HMQC and HMBC experiments, particularly the HMBCs from H-2a to C-1, C-2 and C-3, from H-4a to C-3, C-4 and C-5, from H-6a to C-5, C-6 and C-7, and from H-9 to C-7 and C-8. The down field shift of δ_C 88.2 (C-4), 80.4 (C-5) and 74.8 (C-7) indicated O-bearing C-atoms or hydroxyl substitution. The possibility of γ-lactone was induced according to the HR-MS with the ion peaks at m/z 231.1592 [M + H]^+ and 253.1409 [M + Na]^+. Therefore, the structure of 1 was determined as 5,7-dihydroxy-2,4,6-trimethylnonanoic acid. The relative configuration of 1 was established from the NOE spectra. The presence of NOE correlations between H-2/H-4a/H-5 indicated that H-2 and H-4a were in β-orientation, while H-2a was in α-oriented (Figure 1). Since the easy rotation of C–C bonds in the linear side chain, the relative configurations of C-5, C-6 and C-7 were refractory to determination.

![Figure 1](image-url) Lucentides A (1), B (2) and 19-hydroxyprotylonolide (3) isolated from *Nocardiopsis lucentensis* DSM 44048. (A) structures of 1, 2 and 3. (B) key ^1H–^1H COSY(—) and HMBC correlations (—) for 1 and 2 supporting their structural assignments.
by spectroscopic analysis (Hu et al. 2010). Therefore, compound 1 was determined to be
5-(1,3-dihydroxy-2-methylpentyl)-3,5-dimethylidihydrofuran-2(3H)-one.

Compound 2 was obtained as a colourless oil. The molecular formula was determined to be
C_{12}H_{22}O_{4} on the basis of the positive-ion-mode HRESIMS data (m/z 231.1592 [M + H]^{+}).
The $^{1}$H- and $^{13}$C-NMR spectra along with HMQC experiments revealed the signals of four Me,
three CH$_{2}$, two CH groups and three quaternary C-atoms, including an ester C=O group ($\delta_{C}$
179.2 (C-1)), similar to those of 1. The same C12 unit was determined by the HMBC corre-
lations from four Me to corresponding carbons. Whereas, the 5,6-dihydroxyl substitutions
and a O-bearing quaternary C-atoms were confirmed by the down field shift of 79.4 (C-5),
74.6 (C-6) and 85.9 (C-4). The relative configuration of 2 was determined to be the same
as that of 1 because of the same NOE correlations. Compound 2 was determined to be
5-(1,2-dihydroxy-2-methylpentyl)-3,5-dimethylidihydrofuran-2(3H)-one.

In addition to the two polyketides, compound 3 was isolated as a colourless oil. It was iden-
tified as 19-hydroxyprotylonolide (Figure 1) by comparison of the MS and NMR data obtained
with those reported in the literature (Sadakane et al. 1983; Yasumuro et al. 1994), and this
kind of structures have been isolated from many other organisms, including Strep
tomyces fradiae KA-427. (Omura et al. 1980) Streptomyces sp. strain KA-464(Sadakane et al. 1983) and
Micromonospora sp. YS-02930K1 (Yasumuro et al. 1994). DSM44048 now joins a growing
list of producers of 3.

The antimicrobial activities of compounds 1–3 were performed by paper disc diffusion
assay. The sterile filter paper disks impregnated with 50 μg of the compounds were placed
on agar plates previously inoculated with Mycobacterium smegmatis mc^2 155, Bacillus sub-
tilis ATCC 6051 and Candida albicans 5314. But all of them showed no evident antimicrobial
activities against the tested strains.

The linear carbon scaffolds represented by 1 and 2 are rare among known natural prod-
ucts. We previously reported seven linear polyketides from Xylaria sp.NCY2 (Hu et al. 2010).
Their structures could be classified to be a tetraketide. A proposed pathway for 1 and 2 could
be from condensation of a propionate starter unit with three methyl malonate extender units,
followed by varying modifications, such as hydroxylations at C-5 or C-6/C-7 and esterifica-
tion at C-1. Recently, some new polyketides were discovered such as cytotoxic polyketides
Plakortis simplex (Zhang et al. 2013), amphirionin-4 from Amphidinium species (Minamida
et al. 2014), actinopolysporins A-C from Actinopolyspora erytharea YIM 90600 (Zhao et al.
2011). Compounds of this class have characteristic linear polyketide structures not exceeding
600 molecular units with some oxygen functionalities (e.g. hydroxyl group, ketone, epoxide
and/or tetrahydrofuran ring) and a few methyl and/or exomethylene branches. These fea-
tures emphasise the plasticity and promiscuity of linear polyketide biosynthetic machinery
which produce natural products of structural diversity.

3. Experimental

3.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance DRX-400 NMR spectrometer operating at
400 MHz for $^{1}$H and 100 MHz for $^{13}$C in the indicated solvents. Chemical shifts are expressed
in ppm ($\delta$ units) with TMS as the internal reference. HRESIMS was obtained on a LTQ Velos
Pro HRMS instrument (Thermo Scientific). All solvents were analytical grade purchased from
3.2. Fermentation and extraction

The strain *N. lucentensis* DSM 44048 was inoculated on MP agar media (mannitol 2%, peptone 2%, sea salt 10%, agar 1.5%, pH 7.2) plates and cultured for 14 days at a total volume of 12 L at 38 °C. The fermented cultures were diced and then extracted three times overnight with EtOAc–MeOH–AcOH (80:15:5, v/v/v) at room temperature. After the concentration under vacuum, the crude extract was partitioned between EtOAc and H₂O. EtOAc-soluble partition was dissolved in 100 mL of 95% methanol and extracted five times with an equal volume of petroleum ether (PE) to afford the defatted MeOH extract (3.9 g).

3.3. Isolation and purification of compounds 1–3

The MeOH extract was purified by column chromatography (CC) over Sephadex LH-20 eluted with MeOH to give fractions 1−8. Among them Fr. 3 (1.793 g) was subjected to medium pressure liquid chromatography over RP-18 silica gel (30 g) eluted with gradient aqueous methanol (30, 50, 70 and 100% MeOH, 450 mL each) to afford Fr. 3A-3J. Fr. 3f was separated by CC (SiO₂) to yield 2 (2.9 mg) at the gradient of Pe /acetone 30:1, and 1 (1.0 mg) at the gradient of PE/acetone 27:1. Fr. 3i was also separated by CC (SiO₂, PE/acetone 11:1) to yield 3 (2.0 mg).

3.4. Structure and identification

Lucentide A (1). Colourless oil. \([\alpha]_D^{25}−7.8 (c = 0.05, CH₃OH)\); HRESIMS m/z 231.1592 [M + H]⁺ (calcd. for C₁₂H₂₃O₄⁺, 231.1591). ¹H NMR (400 MHz in CD₃OD): δ 2.93 (1H, m, H-2), 1.24 (3H, d, J = 7.1 Hz, H-2a), 2.29 (1H, dd, J = 9.2, 12.6 Hz, H-3α), 2.09 (1H, t, J = 12.6 Hz, H-3β), 1.36 (3H, s, H-4a), 3.58 (1H, m, H-5), 2.09 (1H, dd, J = 1.9, 7.1, 13.9 Hz, H-6), 1.01 (3H, d, J = 7.1 Hz, H-6a), 3.85 (1H, ddd, J = 1.8, 5.4, 8.2 Hz, H-7), 1.57 (1H, m, H-8α), 1.47 (1H, m, H-8β), 0.95 (3H, t, J = 7.4 Hz, H-9); ¹³C NMR (100 MHz in CD₃OD): δ 181.8 (C-1), 35.8 (C-2), 15.9 (C-2a), 40.0 (C-3), 88.2 (C-4a), 21.4 (C-4a), 80.4 (C-5), 74.8 (C-6), 11.7 (C-6a), 38.8 (C-7), 28.2 (C-8), 11.1 (C-9).

Lucentide B (2). Colourless oil. \([\alpha]_D^{25}−14.2 (c = 0.16, CH₃OH)\); HRESIMS m/z 231.1592 [M + H]⁺ (calcd. for C₁₂H₂₃O₄⁺, 231.1591). ¹H NMR (400 MHz in CDCl₃): δ 2.82 (1H, m, H-2), 1.29 (3H, d, J = 7.1 Hz, H-2a), 2.29 (1H, dd, J = 8.9, 12.9 Hz, H-3α), 2.15 (1H, t, J = 12.4 Hz, H-3β), 1.47 (3H, s, H-4a), 3.46 (1H, s, H-5), 1.31 (3H, s, H-6a), 1.71 (1H, m, H-7), 1.55 (1H, m, H-8α), 1.43 (1H, m, H-8β), 0.97 (3H, d, J = 7.2 Hz, H-9); ¹³C NMR (100 MHz in CDCl₃): δ 179.2 (C-1), 34.2 (C-2), 15.2 (C-2a), 40.5 (C-3), 85.9 (C-4), 22.4 (C-4a), 79.4 (C-5), 74.6 (C-6), 23.8 (C-6a), 43.6 (C-7), 17.1 (C-8), 14.6 (C-9).

19-Hydroxyprotylonolide (3). Colourless oil. HRESIMS m/z 411.2743 [M + H]⁺ (calcd. for C₁₂H₂₃O₄⁺, 411.2741). ¹H NMR (400 MHz in CDCl₃): δ 2.51 (dd, J = 10.8, 16.6 Hz, H-2), 1.95 (d, J = 16.6 Hz, H-2), 3.64 (d, J = 10.6 Hz, H-3), 1.50 (m, H-4), 4.14 (d, J = 10.0 Hz, H-5), 1.30 (m, H-6), 2.03, 1.55 (m, H-7), 2.77 (m, H-8), 6.36 (d, J = 15.5 Hz, H-10), 7.31 (d, J = 15.5 Hz, H-11),
5.66 (d, J = 10.4 Hz, H-13), 2.73 (m, H-14), 4.72 (dt, J = 7.3 Hz, H-15), 1.86, 1.59 (m, H-16), 0.95 (t, J = 7.4 Hz, H-17), 1.02 (d, J = 6.7 Hz, H-18), 4.33 (q, J = 6.2 Hz, H-19), 1.30 (d, J = 6.1 Hz, H-20), 1.29(d, J = 6.5 Hz, H-21), 1.80 (s, H-22), 1.09(d, J = 6.4 Hz, H-23); 13C NMR (100 MHz in CDCl3): δ 175.0 9 (C-1), 39.4 (C-2), 66.9 (C-3), 39.8 (C-4), 71.5 (C-5), 40.6 (C-6), 29.3 (C-7), 44.9 (C-8), 204.0 (C-9), 118.4 (C-10), 148.3 (C-11), 133.8 (C-12), 146.0 (C-13), 39.0 (C-14), 78.9 (C-15), 24.9 (C-16), 9.9 (C-17), 9.1 (C-18), 68.1 (C-19), 22.5 (C-20), 17.9 (C-21), 13.3 (C-22), 16.4 (C-23).

**Supplementary material**

Supplementary material including NMR and HRESI-MS spectra of compounds 1 and 2 are available online.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

Al-Zarban SS, Al-Musallam AA, Abbas I, Stackebrandt E, Kroppenstedt RM. 2002. *Saccharomonospora halophila* sp. nov., a novel halophilic actinomycete isolated from marsh soil in Kuwait. Int J Syst Evol Microbiol. 52:555–558.

Cho JY, Williams PG, Kwon HC, Jensen PR, Fenical W. 2007. Lucentamycins A–D, cytotoxic peptides from the marine-derived actinomycete *Nocardiopsis lucentensis*. J Nat Prod. 70:1321–1328.

Chun J, Bae KS, Moon EY, Jung SO, Lee HK, Kim SJ. 2000. *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. Int J Syst Evol Microbiol. 50:1909–1913.

Fu P, Liu P, Qu H, Wang Y, Chen D, Wang H, Li J, Zhu W. 2011. α-pyrones and diketopiperazine derivatives from the marine-derived actinomycete *Nocardiopsis dassonvillei* HR10-5. J Nat Prod. 74:2219–2223.

Hamedi J, Mohammadipanah F, Potter G, Sproer C, Schumann P, Goker M, Klenk HP. 2011. *Nocardiopsis arvandica* sp. nov., isolated from sandy soil. Int J Syst Evol Microbiol. 61:1189–1194.

Hu ZY, Li YY, Lu CH, Lin T, Hu P, Shen YM. 2010. Seven novel linear polyketides from *Xylaria* sp. NCY2. Helv Chim Acta. 93:925–933.

Kim MC, Hwang E, Kim T, Ham J, Kim SY, Kwon HC. 2014. Nocatriones A and B, photoprotective tetracenediones from a marine-derived *Nocardiopsis* sp. J Nat Prod. 77:2326–2330.

Kim MC, Kwon OW, Park JS, Kim SY, Kwon HC. 2013. Nocapyrones H–J, 3,6-disubstituted α-pyrones from the marine actinomycete *Nocardiopsis* sp. KMF-001. Chem Pharm Bull. 61:511–515.

Li YQ, Li MG, Li W, Zhao JY, Ding ZG, Cui XL, Wen ML. 2007. Griseusin D, a new pyranonaphthoquinone derivative from a alkaphilic *Nocardiopsis* sp. J Antibiot. 60:757–761.

Lin C, Lu CH, Shen YM. 2010. Three new 2-pyranone derivatives from mangrove endophytic actinomycete strain *Nocardiopsis* sp. A00203. Rec Nat Prod. 4:176–179.

Meyer J. 1976. *Nocardiosis*, a new genus of the order actinomycetales. Int J Syst Bacteriol. 26:487–493.

Minamida M, Kumagai K, ulanova D, Akakabe M, Konishi Y, Tominaga A, Tanaka H, Tsuda M, Fukushima E, Kawabata J, et al. 2014. Amphirionin-4 with potent proliferation-promoting activity on bone marrow stromal cells from a marine dinoflagellate *Amphidinium* species. Org Lett. 16:4858–4861.

Omura S, Kitao C, Matsubara H. 1980. Isolation and characterization of a new 16-membered lactone, protylonolide, from a mutant of tylosin-producing strain, *Streptomyces fradiae* KA-427. Chem Pharm Bull. 28:1963–1965.
Sabry SA, Ghanem NB, Abu-Ella GA, Schumann P, Stackebrandt E, Kroppenstedt RM. 2004. Nocardiopsis aegyptia sp. nov., isolated from marine sediment. Int J Syst Evol Microbiol. 54:453–456.

Sadakane N, Tanaka Y, Omura S. 1983. Hybrid biosynthesis of a new macrolide antibiotic by a daunomycin-producing microorganism. J Antibiot. 36:921–922.

Schneemann I, Ohlendorf B, Zinecker H, Nagel K, Wiese J, Imhoff JF. 2010. Nocapyrones A–D, γ-pyrones from a Nocardiopsis strain isolated from the marine sponge Halichondria panicea. J Nat Prod. 73:1444–1447.

Sun M, Zhang X, Hao H, Li W, Lu C. 2015. Nocarbenzoxazoles A–G, benzoxazoles produced by halophilic Nocardiopsis lucentensis DSM 44048. J Nat Prod. 78:2123–2127. doi:10.1021/acs.jnatprod.5b00031

Takahashi A, Hottan K, Saito O, Morioka M, Okami Y, Umezawa H. 1986. Production of novel antibiotic, dopisamine, by a new subspecies of Nocardiopsis mutabilis with multiple antibiotic resistance. J Antibiot. 39:175–183.

Tian S, Yang Y, Liu K, Xiong Z, Xu L, Zhao L. 2014. Antimicrobial metabolites from a novel halophilic actinomycete Nocardiopsis terrae YIM 90022. Nat Prod Res. 28:344–346.

Wu ZC, Li S, Nam SJ, Liu Z, Zhang C. 2013. Nocardiamides A and B, two cyclohexapeptides from the marine-derived actinomycete Nocardiopsis sp. CNX037. J Nat Prod. 76:694–701.

Yassin AF, Galinski EA, Wohlfarth A, Jahnke K-D, Schaal KP, Trüper HG. 1993. A new actinomycete species, Nocardiopsis lucentensis sp. nov. Int J Syst Evol Microbiol. 43:266–271.

Yasumuro K, Shibazaki M, Sasaki T, Imai H, Yamaguchi H, Suzuki K, Morioka M, Takebayasi Y. 1994. 16-membered lactone compounds from izenamicins-producing microorganism. J Antibiot. 47:250–252.

Zhang J, Tang X, Li J, Li P, de Voogd NJ, Ni X, Jin X, Yao X, Li P, Li G. 2013. Cytotoxic polyketide derivatives from the South China sea sponge Plakortis simplex. J Nat Prod. 76:600–606.

Zhao LX, Huang SX, Tang SK, Jiang CL, Duan Y, Beutler JA, Henrich CJ, McMahon JB, Schmid T, Blees JS, et al. 2011. Actinopolysporins A–C and tubercidin as a Pdcd4 stabilizer from the halophilic actinomycete Actinopolyspora erythraea YIM 90600. J Nat Prod. 74:1990–1995.