

New Rancinamycin Analogues from *Streptomyces lincolnensis*Mi Ae Kim,^{†,#} Eun La Kim,[†] Kyoung-Ja Kim,[‡] Jongki Hong,[§] Baoquan Bao,[†] and Jee H. Jung^{†,*}[†]College of Pharmacy, Pusan National University, Busan 609-735, Korea. *E-mail: jhjung@pusan.ac.kr[‡]Department of Biotechnology, College of Life Science, Soonchunhyang University, Asan 336-745, Korea[§]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea[#]Korea Food and Drug Administration, Busan 608-829, Korea

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Streptomyces is the largest bacterial genus with antibiotic secondary metabolites. It is also the source of immunosuppressants, anti-cancer, and anti-hyperlipidemic lead compounds. *Streptomyces lincolnensis* produces lincomycin and related antibiotics. Rancinamycins I-IV and gamma-aminobutyric acid (GABA) as antibiotics and a growth related factor, respectively, were also reported from *S. lincolnensis*.^{1,2} As part of an ongoing search for antioxidant compounds from *S. lincolnensis*, protocatechualdehyde (**4**) was isolated from a butanol fraction of the extract.³ In a continuing study on the same strain, two new cyclitol derivatives, which are designated lincitols A (**1**) and B (**2**), along with a benzaldehyde derivative (**3**) were isolated from the butanol fraction (Figure 1). This paper describes the isolation, structure elucidation, and biological evaluation of the isolated compounds.

Lincitol A (**1**) was obtained as a colorless oil. FABMS and HRFABMS revealed a pseudomolecular ion at m/z 269 $[M + Na]^+$, and a molecular formula of $C_{11}H_{18}O_6$, respectively. The precise mass of the $[M + Na]^+$ ion at m/z 269.1021 matched well with the expected formula of $C_{11}H_{18}O_6Na$ ($\Delta +2.0$ mmu). The 1D

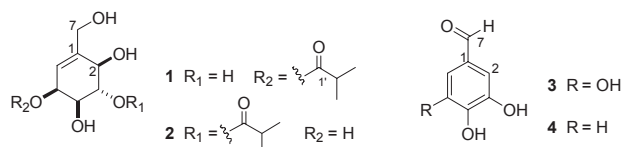


Figure 1. Structures of the compounds isolated from *Streptomyces lincolnensis*.

and 2D NMR data showed the characteristic signals of cyclitol and an isobutyl ester. The ^{13}C and DEPT spectra showed 11 carbons due to two methyl (δ_C 18.2), one methine (δ_C 34.0), four oxymethines (δ_C 73.3, 72.7, 69.8, and 69.2), one oxymethylene (δ_C 61.5), one olefinic (δ_C 117.4), and two quaternary carbons (δ_C 146.0 and 177.3). In the HMBC spectrum, the key correlations from H-7 (δ_H 4.18) to C-1, C-2, and C-6, and from H-5 (δ_H 5.38) to C-1, C-6, and C-1' (δ_C 177.3) were observed. The assigned planar structure was similar to rancinamycin Ia isolated previously from *S. lincolnensis*.⁵

Lincitol A (**1**) is the reduced alcohol form of rancinamycin Ia. The stereochemistry was deduced to be identical to that of another analogous compound, streptol, on the basis of comparison of the coupling constants and specific optical rotation value. Streptol (also known as valienol) is a plant growth regulator that was isolated from a culture of an unidentified *Streptomyces* sp.⁶ Streptol shares the same cyclitol skeleton with **1** (Figure 2) and has the same absolute configuration at the chiral carbons (C-2 to C-5) as α -D-glucose.⁷ Table 1 shows highly identical coupling patterns and constants (Hz) for the ring protons of streptol and lincitol A (**1**). The vicinal couplings between H-2, 3, and 4 (7.5, 10.5 Hz) were significantly strong, indicating the diaxial (anti-face) positioning of each protons. The NOE correlation between H-4 (δ_H 3.63) and H-2 (δ_H 4.0) corroborated the relative configuration at C-2, -3, and -4, which indicates a half-chair conformation rather than a boat conformation (Figure 3). In addition, Lincitol A (**1**) showed the same sign of optical rotation ($[\alpha]_D^{25} +96.5$) to that of streptol ($[\alpha]_D^{25} +91.8$).⁸ On the basis of aforementioned data, the absolute configuration of **1** was

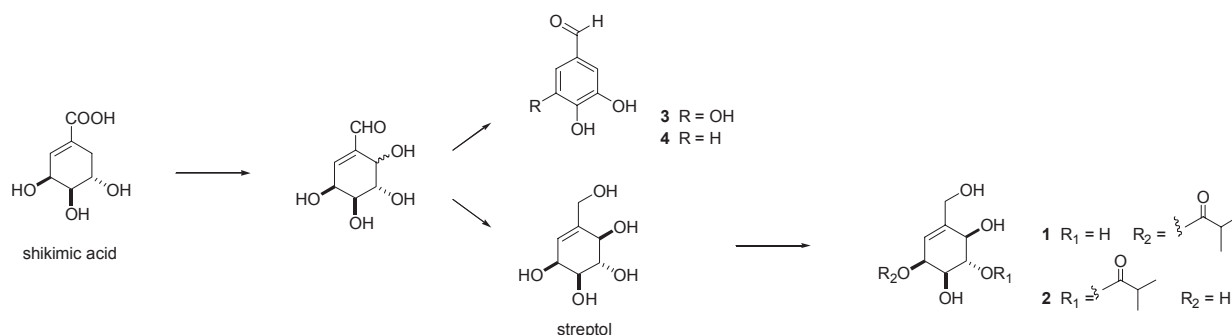


Figure 2. Possible biogenesis of compounds **1-4**.^{5,6}

Table 1. Comparison of the coupling constants (Hz) of lincitols (**1** and **2**) and streptol

position	streptol ^a	lincitol A (1) ^b	lincitol B (2) ^b
2	dq, 8, 1	d, 7.5	d, 8.0
3	dd, 11, 8	dd, 10.5, 7.5	dd, 10.5, 8.0
4	dd, 11, 4	dd, 10.5, 4.0	dd, 10.5, 5.0
5	dd, 4, 5	dd, 4.0, 5.5	dd, 5.0, 5.0
6	dq, 5, 1	dd, 5.5, 1.5	dd, 5.0, 1.5
7	d, 14 dq, 14, 1	s	s

^aMeasured at 400 MHz, in D₂O.⁶ ^bMeasured at 500 MHz, in CD₃OD.

defined as (1*S*,2*R*,3*S*,4*R*).

Lincitol B (**2**) was obtained as a light gray oil. The precise mass of the [M + Na]⁺ ion at *m/z* 269.1019 matched well with the expected formula of C₁₁H₁₈O₆Na ($\Delta +1.8$ mmu), which is identical to that of **1**. The ¹H and ¹³C NMR data of **2** were also similar to those of **1** except for the substitution position of an isobutyl ester group. The upfield shift at δ_{H} 5.19 compared with δ_{H} 5.38 of the proton belonging to carbon bearing an isobutyl ester group in **2** and **1**, respectively suggested that the location of the isobutyl ester group in **2** is far from the double bond of the ring. The HMBC spectrum revealed the key correlation from H-3 to C-1', indicating that the isobutyl ester group in **2** is located at C-3. The coupling constants of the ring protons were similar to those of streptol (Table 1) suggesting the same relative stereochemistry with streptol. The absolute configuration was defined to be the same as streptol and lincitol A (**1**) on the basis of the positive optical rotation value ($[\alpha]_{\text{D}}^{25} +52.0$).

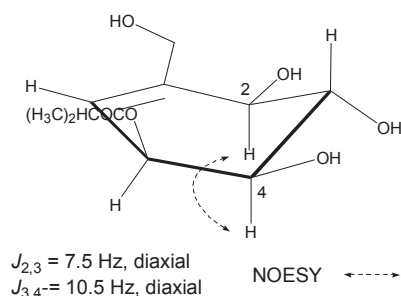
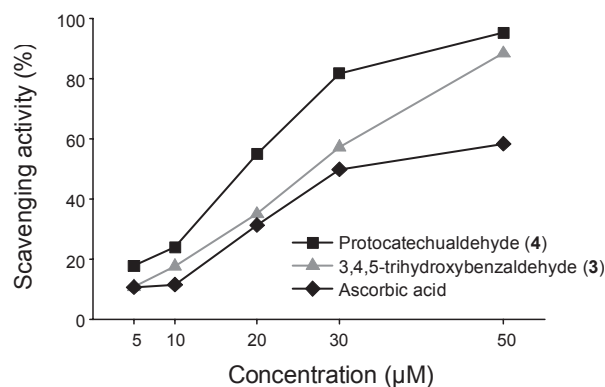
Shikimic acid was suggested to be a biogenetic precursor of rancinamycins, according to their structural similarities. The reduction and hydroxylation of shikimic acid would give rancinamycin III which can afford rancinamycin I and II (by acylation) and rancinamycin IV (by dehydration).⁵ The same configuration of the three hydroxyl groups of streptol and shikimic acid suggests that the biosynthesis of streptol might occur *via* the shikimate pathway.⁶ Therefore, the shikimate pathway may also be possible for the biogenesis of lincitols (**1** and **2**) (Figure 2).

Compound **3** was obtained as a colorless crystalline powder, and identified as 3,4,5-trihydroxybenzaldehyde based on comparison of spectral data with those reported.⁹

Two benzaldehyde derivatives (**3** and **4**) are potent antioxidants.^{10,11} Compounds **3** and **4** showed higher DPPH radical scavenging activity than the positive control, ascorbic acid (Figure 4). In contrast to expectation, the new cyclitol derivatives (**1** and **2**) did not exhibit any antioxidant activity in the DPPH assay. Accordingly, the antioxidant activity of the BuOH fraction of a culture of *S. lincolnensis* can be attributed to the two phenolic compounds (**3** and **4**).

Experimental Section

General procedures. The ¹H and ¹³C NMR spectra were recorded on Varian UNITY 400 MHz and Varian INOVA 500 MHz spectrometers. The chemical shifts were reported with

**Figure 3.** NOESY correlation between H-2 and H-4 for the half-chair conformation of compound **1**.**Figure 4.** DPPH radical scavenging activity of compounds **3** and **4**. Each value represents the mean \pm S. D. (*n* = 3).

reference to the respective residual solvent or deuterated solvent peaks (δ_{H} 3.30 and δ_{C} 49.0 for CD₃OD; δ_{H} 4.82 for D₂O). The HRFABMS and LRFABMS data was obtained on JEOL JMS-700 mass spectrometer (JEOL LTD, Japan). HPLC was performed with a Shodex C18M 10E column (preparative, 250 \times 10 mm, 5 μm , and 100 Å) using a RI-71 detector and YMC packed J'sphere ODS-H80 column (250 \times 10 mm, 4 μm , and 80 Å). The optical rotation was measured on a Jasco P-1020 polarimeter using a 1 dm path length cell. DPPH and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The colorimetric absorbance was measured on a UVmini 1240 UV-vis spectrophotometer (Shimadzu, Japan).

Collection and taxonomic identification. The bacterial strain was isolated from Mongolian soil, and was identified by Kyoung-Ja Kim of Soonchunhyang University on the basis of morphological and biochemical analysis.⁴

Extraction and isolation. After filtration, the culture media of *Streptomyces lincolnensis* was freeze dried, and then partitioned between water and *n*-BuOH. The *n*-BuOH layer was subjected to Sephadex LH-20 (MeOH), and 4 fractions (LHS1-LHS4) were collected. Subfraction LHS1 (5.6 g) was subjected to MPLC on Combiflash RetrieveTM using a Rediseq C-18 column, with stepped-gradient elution and a solvent system of 10 to 100% AcCN/H₂O to yield 11 fractions. LHS1-2 was selected for further separation because of its good antioxidant activity in the DPPH free radical scavenging assay. It was subjected to reversed-phase HPLC on Shodex C18M 10E with 16% MeOH. Compound **3** (3.5 mg) was obtained by purification of subfraction LHS 1-2-7. The purification of LHS 1-2-12 and

1-2-10 yielded lincitols A (**1**, 2.0 mg) and B (**2**, 1.8 mg).

Lincitol A (1). Colorless oil; $[\alpha]_D^{25} + 96.5$ (c 0.21, H₂O); ¹H NMR (CD₃OD, 500 MHz) δ 5.79 (1H, dd, $J = 5.5, 1.5$ Hz, H-6), 5.38 (1H, dd, $J = 4.0, 5.5$ Hz, H-5), 4.18 (2H, s, H-7), 4.00 (1H, d, $J = 7.5$ Hz, H-2), 3.79 (1H, dd, $J = 10.5, 7.5$ Hz, H-3), 3.63 (1H, dd, $J = 10.5, 4.0$ Hz, H-4), 2.57 (1H, m, H-2'), 1.15 (6H, d, $J = 9.5$ Hz, H-3', H-4'); ¹³C NMR (CD₃OD, based on HSQC and HMBC experiments, 500 MHz) δ 177.3 (C-1'), 146.0 (C-1), 117.4 (C-6), 73.3 (C-3), 72.7 (C-2), 69.8 (C-4), 69.2 (C-5), 61.5 (C-7), 34.0 (C-2'), 18.2 (C-3', C-4'); LRFABMS m/z 269.21 [M + Na]⁺; HRFABMS m/z 269.1021 [M + Na]⁺ (calcd for C₁₁H₁₈O₆Na, 269.1001).

Lincitol B (2). Light gray oil; $[\alpha]_D^{25} + 52.0$ (c 0.23, H₂O); ¹H NMR (CD₃OD, 500 MHz) δ 5.85 (1H, dd, $J = 5.0, 1.5$ Hz, H-6), 5.19 (1H, dd, $J = 10.5, 8.0$ Hz, H-3), 4.19 (1H, dd, $J = 5.0$ Hz, H-5), 4.18 (2H, s, H-7), 4.15 (1H, d, $J = 8.0$ Hz, H-2), 3.58 (1H, dd, $J = 10.5, 5.0$ Hz, H-4), 2.63 (1H, m, H-2'), 1.19 (6H, d, $J = 7.0$ Hz, H-3', H-4'); ¹³C NMR (CD₃OD, 100 MHz) δ 179.0 (C-1'), 143.9 (C-1), 123.1 (C-6), 76.2 (C-3), 71.6 (C-2), 71.0 (C-4), 67.8 (C-5), 62.7 (C-7), 35.4 (C-2'), 19.4 (C-3', C-4'); LRFABMS m/z 269.22 [M + Na]⁺; HRFABMS m/z 269.1019 [M + Na]⁺ (calcd for C₁₁H₁₈O₆Na, 269.1001).

3,4,5-Trihydroxybenzaldehyde (3). Colorless, crystalline powder; ¹H NMR (CD₃OD, 500 MHz) δ 9.60 (1H, s), 6.91 (2H, s, H-2, H-6), ¹³C NMR (CD₃OD, 100 MHz) δ 193.2 (C-7), 147.3 (C-3, C-5), 142.4 (C-4), 129.2 (C-1), 110.2 (C-2, C-6).

DPPH radical scavenging activity assay. The antioxidant activity was measured using a DPPH radical scavenging assay method. A 0.1 mL sample was mixed with 0.9 mL of a methanolic 0.15 mM DPPH solution. After vortexing thoroughly and leaving to stand at room temperature for 30 min, the absorbance of the mixture was measured at 517 nm using a UVmini 1240

UV-vis spectrophotometer. The mean of three measurements was taken. Ascorbic acid was used as the positive control. The scavenging activity was determined using the following equation:

$$\% \text{ Scavenging Activity} = 100 \times (1 - \text{Abs. sample} / \text{Abs. control})$$

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