

1 **Isolation and Anti-HIV-1 Integrase Activity of Lentzeosides A-F from Extremotolerant**

2 ***Lentzea* sp. H45, a strain isolated from a high altitude Atacama Desert soil**

3 **Running head: Lentzeosides A-F from Extremotolerant *Lentzea* sp. H45**

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18 The extremotolerant isolate H45 was one of several actinomycetes isolated from a high altitude
19 Atacama Desert soil collected in northwest Chile. The isolate was identified as a new *Lentzea* sp.
20 using a combination of chemotaxonomic, morphological and phylogenetic properties. Large scale
21 fermentation of the strain in two different media followed by chromatographic purification led to
22 the isolation of six new diene and monoene glycosides named lentzeosides A–F, together with the
23 known compound (Z)-3-hexenyl glucoside. The structures of the new compounds were confirmed
24 by HRESIMS and NMR analyses. Compounds **1-6** displayed moderate inhibitory activity against
25 HIV integrase.

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1 INTRODUCTION

2 Natural products are known to be a rich source of diverse chemical scaffolds for drug discovery.
3 However, their use has diminished in the past two decades, mainly due to technical barriers when
4 screening natural products in high-throughput assays against molecular targets and to their limited
5 availability for clinical trials.¹ Additionally, the discovery of new bioactive natural products is
6 challenging due to the high rate of re-discovery of known metabolites, a problem that can be
7 addressed by isolating and screening novel microorganisms from underexplored habitats, such as
8 desert biomes^{2,3}, and by incorporating rigorous dereplication procedures into all stages of the
9 discovery process. In addition, industry has realized that phenotypic screening is more effective at
10 discovering new bioactive compounds than narrow screening of molecular targets.

11 The Atacama Desert in Chile is known for its extreme aridity which has persisted for at least ~15
12 million years.⁴ Some regions in the desert were once thought to have “Mars-like” soils deemed too
13 extreme for life to exist given high levels of UV radiation, the presence of inorganic oxidants, areas
14 of high salinity, and very low concentrations of organic carbon.⁵ However, recent research has
15 revealed extraordinary bacterial diversity across a range of Atacama environments^{6,7}, and many
16 novel actinomycetes have been isolated from hyper – and extreme hyper-arid soils.^{8,9} Biological
17 and genome-guided screening of some of these actinomycetes has led to the isolation and
18 characterization of new natural products belonging to diverse structural classes and exhibiting
19 various biological activities, as exemplified by the antimicrobial chaxamycins and chaxalactins
20 isolated from *Streptomyces leeuwenhoekii* C34^T, the abenquines from *Streptomyces* sp. DB634, the
21 antitumor atacamycins from *Streptomyces* sp. C38, and the cell invasion inhibitor chaxapeptin from
22 *S. leeuwenhoekii* strain C58.¹⁰⁻¹⁶

1 As part of our ongoing program to investigate the extremobiosphere as a source of new natural
2 products, we have focused our attention on *Lentzea* sp. strain H45 which was isolated from a high
3 altitude Atacama Desert soil and shown to produce a specific pattern of secondary metabolites
4 based on its LCMS profile and associated NMR data. Chemical screening of the strain on two
5 different cultivation media led to the isolation of six new and one known diene, as well as monoene
6 glycosides (Figure 1). Structure elucidation of these compounds was based on HRESIMS, 1D and
7 2D NMR analyses. The isolated compounds were screened for their inhibitory activity against HIV-
8 1 integrase, an enzyme that is critical for the integration of the HIV genome into the host genome.¹⁷
9 This target is very attractive for the development of new anti-HIV therapy as it is selective for the
10 virus.

11 **RESULTS**

12 In this study, actinobacterial isolate H45 was obtained from subsurface soil sample collected at an
13 altitude > 5000 m in the vicinity of the ALMA Observatory in the Atacama Desert, Chile. The
14 chemotaxonomic, morphological, physiological and phylogenetic properties of strain H45 are in
15 line with its classification in the genus *Lentzea*.^{18,19} The strain was found to be an aerobic, Gram-
16 positive actinomycete that formed a branched substrate mycelium and aerial hyphae that
17 fragmented into rod-shaped elements (Figure 2), while chemical analysis of whole-organism
18 hydrolysates revealed the presence of *meso*-diaminopimelic acid and galactose, mannose and
19 ribose, and the predominant isoprenologue was tetrahydrogenated menaquinone with nine isoprene
20 units (MK9[H4]). The organism formed a distinct branch at the periphery of the *Lentzea* 16S rRNA
21 gene tree, a position that was supported by all of the tree-making algorithms and by a 69% bootstrap
22 value (Figure 3). The strain was most closely, albeit loosely, related to *Lentzea kentuckyensis*^T

1 NRRL B-34416. These results clearly show that the isolate forms a new centre of taxonomic
2 variation in the genus *Lentzea* consistent with its recognition as a putative new species.

3 Large scale fermentation of *Lentzea* sp. strain H45 on two different media supplemented with
4 Diaion HP-20 resin was followed by methanolic extraction of the resin beads; subsequently, the
5 crude extract was subject to multiple steps of medium and high pressure preparative C-18
6 chromatography which resulted in the isolation of six new and one known natural product based
7 on HRESIMS and NMR data (Figure 1).

8 HRESIMS analysis of compound **1** yielded a $[M+Na]^+$ ion at m/z 281.1351 indicating a molecular
9 formula of $C_{13}H_{22}O_5$. The analysis of 1H , ^{13}C and multiplicity-edited HSQC NMR spectra revealed
10 the presence of one methylene (δ_C/δ_H 20.5/2.16) and one oxymethylene group (δ_C/δ_H 68.3/4.26,
11 4.08), one methyl doublet (δ_C/δ_H 17.9/1.15), one methyl triplet (δ_C/δ_H 14.2/0.94), four olefinic
12 resonances (δ_C/δ_H 133.7/5.42, 129.6/5.71, 127.4/5.94, 127.0/6.58) and five oxymethine groups
13 (δ_C/δ_H 102.1/4.15, 76.4/3.09, 75.3/2.80, 73.7/2.98, 71.5/3.15). Furthermore, the 1H NMR spectrum
14 showed 3 hydroxy groups resonating at δ_H 4.8-5.1. The COSY correlations of H₂-1 through H₃-7
15 indicated a spin system comprising two conjugated olefins with a terminal ethyl group and
16 established a (2*E*,4*Z*)-heptadien-1-ol substructure (Figure 4). This was corroborated by the HMBC
17 correlations of H₂-1/C-3, H-2/C-4, and H₃-7 to both C-5 and C-6 (Figure 4). The COSY spectrum
18 indicated a second spin system which included the signals H-1' to H₃-6', which in combination with
19 the HMBC correlation of H-1'/C-5' suggested a β -L-quinovopyranose (β -L-6-
20 deoxyglucopyranose), which was confirmed by the optical rotation, the coupling constant of the
21 anomeric proton ($J = 7.8$ Hz), axial-axial proton couplings for H1'/H2', H2'/H3', H3'/H4', H4'/H5'
22 (Table 2) as well as the agreement of the ^{13}C NMR data and the ROESY correlations with reported
23 data.²⁰ The sugar moiety was further confirmed by acid hydrolysis of **1** upon which α -L-quinovose

1 was detected by co-chromatography on TLC in comparison with authentic sugar samples. Finally,
2 the connectivity of the two substructures was confirmed by the HMBC correlation of H-1'/C-1.
3 The geometry of the two double bonds was established as (2*E*,4*Z*) based on coupling constant data
4 of H-2 and H-5 with values of 16.1 Hz and 10.1 Hz, respectively which was further supported by
5 ROESY correlations of H-2/H-4, H-3/H₂-1, H-4/H-5 and H-3/H₂-6 (Figure 4). Based on this
6 evidence, **1** was identified as a new natural product for which the name lentzeoside A is proposed.

7 Compound **2** shared the chemical formula with **1** as C₁₃H₂₂O₅ based on the HRESIMS analysis that
8 gave an [M+Na]⁺ ion at *m/z* 281.1351. Comparison of the ¹H and ¹³C NMR data of **2** with those
9 obtained for **1** revealed that **2** was the 1'-epimer of **1**, and the presence of an α-L-rhamnopyranose
10 was corroborated by the optical rotation, the very small coupling of the anomeric proton and the
11 agreement of the ¹³C NMR data and the ROESY correlations with the reported data.²¹ The sugar
12 moiety was further confirmed by acid hydrolysis of **2** upon which α-L-rhamnose was detected by
13 co-chromatography on TLC in comparison with authentic sugar samples. Thus, compound **2** was
14 identified as a new secondary metabolite for which we propose the name lentzeoside B.

15 The molecular formula C₁₃H₂₂O₆ for compound **3** was established on the basis of HRESIMS
16 analysis that afforded an [M+Na]⁺ ion at *m/z* 297.1299 indicating one more oxygen atom than in
17 **1**. The ¹H, ¹³C NMR and multiplicity-edited HSQC spectra showed a close similarity to those
18 obtained for **1**, but indicated that the β-L-rhamnopyranose unit in **1** had been replaced by a
19 β-D-glucopyranose. This assumption was confirmed by the optical rotation, the magnitude of the
20 coupling constant analysis of the anomeric proton (*J*=7.9 Hz) as well as the agreement of the ¹³C
21 NMR data and the ROESY correlations with the reported data.²² The sugar moiety was further
22 confirmed by acid hydrolysis of **3** upon which β-D-glucose was detected by co-chromatography

1 on TLC in comparison with authentic sugar samples. Therefore, the structure of **3** was established
2 as depicted, representing a new natural product for which the name lentzeoside C is proposed.

3 The HRESIMS analysis of compound **4** provided a $[M+Na]^+$ ion at m/z 281.1349 indicating it to
4 be an isomer of **2**. Analysis of the NMR spectra and the optical rotation indicated that the two
5 compounds contained a α -L-rhamnopyranose, but differed in the geometry of the diene moiety.
6 The coupling constant data of H-2 and H-5 with values of 16.1 Hz and 15.5 Hz, respectively
7 indicated *E*-configurations for both olefinic groups in **4**. This was further supported by the ROESY
8 correlations of H₂-1/H-3, H-3/H-5, H-2/H-4 and H-4/H₂-6 (Figure 4). On that basis, **4** was
9 identified as a new secondary metabolite for which the name lentzeoside D is proposed.

10 The molecular formula of compounds **5** and **6** was deduced as C₁₂H₂₂O₅ as HRESIMS analysis of
11 $[M+Na]^+$ and $[M+H]^+$ ions at m/z 269.1351 and 247.1545, respectively indicating one carbon atom
12 less than **1**. NMR analysis and optical rotation of **5** and **6** indicated that they contained an
13 α -L-rhamnopyranose and a β -L-quinovopyranose as described above for **2** and **1**, respectively,
14 which was connected to the same (*Z*)-3-hexenyl side chain. The presence of the latter was evident
15 from COSY correlations of H₂-1 through H₃-6. As the signals for H-3 and H-4 exhibited non-first
16 order coupling, the *E*-configuration of the double bond was established based on ROESY
17 correlations of H₂-1/H-3, H-4/H₃-6 and H₂-2/H₂-5 (Figure 4). Based on this information,
18 compounds **5** and **6** were identified as new natural products for which the names lentzeoside E and
19 F, respectively, are proposed.

20 Based on the NMR and accurate mass analyses, compound **7** was identified as (*Z*)-3-hexenyl
21 glucoside, this compound has been isolated from several plant sources, such as *Epimedium*
22 *grandiflorum*.²³

1 Compounds **1-6** were evaluated for their anti-HIV integrase activity at different concentrations.
2 Compounds **3, 4** and **5** inhibited HIV integrase with IC₅₀ values of 21 μM, 16 μM and 21 μM
3 respectively. Compounds **1, 2** and **6** were only weakly active, they did not give 50 % inhibition of
4 the enzyme activity up to a concentration of 100 μM.

5 **DISCUSSION**

6 A major draw of filamentous actinomycetes, especially streptomycetes, is their unrivalled capacity
7 to synthesize structurally diverse bioactive metabolites. In this context, the present study provides
8 further evidence that taxonomically novel actinomycetes isolated from Atacama Desert soils are a
9 rich source of new chemical entities. It is especially interesting that the new compounds,
10 lentzosides A-F, are derived from a novel *Lentzia* strain as previously new specialized metabolites
11 from Atacama Desert actinomycetes have come from novel *Streptomyces* strains,⁷ notably from a
12 deep seated 16S rRNA gene clade that can be equated with the species, *Streptomyces*
13 *leeuwenhoekii*.¹⁰⁻¹⁶ The genus *Lentzia* was validly published in 1995,²⁴ but to date does not appear
14 to have been studied in terms of its natural product chemistry, though *Lentzia* sp. 7887 effects the
15 biotransformation of FR901459, a novel derivative of cyclosporin.²⁵

16 Chemical screening of this novel *Lentzia* sp. strain H45 on two different media led to the isolation
17 of six new diene and monoene glycosides and the known compound (*Z*)-3-hexenyl glucoside. It is
18 interesting to note that this class of metabolites was not traced before from microbial sources and
19 the most closely related known compound **7** which was also encountered in the present study had
20 previously been obtained from plant sources. Due to the close similarity of lentzeosides, it could
21 be argued that they were artifacts rather than natural products. To preclude this assumption and
22 confirm them as natural products, they were directly detected by LCMS analysis of the fresh

1 bacterial culture broth before the inclusion of the Diaion HP20, extraction and purification process
2 with the exact retention times, UV absorption and accurate mass.

3 Compounds **1-6** were found to inhibit HIV-1 integrase *in vitro*. Treatment of HIV usually involves
4 a combination therapy of different drugs that target different stages of the viral replication cycle, a
5 procedure that overcomes the development of resistance in the virus due to its high mutation rate²⁶.
6 HIV integrase is one of the key enzymes in the virus replication cycle as it is responsible for the
7 integration of the reverse transcribed viral cDNA into the host cell genome.²⁷ Raltegravir[®] is the
8 first FDA clinically approved HIV integrase inhibitor used to treat both HIV-1 infections in
9 treatment-experienced adult patients who have evidence of viral replication and HIV-1 strains
10 resistant to multiple antiretroviral agents.²⁸

11 **EXPERIMENTAL PROCEDURES**

12 **General Experimental Procedures.** NMR data were acquired on a Varian VNMRS 600 MHz NMR
13 spectrometer and LC-HRESIMS data obtained by using a LTQ/XL Orbitrap coupled to the HPLC
14 system (PDA detector, PDA autosampler, and pump) under the following conditions: capillary
15 voltage of 45 V, capillary temperature of 260 °C, auxiliary gas flow rate of 10–20 arbitrary units,
16 sheath gas flow rate of 40–50 arbitrary units, spray voltage of 4.5 kV with a mass range of
17 100–2000 amu (maximal resolution of 30000). For LC/MS, a C18 analytical HPLC column (5 µm,
18 4.6 mm × 150 mm) was used with a mobile phase of 0 to 100% MeOH over 30 min at a flow rate
19 of 1 mL.min⁻¹. Optical rotations were recorded using a Bellingham + Stanley ADP410 polarimeter.
20 Medium Pressure Liquid Chromatographic separations were carried out using a Biotage SP1 flash
21 system fitted with a reversed phase 40iM cartridge (KP-C18-HSTM, 40 x 150 mm), the detection
22 was carried out at 220 and 254 nm. For RP-HPLC separations, a SunFire (C₁₈, 250 x 10 mm, 5 µm
23 i.d.) column connected to an Agilent 1200 series binary pump was used and monitored with an

1 Agilent photodiode array detector, the detection was carried out at 220, 254, 280, and 320 nm. Acid
2 hydrolysis of the isolated compounds was performed as reported before.²⁹ HPLC solvents and the
3 authentic sugar samples were obtained from Sigma-Aldrich, the ingredients for fermentation were
4 from Oxoid UK and Sigma-Aldrich and the Diaion HP-20 resin from Resindion S.R.L., a
5 subsidiary of Mitsubishi Chemical Co., Binasco, Italy.

6 ***Microbial taxonomy and fermentation conditions.*** Strain H45 was isolated from a subsurface soil
7 sample collected at 5048 meters above sea level on the Chajnantor plateau, in northeast Chile
8 (23°00'48.8"S/67°45'30.8"W) by one of us (MG) in November 2012. The strain was isolated on
9 Gauze's No. 1 agar³⁰ supplemented with the antifungal antibiotics cycloheximide and nystatin
10 (each at 25 µg.mL⁻¹) using the procedure described by Okoro *et al.*⁸ The strain has phenotypic
11 properties consistent with its classification in the genus *Lentzea* and forms a distinct phyletic line
12 in the *Lentzea* 16S rRNA gene tree. The *Lentzea* isolate has been deposited in the NCIMB and
13 NRRL public service collections under the accession numbers 14966 and B-65282 respectively,
14 the GenBank accession number of the 16S rRNA gene sequence of the strain is LT009512. Two
15 cultivation media were used for large scale fermentation of the strain: Medium 410 contained
16 glucose (10.0 g), glycerol (10.0 g), casamino acids (15.0 g), oatmeal (5.0 g), peptone (10.0 g),
17 yeast extract (5.0 g), CaCO₃ (1.0 g) and distilled water to 1 L, pH 7.0; and modified ISP2 medium
18 malt extract (4.0 g), yeast extract (10.0 g), dextrose (10.0 g), glycerol (10.0 g), and distilled water
19 to 1 L, pH 7.0. The strain was grown in 4 L of each of these media by shaking at 180 rpm in a
20 shaker incubator at 30 °C for 7 days when HP-20 resin beads was added followed by shaking at
21 180 rpm for 6 h prior to centrifugation at 3000 rpm for 15 min.

22 ***Extraction and isolation.*** Biomass of the strain H45 and HP-20 resin beads were extracted with
23 methanol (MeOH × 2) and acetone (×1) and the resultant extracts combined and concentrated *in*

1 *vacuo*. The combined extracts were purified using the Biotage flash system with a gradient of
2 MeOH in water (5-100% in 12 column volumes). The eluted fractions of both media were screened
3 using LCMS and ¹H NMR and the interesting fractions further purified using RP-HPLC. For
4 medium 410, the second fraction was purified using a gradient of MeOH in H₂O/MeOH (95:5)
5 (25-100% in 25 min, flow rate 2.0 mL/min) to obtain compounds **1** (3.1 mg), **2** (1.0 mg), **3**
6 (2.2 mg), **4** (1.0 mg), and **5** (2.0 mg). In the case of the modified ISP2 medium, the fourth fraction
7 was purified using a gradient of MeOH in H₂O/MeOH (95:5) (50-100% in 25 min, flow rate
8 2.2 mL/min) to obtain compounds **1** (3.1 mg), **2** (1.1 mg), **5** (3.4 mg), **6** (3.4 mg), and **7** (2.2 mg).

9 Lentzeoside A (**1**): Light yellow powder; [α]²⁰_D -25 (c 0.15, MeOH); UV (MeOH) λ_{max} (log ε) at
10 258 (3.2), 268 (3.6) and 280 (2.7) nm; ¹H and ¹³C NMR data (Tables 1-2); HRESIMS *m/z* [M+Na]⁺
11 at 281.1351 indicating the molecular formula C₁₃H₂₂O₅ (calculated [M+Na]⁺ ion at *m/z* 281.1359).

12 Lentzeoside B (**2**): Light yellow powder; [α]²⁰_D -31 (c 0.18, MeOH); UV (MeOH) λ_{max} (log ε) at
13 258 (3.1), 270 (3.4) and 280 (2.5) nm; ¹H and ¹³C NMR data (Tables 1-2); HRESIMS *m/z* [M+Na]⁺
14 at 281.1351 indicating the molecular formula C₁₃H₂₂O₅ (calculated [M+Na]⁺ ion at *m/z* 281.1359).

15 Lentzeoside C (**3**): Light yellow powder; [α]²⁰_D +38 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) at
16 258 (3.2), 274 (3.8) and 282 (2.9) nm; ¹H and ¹³C NMR data (Tables 1-2); HRESIMS *m/z* [M+Na]⁺
17 at 297.1299 indicating the molecular formula C₁₃H₂₂O₆ (calculated [M+Na]⁺ ion at *m/z* 297.1309).

18 Lentzeoside D (**4**): Light yellow powder; [α]²⁰_D -22 (c 0.18, MeOH); UV (MeOH) λ_{max} (log ε) at
19 258 (3.1), 270 (3.4) and 280 (2.5) nm; ¹H and ¹³C NMR data (Tables 1-2); HRESIMS *m/z* [M+Na]⁺
20 at 281.1349 indicating the molecular formula C₁₃H₂₂O₅ (calculated [M+Na]⁺ ion at *m/z* 281.1359).

1 Lentzeoside E (**5**): colorless powder; $[\alpha]^{20}_{\text{D}} -29$ (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) at 239
2 (3.6) nm; ^1H and ^{13}C NMR data (Tables 1-2); HRESIMS m/z $[\text{M}+\text{Na}]^+$ at 269.1351 indicting the
3 molecular formula $\text{C}_{12}\text{H}_{22}\text{O}_5$ (calculated $[\text{M}+\text{Na}]^+$ ion at m/z 269.1359).

4 Lentzeoside F (**6**): colorless powder; $[\alpha]^{20}_{\text{D}} -25$ (c 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) at 241
5 (3.4) nm; ^1H and ^{13}C NMR data (Tables 1-2); HRESIMS m/z $[\text{M}+\text{H}]^+$ at 247.1545 indicting the
6 molecular formula $\text{C}_{12}\text{H}_{22}\text{O}_5$ (calculated $[\text{M}+\text{H}]^+$ ion at m/z 247.1540).

7 ***Anti-HIV-1 integrase activity.*** The HIV-1 integrase inhibitory activities of compounds 1-6 were
8 evaluated using a kit purchased from XpressBio Life Science Products (Frederick, MD 21705,
9 USA) and by following the manufacturer's protocol. Briefly, biotin-linked HIV-1 LTR U5 donor
10 substrate (DS) DNA was applied to a streptavidin-coated 96-well plate, the test compounds were
11 then added along with target substrate DNA and HIV integrase. The integrase processes the HIV-1
12 LTR U5 and catalyzes the strand transfer recombination reaction to integrate the DS DNA into the
13 target substrate DNA. The products of these reactions were detected colorimetrically using an
14 HRP-labeled antibody, sodium azide was used as a positive inhibitory control. Each compound
15 was tested at 4 different concentrations (100, 50, 12.5 and 3.1 μM) in triplicate.

16 **CONFLICT OF INTEREST**

17 The authors declare no conflict of interest.

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1 **Figure legends**

2 **Figure 1.** Compounds isolated from *Lentzea* sp. H45.

3 **Figure 2.** Scanning electron micrograph of *Lentzea* sp. H45 showing fragmentation of aerial
4 hyphae into rod-shaped elements following growth on ISP 3 agar after incubation at 28 °C for 10
5 days. Bar 1µm.

6 **Figure 3.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing
7 relationships between isolate H45 and the type strains of *Lentzea* and *Lechevalieria* species.
8 *Asterisks* indicate branches of the tree that were also recovered using the maximum-likelihood and
9 maximum-parsimony tree-making methods. Numbers at the nodes indicate levels of bootstrap
10 support based on a neighbour-joining analysis of 1000 resampled datasets, only values above 50%
11 are shown.

12 **Figure 4.** Key COSY, HMBC and ROESY correlations of compounds **1**, **4**, and **5**.

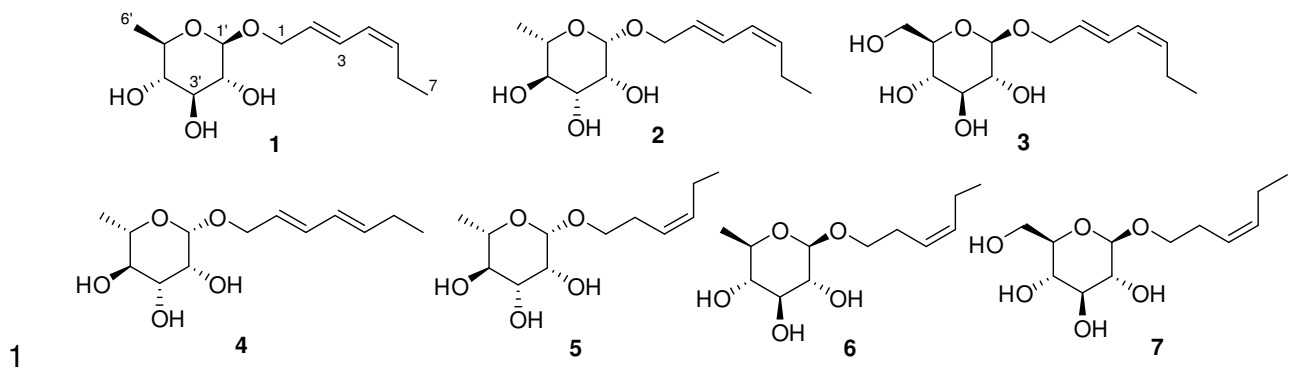
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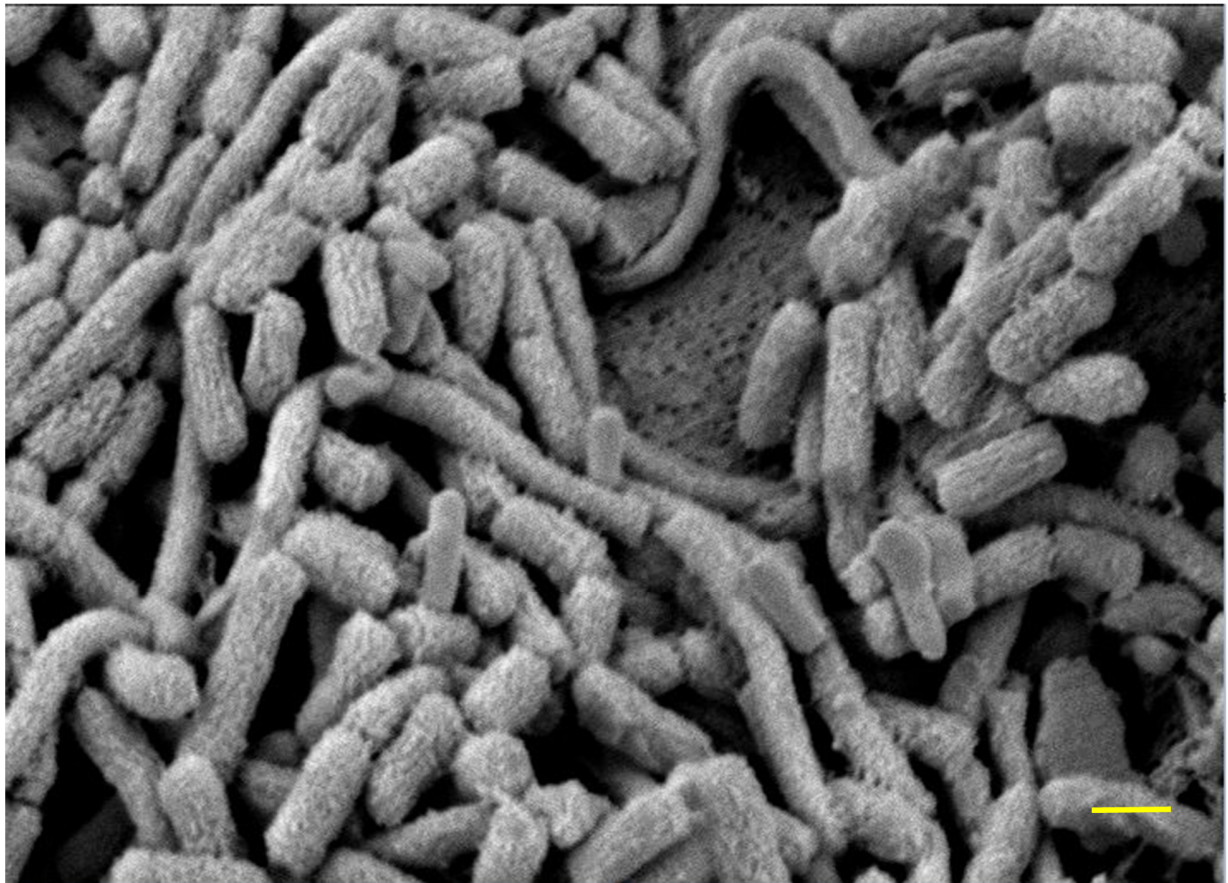
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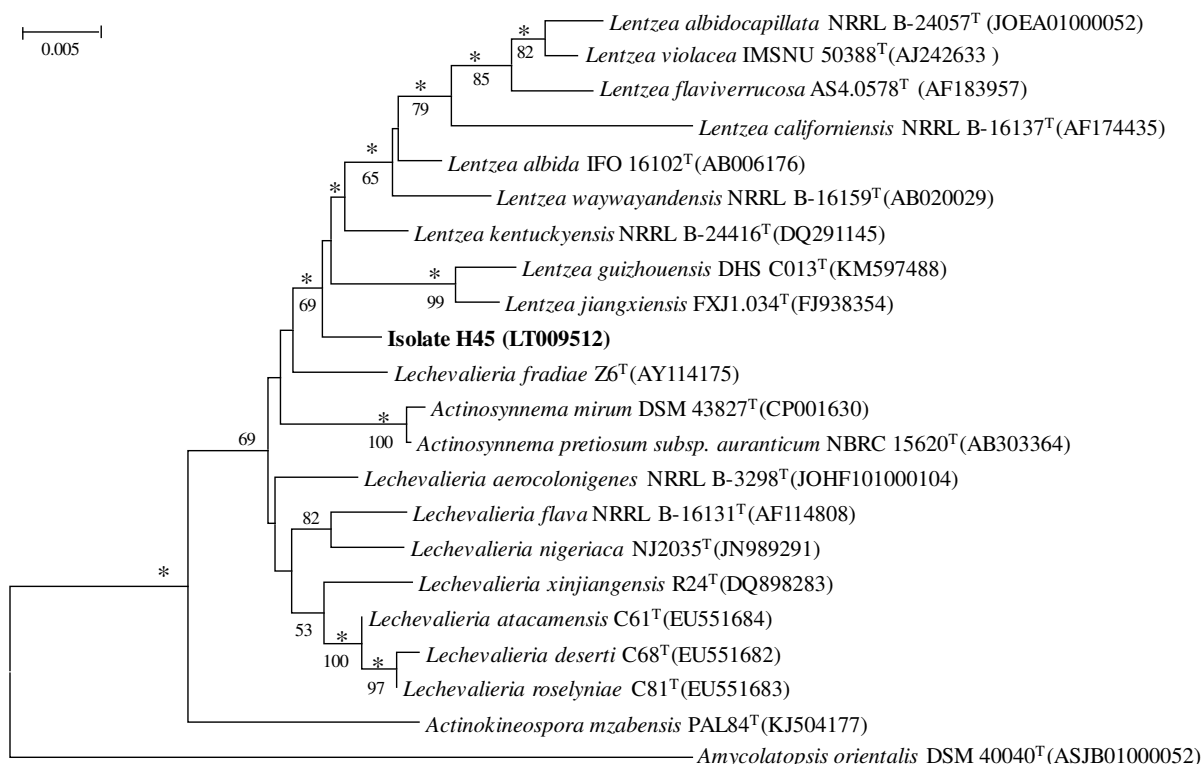
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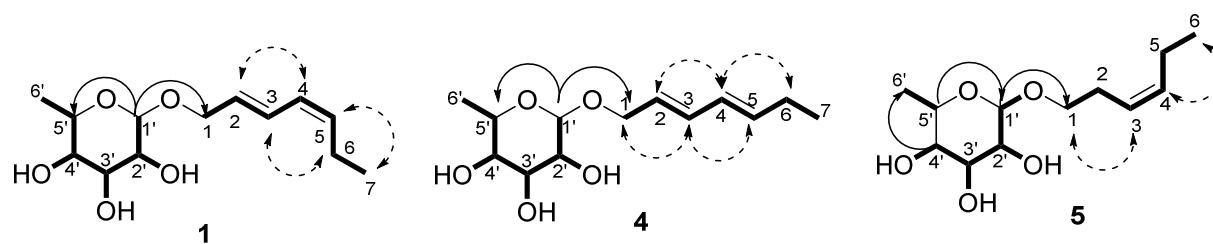
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4 **Figure 2.** Scanning electron micrograph of *Lentzea* sp. H45 showing fragmentation of aerial
 5 hyphae into rod-shaped elements following growth on ISP 3 agar after incubation at 28 °C for 10
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 2 **Figure 3.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing
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 4 Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood and
 5 maximum-parsimony tree-making methods. Numbers at the nodes indicate levels of bootstrap
 6 support based on a neighbour-joining analysis of 1000 resampled datasets, only values above 50%
 7 are shown.



9 **Figure 4.** Key COSY (—), HMBC (↷) and ROESY (↻) correlations of
 10 compounds 1, 4, and 5.

1 **Table 1.** ^{13}C (150 MHz) NMR spectroscopic data for lentzeosides A–F (**1–6**) in $\text{DMSO-}d_6$

Position	1	2	3	4	5	6
	$\delta\text{C, mult.}$	$\delta\text{C, mult.}$	$\delta\text{C, mult.}$	$\delta\text{C, mult.}$	$\delta\text{C, mult.}$	$\delta\text{C, mult.}$
1	68.3, CH ₂	66.5, CH ₂	68.2, CH ₂	68.3, CH ₂	66.1, CH ₂	68.2, CH ₂
2	129.6, CH	129.5, CH	129.6, CH	127.1, CH	27.2, CH	27.6, CH
3	127.0, CH	127.2, CH	127.1, CH	132.5, CH	125.4, CH	125.2, CH
4	127.4, CH	127.3, CH	127.4, CH	128.5, CH	133.0, CH	132.9, CH
5	133.7, CH	133.9, CH	133.7, CH	136.5, CH	20.1, CH ₂	20.1, CH ₂
6	20.5, CH ₂	20.5, CH ₂	20.5, CH ₂	25.0, CH ₂	14.2, CH ₃	14.2, CH ₃
7	14.2, CH ₃	14.1, CH ₃	14.2, CH ₃	13.4, CH ₂		
1'	102.1, CH	99.3, CH	102.0, CH	99.1, CH	99.9, CH	102.7, CH
2'	73.7, CH	70.7, CH	73.5, CH	70.7, CH	70.7, CH	73.6, CH
3'	76.4, CH	70.5, CH	76.7, CH	70.5, CH	70.5, CH	76.5, CH
4'	75.3, CH	72.0, CH	68.2, CH	72.0, CH	72.0, CH	75.3, CH
5'	71.5, CH	68.5, CH	76.9, CH	68.5, CH	68.4, CH	71.5, CH
6'	17.9, CH ₃	17.9, CH ₃	61.1, CH ₂	17.9, CH ₃	17.9, CH ₃	17.9, CH ₃

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1 **Table 2.** ^1H (600 MHz) NMR spectroscopic data for lentzeosides A–F (**1-6**) in $\text{DMSO-}d_6$

Position	1	2	3	4	5	6
	δ_{H} , mult. (J in Hz)	δ_{H} , mult. (J in Hz)	δ_{H} , mult. (J in Hz)	δ_{H} , mult. (J in Hz)	δ_{H} , mult. (J in Hz)	δ_{H} , mult. (J in Hz)
1	4.26 (dd, 13.2, 5.4)	4.11 (dd, 12.9, 5.3)	4.32 (dd, 13.7, 5.8)	4.06 (dd, 12.2, 5.3)	3.51 (m)	3.66 (m)
	4.08 (dd, 13.1, 5.2)	3.97 (dd, 12.7, 5.2)	4.10 (dd, 13.6, 6.5)	3.91 (dd, 12.4, 5.1)		3.42 (m)
2	5.71 (dt, 16.1, 6.3)	5.73 (dt, 15.9, 6.1)	5.73 (dt, 15.7, 5.9)	5.63 (dt, 16.1, 5.8)	2.24 (m)	2.26 (m)
3	6.58 (t, 14.5)	6.51 (t, 14.2)	6.59 (dd, 15.2, 11.2)	6.18 (t, 15.3)	5.32 (m)	5.33 (m)
4	5.94 (t, 11.2)	5.96 (t, 11.3)	5.96 (t, 11.1)	6.04 (t, 15.1)	5.42 (m)	5.42 (m)
5	5.42 (dt, 10.1, 6.7)	5.43 (dt, 10.5, 6.2)	5.42 (dt, 10.9, 6.5)	5.75 (dt, 15.5, 6.5)	2.01 (m)	2.01 (m)
6	2.16 (m)	2.16 (m)	2.16 (m)	2.07 (m)	0.92 (t, 7.6)	0.92 (t, 7.6)
7	0.94 (t, 7.5)	0.95 (t, 7.5)	0.94 (t, 7.5)	0.96 (t, 7.4)		
1'	4.15 (d, 7.8)	4.57 (br s)	4.15 (d, 7.9)	4.56 (br s)	4.54 (br s)	4.12 (d, 7.8)
2'	2.98 (t, 7.5)	3.41 (m)	2.97 (m)	3.40 (m)	3.39 (m)	2.94 (t, 7.9)
3'	3.09 (t, 8.6)	3.60 (br s)	3.12 (t, 7.6)	3.59 (br s)	3.57 (br s)	3.08 (t, 8.8)
4'	2.80 (t, 7.6)	3.18 (m)	3.04 (m)	3.17 (m)	3.17 (t, 8.9)	2.79 (t, 8.6)
5'	3.15 (m)	3.38 (m)	3.05 (m)	3.38 (m)	3.37 (m)	3.15 (m)
6'	1.15 (d, 6.1)	1.14 (d, 6.4)	3.66 (dd, 11.5, 5.6)	1.13 (d, 6.1)	1.12 (d, 6.3)	1.14 (d, 6.3)
			3.43 (m)			
OH-2'	5.07 (br s)		5.08 (d, 4.4)	4.74 (br s)	4.72 (br s)	4.91 (d, 18.1)
OH-3'	4.96 (br s)		4.91 (d, 3.9)		4.51 (br s)	4.94 (br s)
OH-4'	4.96 (br s)		4.96 (br s)	4.47 (br s)	4.70 (br s)	4.91 (br s)
OH-6'			4.49 (t, 5.7)			

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