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NOTE

Lorneic acids C and D, new trialkyl-substituted aromatic acids isolated from a terrestrial *Streptomyces* sp.

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Our ongoing research into the chemical diversity of terrestrial actinomycetes associated with the plant rhizosphere has led to the discovery of rare metabolites, such as leopolic acid A[1] and juniperolide A,[2] which were isolated from *Streptomyces* sp. cultured from the rhizosphere of the plant *Juniperus excelsa*. The discovery of such rare and chemically diverse structures further prompted us to investigate this new source of biodiversity in order to expand and enhance our chances of discovering novel secondary metabolites. During our recent investigation into the secondary metabolism of rare terrestrial actinomycetes, we recovered a *Streptomyces* sp. (Lv-4-15) from the root zone of the plant *Phyllostachys viridi-glaucescens*. HPLC-DAD-MS analysis of a small-scale liquid cultivation (100 ml) revealed biosynthetically related compounds (*m/z* 260 (**1**) and 232 (**2**)) sharing a common UV spectrum ($\lambda_{max} = 210$ and 254 nm).

The *Streptomyces* sp. (Lv-4-15) was cultivated in M medium (6 l) for 8 days at 30 °C and then extracted with ethyl acetate (5 l) to give a crude extract of 112.6 mg. The crude extract was fractionated by sequential trituration with 20 ml each of hexane, CH_2Cl_2 and MeOH to afford 39.6, 40.7 and 11.6 mg fractions, respectively. The CH_2Cl_2 fraction was subsequently purified by semi-preparative reverse phase HPLC to yield compounds **1** ($t_R = 26.5$ min, 0.9 mg) and **2** ($t_R = 24.6$ min, 0.6 mg). An account of the spectroscopic analysis leading to the assignment of structures to lorneic acids C (**1**) and D (**2**) is presented below.

HRESI(+)MS analysis of lorneic acid C (**1**) (Table 1, Supplementary Figure S3) revealed a pseudomolecular ion ($[M+Na]^+$) indicative of a molecular formula ($C_{17}H_{24}O_2Na$) requiring six double bond equivalents. The NMR (methanol-d_4) (Table 2) data (Supplementary Figures S1a-d) revealed a 1,2,4-trisubstituted benzene ring as well as a (*E*)-1,2-disubstituted double bond. Analysis of the 2D NMR COSY data (Table 2) (Figure 1) identified three isolated spin systems. These included (i) a 1-substituted (*E*)-but-1-ene (C-13 to C-16); (ii) a 3,4-disubstituted toluene (C-7 to C-12, including C-17) and (iii) a 1,5-disubstituted pentane (C-2 to C-6). HMBC correlations from H-11 (δ_H 7.21) to C-13 (δ_C 127.8) and H_2-6 (δ_H 2.62) to C-8 (δ_C 128.4) established the point of attachment of the but-1-ene and the pentane side chains onto the 3,4-disubstituted toluene. The carboxylic acid functionality was positioned on the methylene H_2-2 (δ_H 2.26) of the 1,5-di-substituted pentane side chain based on HMBC correlations from both H_2-2 and H_2-3 (δ_H 1.62) to C-1 (δ_C 178.1).

HRESI(+)MS analysis of lorneic acid D (**2**) (Table 1, Supplementary Figure S4) revealed a pseudomolecular ion ($[M+Na]^+$) indicative of a molecular formula ($C_{15}H_{20}O_2Na$). A high degree of similarity between the ^1H NMR spectrum of **2** and **1** followed by a close examination of the NMR data (Supplementary Figures S2a-d) revealed that the 3,4-disubstituted toluene and the but-1-ene substituents remained unchanged, with the only change observed for the side chain now identified as butyric acid (C-1–C-4). The trialkyl-substituted aromatic acids belong to the rare class of

Table 1 Physico-chemical properties of 1 and 2

	1	*2*
Appearance	White solid	White solid
HR-ESI-MS (m/z)		
Found	283.1668 $[M+Na]^+$	255.1352 $[M+Na]^+$
Calcd	283.1674 ($C_{17}H_{24}O_2Na$)	255.1360 ($C_{15}H_{20}O_2Na$)
UV λ_{max} nm (log ε) (MeOH)	210 (4.10), 254 (3.82)	210 (4.10), 254 (3.82)

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Table 2 NMR (500 MHz, methanol-d_4) data for lorneic acids C (1) and D (2)

Position	δ_H, *mult (J in Hz) (**1**)*	δ_C[a]	*COSY*	*HMBC*	δ_H, *mult (J in Hz) (**2**)*	δ_C[a]	*COSY*	*HMBC*
1		178.1				178.6		
2	2.26, m[b]	35.5	3	1, 3, 4	2.27, m[b]	35.4	3	1, 3, 4
3	1.62, m	26.3	2, 4	1, 2, 4, 5	1.82, m	27.5	2, 4	1, 2, 4, 5
4	1.30–1.37, m	30.4	3, 5		2.66, dd (8.1, 7.0)	33.1	3	2, 3, 5, 6, 10
5	1.53, m	31.9	4, 6	3, 6, 7		136.7		
6	2.62, dd (7.4, 7.0)	33.6	5	4, 5, 8, 12	6.99, d (7.6)	130.1	7	4, 8, 10
7		137.2			6.94, d (7.6)	128.4	6	9, 15
8	6.97, d (7.7)	130.4	9	6, 10, 12		136.6		
9	6.92, d (7.7)	128.4	8	7, 11, 17	7.23, s	127.2		5, 7, 11, 15
10		136.1				137.8		
11	7.21, s	127.2		9, 13, 17	6.63, d (15.4)	127.6	12	5, 9, 13
12		137.1			6.13, dt (15.4, 6.5)	134.4	11, 13	10, 13, 14
13	6.61, d (15.6)	127.8	14	7, 11, 15	2.25, m[b]	27.2	12, 14	11, 12, 14
14	6.11, dt (15.6, 6.6)	134.3	13, 15	12, 15, 16	1.11, t (7.5)	14.1	13	12, 13
15	2.24, m[b]	27.3	14, 16	13, 14, 16	2.28, s	20.9		7, 8, 9
16	1.11, t (7.5)	14.3	15	14, 15				
17	2.27, s	21.1		9, 10, 11				

[a]Assignments supported by HSQC and HMBC.
[b]Overlapping signals.

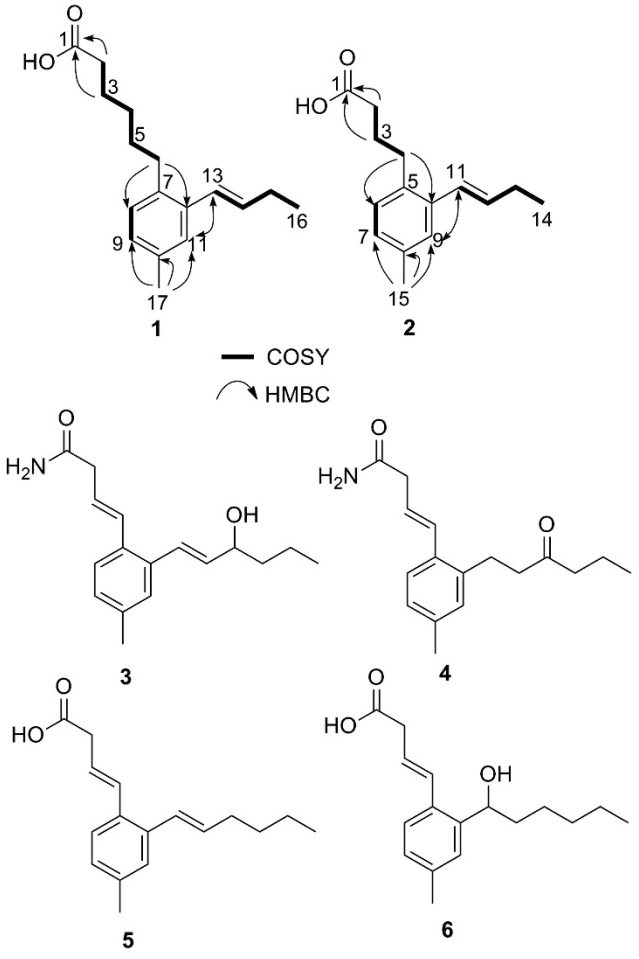

Figure 1 Key 2D NMR correlations (500 and 700 MHz, methanol-d_4) for lorneic acids C (**1**) and D (**2**) and structures of **3**–**6**.

secondary metabolites, known examples that are limited to lorneamides A (**3**) and B (**4**) isolated from an Australian marine-derived *Streptomyces* sp.,[3] and the recently described phosphodiesterase 5 inhibitors, lorneic acids A (**5**) and B (**6**) from a Japanese marine-derived *Streptomyces* sp.[4] Lorneic acids C (**1**) and D (**2**) exhibited no cytotoxic and antibacterial activity. In summary, the diversity of this rare class of trialkyl-substituted aromatic amides/acids has been further expanded, warranting further investigation of the role of these rare metabolites in nature.

EXPERIMENTAL PROCEDURE

NMR spectra were obtained on a Bruker Ascend 500 MHz spectrometer equipped with a cryoprobe system (Bruker Biospin GmbH, Rheinstetten, Germany) in the solvents indicated and referenced to residual ^{1}H signals in deuterated solvents. ESI-MS images were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector (Agilent, Waldbronn, Germany) in both positive and negative ion modes under the following conditions: Zorbax (Bremen, Germany) C_8 column, 150 × 4.6 mm, eluting with 0.4 ml min^{-1} 95% H_2O/MeCN to 5% H_2O/MeCN (with isocratic 0.01% TFA) over 22 min, and then held for 5 min. HR-MS was carried out using an UltiMate 3000 rapid separation liquid chromatography system (Dionex RSLC, Idstein, Germany) coupled to an UHR-TOF mass spectrometer (Bruker Daltonik maxis, Bremen, Germany) operating in the positive ESI mode.

Sampling was performed in the Nikitsky Botanical Garden of Crimea (Ukraine). The soil was collected from the root zone of *Phyllostachys viridi-glaucescens*. One gram of the collected soil was resuspended in 10 ml of sterile water. Serial dilutions of the soil suspension were prepared in sterile water and inoculated onto the oatmeal agar (oatmeal—40 g l^{-1}, agar—15 g l^{-1}, pH 7.5). The plates were incubated for 20 days at 28 °C. Individual colonies were transferred onto new oatmeal agar plates for further analysis and maintenance. To sequence the 16s rDNA, the chromosomal DNA of *Streptomyces* sp. Lv 4–15 was isolated according to the protocol described in the literature.[5] Based on 16s rDNA sequence analysis, strain Lv 4–15 was classified as a *Streptomyces* genus. The strain *Streptomyces* sp. Lv 4–15 is deposited in the microorganism collection of Ivan Franko Lviv National University.

Strain Lv 4–15 was cultivated in a 250-ml Schott flask containing M1 (1% starch, 0.4% yeast extract, and 0.2% peptone) prepared in distilled water (80 ml). The strains were shaken at 145 r.p.m. for 8 days at 30 °C, extracted with EtOAc (50 ml), and the organic phase concentrated *in vacuo* to yield a crude extract of 3.7 mg. The crude extracts were redissolved in MeOH, yielding a concentration of 1 mg ml^{-1}, and analyzed by HPLC-DAD-ESI(±)MS.

Five 5-l Erlenmeyer flasks containing M1 broth (1.2 l) were inoculated with starter culture (20 ml) of *Streptomyces* sp. The flasks were incubated at 30 °C on a rotary shaker at 150 r.p.m. for 8 days, extracted with EtOAc (2 × 500 ml per flask), and the organic phases concentrated *in vacuo* to yield a combined EtOAc extract (145.5 mg). The EtOAc extract was sequentially triturated with hexane, CH_2Cl_2 and MeOH (40 ml aliquots), which were concentrated

in vacuo, to yield 43.3, 56.7 and 13.3 mg partitions, respectively. The CH_2Cl_2 soluble material was further fractionated by HPLC (Zorbax, C_8 column, 250×9.4 mm, 5 µm, 3 ml min^{-1}, gradient from 10 to 100% ACN–H_2O over 30 min, with a 0.01% FA modifier) to afford lorneic acid C (**1**) ($t_R = 25.5$ min, 0.9 mg) and lorneic acid D (**2**) ($t_R = 23.8$ min, 0.6 mg).

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