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Structure Assignment of Lucentamycin E and Revision of the Olefin Geometries of the Marine-Derived Lucentamycins

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Abstract

A new lucentamycin analog, lucentamycin E (**5**), was isolated from the culture broth of the marine-derived actinomycete *Nocardiopsis lucentensis*, strain CNR-712. The absolute stereostructure of **5** was assigned by comprehensive analyses of NMR data and by application of the advanced Marfey's method. The planar structure of **5** was analogous to lucentamycins A–D, whereas the olefin geometry of the 3-methyl-4-ethylideneproline moiety was found to be *E*, opposite to that previously reported. Consequently, a reinvestigation of the olefin geometries of the 3-methyl-4-ethylideneproline residues of lucentamycins A–D showed that the olefin geometries of the substituted proline functionalities must be revised to (2*S*, 3*R*, *E*)-3-methyl-4-ethylideneproline.

In an earlier publication the structures of the lucentamycins A–D (**1–4**), peptide metabolites produced by the marine-derived actinomycete *Nocardiopsis lucentensis*, were reported.¹ Subsequently, several research groups have attempted a total synthesis of lucentamycin A,^{2–5} based on the structural novelty of the 3-methyl-4-ethylideneproline unit and their potent cytotoxicities toward human colon carcinoma cells. Recently, Del Valle and co-workers accomplished the total synthesis of the reported structure of lucentamycin A.² However, in that report, a discrepancy in the NMR data between synthetic lucentamycin A and natural lucentamycin A was observed with most of the differences attributed to resonances derived from the ethylideneproline moiety. A total synthesis of 8-*epi*-lucentamycin A was concurrently reported by Lindsley and co-workers³ and subsequent publications from the groups of Del Valle⁴ and Sim⁵ described the synthesis of all four possible stereoisomers of 3-methyl-4-ethylideneproline and their incorporation into target tripeptides. They also found that all of their synthetic compounds did not match natural lucentamycin A. In this paper, we correct the structures of the lucentamycins, specifically revising the structure of the substituted proline residue to (2*S*, 3*R*, *E*)-3-methyl-4-ethylideneproline and also adding another metabolite, lucentamycin E (**5**), to this group.

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Based on the discrepancies defined by synthetic studies, we decided to reisolate the lucentamycins from *N. lucentensis*, strain CNR-712. During this re-examination, we isolated a new metabolite, lucentamycin E (**5**). Re-evaluation of the ROESY NMR data derived from this compound, coupled with Marfey's amino acid analysis of the acid hydrolysates of **5** and lucentamycin A (**1**) and syntheses of the four stereoisomers of 3-methyl-4-ethylideneproline, allowed the full stereostructures of **1–5** to be defined. When the original ROESY spectroscopic data of lucentamycin A were re-examined, it was clear that the incorrect *Z*-olefin assignment¹ was based upon observed 2D NMR contours that were non-reproducible.

Lucentamycin E (**5**), obtained as a colorless gum, analyzed for the molecular formula $C_{31}H_{43}N_7O_5$ by interpretation of HRFABMS data. Interpretation of 1H , ^{13}C and 2D NMR data revealed that **5** was composed of three amino acid residues [tryptophan (Trp), homoarginine (Har), 3-methyl-4-ethylideneproline (Mep)] and an isopentenoic acid moiety. The structure of **5** was found to be analogous to lucentamycin B but was composed of an isopentenoic acid residue instead of an isopentanoic acid. The structure of the isopentenoic acid group was assigned by interpretation of HMBC correlations from the geminal methyl groups (δ_H 1.75 and 2.05) to the quaternary carbon (C-29) and to the sp^2 carbon resonance (C-28), as well as from the olefinic proton signal (H-28) to the carbonyl carbon (C-27).

The secondary amide proton signal (21-NH) at δ_H 7.94 exhibited a strong COSY correlation with a methine proton signal at δ_H 4.65 (H-21), which is the α -proton of the homoarginine (Har) residue of **5**. A serial COSY correlation was observed from H-21 to the methylene proton signals at δ_H 3.01 and 3.15 (H₂-25) which were connected to 25-NH [δ_H 10.47] of the guanidine unit. An HMBC correlation of the α -proton signal at δ_H 4.65 (H-21) to the carbonyl carbon at δ_C 165.3 (C-27) in the isopentenoic acid moiety indicated the connectivity of the Har and isopentenoic acid residues.

Comprehensive analysis of 1D and 2D NMR data (Table 1) showed that the C-1 to C-21 core of lucentamycin E was identical to that of lucentamycin B (**2**), thus establishing their relationship. However, the geometrical configuration of the exocyclic double bond in the Mep moiety of **5** was determined as *E* on the basis of clear ROESY correlations between H₃-18 and H-14 and between H₃-18 and H-19, coupled with a ROESY correlation between H-17 and H₂-16 (Figure 1). The relative configuration at C-13 and C-14 in the Mep moiety of **5** was determined to be *syn* based on the analysis of ROESY correlations and the value of the coupling constant ($^3J = 8.5$ Hz) between H-13 and H-14 in comparison with the previously reported NMR data for the four synthetic stereoisomers of lucentamycin A (with *Z*-olefin).⁵ Because the *E* double-bond geometry of **5** was opposite to that previously assigned to **1–4**, the alkene geometry of lucentamycin A (**1**) was reinvestigated by careful analysis of ROESY NMR data under several conditions. A strong ROESY correlation between H₃-13 and H₃-14 revealed that the double-bond geometry of Mep of lucentamycin A was also in the *E* configuration.

The absolute configurations of the amino acid residues in **5** were defined by applying the advanced Marfey's method.⁶ The acid hydrolysate of **5** was derivatized, in separated experiments, with 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (1-FDLA) and d-FDLA, and analyzed by C8 RP HPLC LC-MS. The retention times, molecular ion peaks and UV spectra of Trp and Har derivatives were identical with those of authentic L-Trp and L-Har derivatives. In addition, the HPLC elution time of 1-FDLA-derivatized Mep (33.40 min) was earlier than d-FDLA derivatized Mep (36.20 min). For the determination of the absolute configuration of Mep, Marfey's analysis was performed with four stereoisomers of Mep, which were obtained from Taebo Sim at the Korea Institute of Science and Technology (unpublished data). The Mep moiety of **5** can be in two stereoisomeric forms (2*R*, 3*S* and 2*S*, 3*R* configuration) based on the analysis of ROESY correlations and the value of the coupling constant between H-13 and H-14. The HPLC retention time of the 1-FDLA derivative of (2*S*, 3*R*)-Mep was 34.97 min (run at a different time) and the 1-FDLA derivative of (2*R*, 3*S*)-Mep was 37.17 min, whereas the L-FDLA-derivatized Mep in the acid hydrolysate of **5** (33.40 min, run earlier) matched best with that of (2*S*, 3*R*)-Mep. Thus, the absolute configuration of the Mep moiety in **5** was defined as 2*S*, 3*R*, which is the same absolute configuration defined earlier for lucentamycin A(**1**). By extensive NMR studies and application of the advanced Marfey's method, the absolute configurations of **5** and **1** can be assigned as 2*S*, 13*S*, 14*R*, 15*E*, 21*S* and 2*S*, 8*S*, 9*R*, 10*E*, 16*S*, respectively.

Experimental Section

General Experimental Procedures

The optical rotation was measured on a PerkinElmer Model 343 polarimeter. UV and IR spectra were recorded using a Perkin Elmer Lambda 35 UV/VIS Spectrometer and a Thermo Scientific Nicolet iS10 spectrometer, respectively. High-resolution FAB/MS data were obtained using a JEOL/JMS-AX505WA instrument. ¹H, ¹³C, and 2D NMR spectral data were obtained in DMSO-*d*₆ on a Varian UNITY 500 MHz NMR spectrometer. Low-resolution ESI-MS data were acquired by an Agilent 1200 series HPLC system/6120 quadrupole MSD. A Waters 1525 HPLC-PDA system with a Phenomenex Luna C18 (2) 5 μm column (150 X 4.6 mm) was used for the analysis of the extract and chromatography fractions. Diaion™ HP-20 (SUPELCO) resin was used for flash column chromatography. HPLC separation was performed with a Gilson 321 HPLC system with a Phenomenex Luna 10 μm C18(2) (250 • 10 mm) column for semi-preparative separation and a YMC-Pack ODS-A 5 μm (250 • 10 mm) column for final purification.

Collection, Identification and Cultivation of Strain CNR-712—Actinomycete strain CNR-712, was isolated from a sediment sample collected in a shallow saline pond on the island of Little San Salvador in the Bahamas. The strain shares 99.5% sequence identity with *Nocardiopsis lucentensis*. The 16S rRNA gene sequence has been deposited in GenBank (accession number EF392847). Actinomycete strain CNR-712 was cultured in 32 replicates of 500 mL volume using an A1 liquid culture medium (10 g of starch, 4 g of peptone, 2 g of yeast extract in 1L of natural seawater, SIO Pier) for 7 days at 27 °C while shaking at 200 rpm. After 7 days, the culture media was extracted with EtOAc (16 L overall). Concentration afforded 1.06 g of EtOAc extract.

Isolation and Purification of Lucentamycin E (5**)**—The extract (1.06 g) was absorbed on Celite (1.5 g) and subjected to flash column chromatography using Diaion HP-20 (SUPELCO) resin as the adsorbent phase. The column was eluted with aqueous MeOH using a step gradient approach (20%, 50%, 70%, 100% MeOH in H₂O) to yield four fractions. The 70% aqueous MeOH fraction was dried under vacuum and re-fractionated by reversed-phase HPLC (Gilson 321; Phenomenex Luna 10 μm C18(2) (250 × 10 mm)

column; at a flow rate of 4 mL/min) using isocratic elution with 35% acetonitrile in H₂O. The sub-fraction containing **5** was then finally purified by reversed-phase HPLC by isocratic elution with 35% MeCN in H₂O [(YMC-Pack ODS-A 5 μ (250 $\times$ 10 mm) column; at a flow of 2 mL/min)] to yield **5** (0.8 mg).

Lucentamycin E (5)—The new compound, a white amorphous powder: $[\alpha]_D^{20}$ -19 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 206 (4.34), 220 (4.33), 280 (3.62) nm; IR (KBr) $\nu_{\max}$ 3273, 1635, 1180 cm⁻¹; See Table 1 and Supporting Information for ¹H NMR data and ¹³C NMR data. HRFABMS $[M+H]^+ m/z$ 594.3407 (calcd C₃₁H₄₄N₇O₅, 594.3404).

Acid Hydrolysis and Advanced Marfey's Analysis—Lucentamycin E (**5**, 0.7 mg) was hydrolyzed with 6*N*HCl at 110 °C for 2 h and the hydrolysate was divided into two equal portions. Each portion was treated with 100 μ L of 1*N*NaHCO₃ and 50 μ L of 1% w/v 1-fluoro-2,4-dinitrophenyl-L-5-leucinamide (l-FDLA or d-FDLA) solution in acetone, followed by heating at 80 °C for 3 min. The reaction mixtures were cooled at room temperature, neutralized by adding 30 μ L of 2*N*HCl and diluted with 300 μ L of 50% aqueous MeCN solution. The resulting solution was analyzed by LC-MS [Agilent 6120 quadrupole ESI-MS (positive mode)] using a Phenomenex Luna C18 (2) 5 μ m column (150 $\times$ 4.6 mm) and an aqueous MeCN solution applied in a linear gradient (10–100% for 60 min at a flow 0.7 mL/min). The retention times (l-FDLA derivatives, d-FDLA derivatives) of the l- and d-FDLA derivatized amino acids in the acid hydrolysate of **5** were 19.65 and 19.55 min for L-Har, 33.00 and 34.76 min for L-Trp, and 33.40 and 36.20 min for L-Mep, respectively. The retention times of the l and d-FDLA derivatives of authentic L-Har were 19.74 and 19.50 min, respectively, whereas those of an authentic L-Trp were 33.24 and 34.73 min. The HPLC retention times for the l-FDLA derivatives of four synthetic isomers of 3-methyl-4-ethylideneproline, run at a different time, were 37.82 min for 2*R*, 3*R*-Mep, 37.17 min for 2*R*, 3*S*-Mep, 34.97 min for 2*S*, 3*R*-Mep and 36.11 min for 2*S*, 3*S*-Mep.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Key ROESY correlations for the 3-methyl-4-ethylidineproline moieties in lucentamycins A (1) and E (5).

Table 1NMR Spectroscopic Data (DMSO-*d*₆) for Lucentamycin E (**5**) (500 MHz for ¹H, 125 MHz for ¹³C).

Lucentamycin E (5)		
position	δ _C ^a type	δ _H (J in Hz)
1	175.5, C	
2	54.2, CH	4.19, m
3	28.6, CH ₂	3.03, m ^b
4	111.0, C ^c	
5	127.9, C	
6	118.7, CH	7.57, d (7.5)
7	117.9, CH	6.91, t (7.5)
8	120.6, CH	7.02, t (7.5)
9	111.0, C ^c	7.27, d (7.5)
10	135.9, C	
11	123.1, CH	7.05, br, s
12	167.6, C	
13	65.6, CH	4.21, d (8.5)
14	35.0, CH	3.17, m
15	139.4, C	
16a	51.0, CH ₂	4.40, d (14.0)
16b		4.26, d (14.0)
17	116.3, CH	5.34, q (6.5)
18	13.2, CH ₃	1.57, d (6.5)
19	14.9, CH ₃	0.83, d (7.0)
20	171.4, C	
21	49.9, CH	4.65, br, m
22a	31.9, CH ₂	1.80, m
22b		1.47, m
23a	23.0, CH ₂	1.47, m
23b		1.36, m
24a	28.7, CH ₂	1.52, m
24b		1.34, m
25a	41.3, CH ₂	3.12, m
25b		2.99, m
26	157.2, C	
27	165.3, C	
28	118.6, CH	5.74, s
29	149.5, C	
30	27.0, CH ₃	1.75, s
31	19.4, CH ₃	2.05, s

Lucentamycin E (5)		
position	δ_C^a type	δ_H (J in Hz)
2-NH		7.01, br s ^b
10-NH		10.6, s
21-NH		7.94, d (8.5)
25-NH		10.47, s ^b
26-NH		not observed
26-NH ₂		6.89, s

^a Attached protons assigned by DEPT NMR.

^b Multiplicity patterns were unclear due to signal overlap.

^c Signals were overlapped.