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Discovery and antibacterial activity of lucensimycin C from *Streptomyces lucensis*

Sheo B. Singh [a,*], Deborah L. Zink [a], Kithsiri B. Herath [a], Oscar Salazar [b], Olga Genilloud [b]

[a] *Merck Research Laboratories, Rahway, NJ 07065, USA*
[b] *CIBE, Merck Sharp and Dohme de Espana, S. A. Josefa Valcárcel, Madrid, Spain*

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Abstract

Protein synthesis is one of the key and validated antibacterial targets and its inhibition has led to the development of highly successful clinical drugs. Ribosomal protein S4 (RPSD) is a part of ribosomal machinery and a new potential target for antibacterial agents. Screening of microbial extracts using antisense sensitized *rpsD* strain in *Staphylococcus aureus* followed by chemical analysis led to the isolation of a new compound lucensimycin C, which is a key biosynthetic intermediate of the other lucensimycins. Isolation, structure elucidation, antibiotic activity, and biogenesis have been described.

Emergence of drug-resistant bacteria continues to grow and remains a serious threat to human lives.[1] Protein synthesis is one of the highly effective antibacterial drug targets and its inhibition continues to produce clinically useful antibiotics.[2,3] These include chloramphenicol, macrolides, aminoglycosides, tetracyclines, streptogramins, lincosamides, and oxazolidinones. Protein synthesis is catalyzed by the ribosome, which is composed of two asymmetric macromolecular components, the large (50S) and small (30S) subunits. The large subunit consists of two ribosomal RNAs (rRNAs) 23S and 5S, and 34 unique ribosomal proteins (r-proteins), L1–L34.[4–6] The small subunit is composed of 16S rRNA and 21 r-proteins, S1–S21.[4–6] All but protein S1 of the small subunit appear to be essential for normal ribosomal functioning and protein synthesis. The catalytic power of the protein synthesis resides in the ribosome itself. Ribosomal–proteins are known to help the ribosome to maintain its quaternary structure. Most of the drugs that are in clinical use today bind not only to rRNA but also to one or more r-proteins. Alteration of the rRNA binding to r-proteins inactivates the protein synthesis function. Therefore, it is expected that selectively altering the conformation of a particular r-protein, or inhibiting the synthesis of an r-protein, would potentially result in the loss of function, and may lead to the inhibition of protein synthesis. Small ribosomal protein S4 is one of such proteins and is encoded by *rpsD* gene in both Gram-positive and Gram-negative bacteria, conserved across bacterial species and essential for bacterial growth.[7,8]

We recently reported the design and application of a two-plate whole-cell differential sensitivity screening assay using an antisense-sensitized *Staphylococcus aureus* strain in which the FabF target was dialed down.[9,10] This led to the discovery of platensimycin and platencin two novel and potent inhibitors of FabF and FabF/H with in vivo antibiotic properties.[11–14] A similar two-plate assay with a reduced expression of *rpsD* gene by antisense was used to screen natural product extracts. This screening strategy led to the identification of extracts derived from a strain of *Streptomyces lucensis* MA7349 and *Coniothyrium cerealis* MF7209. Bioassay-guided fractionation using *rpsD* assay led to the isolation of lucensimycin A (**1**) and B (**2**)[15] from *S. lucensis* and coniothyrione from *C. cerealis*.[16]

* Corresponding author. Tel.: +1 732 594 3222; fax: +1 732 594 6880.
E-mail address: sheo_singh@merck.com (S. B. Singh).

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Further chemical analysis of the extracts of *S. lucensis* led to the isolation of a new compound lucensimycin C (**3**), which appears to be an ultimate biosynthetic intermediate of lucensimycin A (**1**). The discovery of lucensimycin C points to an interesting biosynthesis of **1** in which a major portion of the molecule is likely derived from polyketide followed by esterification and cyclization of a pyruvate unit. The isolation, structure elucidation, and the biological activity of lucensimycin C along with biosynthetic proposal are herein described.

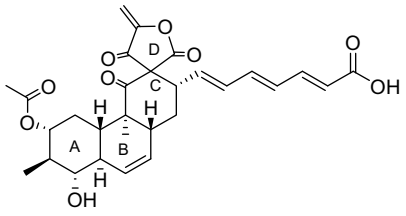
Three liter fermentation broth of *S. lucensis* MA7349[15] was extracted with 3 L acetone and chromatographed on a medium grade Amberchrome reversed-phase column and eluted with a 100 min 5–100% aqueous MeOH gradient followed by chromatography on reversed-phase HPLC using Zorbax RX C_8 column. Elution with aqueous CH_3CN gradient using TFA as a modifier afforded lucensimycin C (**3**, 17.8 mg; 5.9 mg/L) and A (**1**, 19.3 mg, 6.4 mg/L) as amorphous powders. Lucensimycin C (**3**) exhibited the following physical and spectral properties. $[\alpha]_D^{23}$ +78.2 (*c* 1.1, MeOH); UV (MeOH) λ_{max} 294 nm ($\log\varepsilon = 4.79$); IR (ZnSe) ν_{max} 3300, 2931, 1750, 1720, 1700, 1615, 1379, 1239, 1136, 1008, 980, 933, 799, 737 cm^{-1}.

HRESI-FTMS analysis of lucensimycin C (**3**) showed a parent ion at *m*/*z* 560.2485 (calcd M+NH_4: 560.2491) analyzed for ammonium adduct of the molecular ion. The protonated molecular ion was observed at *m*/*z* 543.2227 indicating a molecular weight of 542 and molecular formula of $C_{29}H_{34}O_{10}$ (calcd M+H: 543.2230) suggesting 13 degrees of unsaturation. The molecular formula was identical to lucensimycin B (**2**) and was up by a molecule of H_2O when compared to **1**. However both ^1H and ^{13}C NMR spectra of **3** indicated that it was neither **2** nor just simple water adduct of **1**. The ^{13}C NMR spectrum of **3** showed the presence of 29 carbons (Table 1) and confirmed the molecular formula. Analysis of the DEPT and the HSQC spectra together with the relative positions of the ^{13}C chemical shifts indicated the presence of five carbonyl carbons [one downfield shifted (δ_C 210.2) indicating the presence of a cyclohexanone], eight olefinic methines, an olefinic methylene, an olefinic quaternary, two oxymethines, six aliphatic methines, two aliphatic methylenes, an aliphatic quaternary, and three methyls.

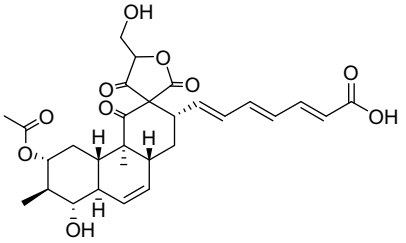
Examination of the ^1H NMR spectrum together with ^1H, ^1H-COSY indicated the presence of C-15–C-21 trienoic acid (λ_{max} 294 nm) and the 6/6/6-tri cyclic system with two oxymethines sandwiching a methine-methyl group located

Table 1
^1H (600 MHz) and ^{13}C (150 MHz) NMR assignments of lucensimycin C (**3**) in CD_3OD

No.	δ_C	δ_H (*J* in Hz)	^1H, ^1H-DQCOSY	^1H, ^1H-TOCSY	HMBC (H→C)
1	32.4	β 1.19, br q, 12	H-1α, H-2, H-10	H-1β, H-2, H-3, H-4, H-5, H-10, H-23	C-2, 5, 10
		α 1.42, m	H-1β, H-2, H-10 (w)	H-1α, H-2, H-3, H-4, H-5, H-10, H-23	C-2, 5, 10
2	76.6	4.60, dt, 4.2, 7.8	H_2-1, H-3	H_2-1, H-3, H-4, H-5, H-10, H-23	C-1, 3, 4, 23, 1′
3	46.5	1.53, m	H-2, H-4, H-23	H-2, H-4, H-5, H-10, H_2-1, H-23	C-1, 2, 4, 5, 23
4	76.7	2.98, t, 10.2	H-3, H-5	H_2-1, H-2, H-3, H-5, H-6, H-7, H-10, H-23	C-2, 3, 5, 6, 10
5	45.6	1.79, br t, 10.2	H-10, H-6 (w)	H_2-1, H-2, H-3, H-4, H-6, H-7, H-10, H-23	C-4, 6, 7, 10
6	126.9	5.99, br d, 10.2	H-5 (w), H-7	H-4, H-5, H-7, H-8, H-10, H-13, H_2-14	C-4, 5, 8, 10
7	130.8	5.77, ddd, 12, 7.2, 4.8	H-5 (w), H-6, H-8,	H-4, H-5, H-7, H-8, H-10, H-12, H-13, H_2-14	C-9
8	46.1	2.24, m	H-7, H-14α, H-14β (w)	H-6, H-7, H-12, H-13, H_2-14, H-15, H-16	C-6, 7, 10, 13, 14, 24
9	51.1	—			
10	34.6	1.99, m	H-1α, H-1β (w), H-5	H-6, H-2, H-4, H-5, H-3, H_2-1	C-1, 2, 4, 5, 6, 8, 9, 24
11	210.2	—			
12	60.1	3.83, d, 12.6	H-13	H-7, H-8, H-13, H_2-14, H-15, H-16, H-17	C-11, 13, 14, 15, 22
13	44.3	2.89, m	H-12, H-14α, H-15	H-7, H-8, H-12, H_2-14, H-15, H-16, H-17, H-18	C-8, 11, 12, 14, 15, 16, 22
14	35.7	β 1.98, m	H-8 (w), H-14α	H-15, H-16, H-13, H-12, H-8, H-14β	C-7, 9, 12, 13, 15
		α 1.71, br q, 12.6	H-8, H-13, H-14β	H-15, H-16, H-13, H-12, H-8, H-14α	C-7, 9, 12, 13, 15
15	139.9	5.95, dd, 15, 7.8	H-13, H-16	H-12, H-13, H-8, H_2-14, H-16, H-17, H-18, H-19, H-20	C-12, 13, 14, 17
16	130.7	6.34, dd, 15, 11	H-15, H-17	H-12, H-13, H-8, H_2-14, H-15, H-17, H-18, H-19, H-20	C-13, 15, 17, 18
17	142.0	6.57, dd, 15, 11	H-16, H-18	H-12, H-13, H-15, H-16, H-18, H-19, H-20	C-15, 16, 18, 19
18	132.1	6.36, dd, 15, 11	H-17, H-19	H-15, H-16, H-17, H-19, H-20	C-16, 19, 20
19	146.5	7.27, dd, 15, 11	H-18, H-20	H-15, H-16, H-17, H-18, H-20	C-17, 18, 20, 21
20	121.8	5.84, d, 15	H-19	H-15, H-16, H-17, H-18, H-19	C-18, 21
21	170.5	—			
22	164.1	—			
23	14.7	1.05, d, 6.6	H-3	H_2-1, H-2, H-3, H-4, H-5, H-10	C-2, 3, 4
24	15.7	1.04, s			C-8, 9, 10, 11
1′	172.5	—			
2′	21.0	2.02, s			C-1′
1″	169.2	—			
2″	146.6	—			
3″	115.0	a 5.50, d, 1.8	H-3″b	H-3″b	C-1″, 2″
		b 6.05, d, 1.8	H-3″a	H-3″a	C-1″, 2″



1 (Lucensimycin A)



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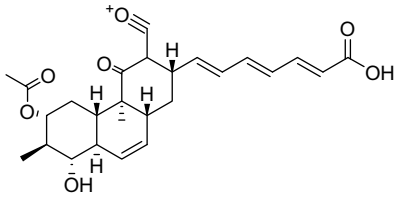
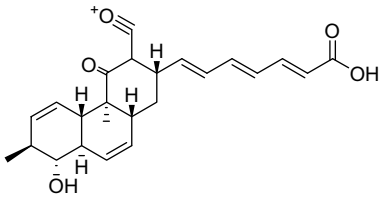


3A



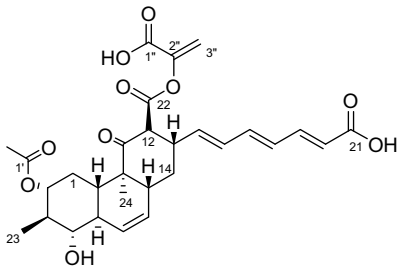
3B

Fig. 1. ESIMS fragmentation of lucensimycin C (**3**).

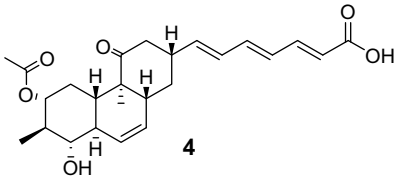
in the A-ring, an extension of the coupling network via a *cis*-olefin (B ring, C-6–C-7) to C-8 methine and to H_2-14 and H-13 and finally back to the trienoic acid unit similar to **1**. The COSY correlations were corroborated by the corresponding TOCSY correlations (delay = 80 ms, see Table 1). H-13 showed an additional COSY correlation to a doublet at δ_H 3.83 with a large coupling ($J = 12.6$ Hz) indicating that this proton was present at C-12 (δ_C 60.1) and thus suggesting that the ring D present in **1** was not present in **3**. C-11 of **3** experienced 8 ppm downfield shift and appeared at δ_C 210.2 compared to **1** (δ_C 202.1) due to the release of the strain caused by the opening of the D-ring. Additionally, the ^{13}C signal assigned for C-22 (δ_C 190.1) of **1** was absent in the ^{13}C NMR of **3** and a new upfield signal at δ_C 169.2 (numbered as C-1″) was present confirming the opening of the ring D. The remaining three carbons of the ring D also experienced significant shifts in their chemical shifts due to the release of the strain after opening of the ring. The olefinic methylene (C-3″) showed about 16 ppm downfield shift and appeared at δ_C 115.0, C-2″ showed about 5 ppm upfield shift and appeared at δ_C 146.6 and the ester carbonyl C-22 showed about 4 ppm upfield shift and appeared at δ_C 164.1 in compound **3**. These assignments were confirmed by HMBC correlations from H-12 and H-13 to the carboxyl C-22 and H_2-3″ to C-2″ and C-1″. These observations suggested that the lucensimycin C was D-ring open version of **1** in which the acid group at C-12 was esterified with enolyl-pyruvate group. The ^1H and ^{13}C NMR assignments were confirmed by HMBC correlations (Table 1).

The mass spectral fragmentation of **3** produced two distinct fragment ions at *m*/*z* 455.2065 (calcd for $C_{26}H_{31}O_7$: 455.2064) and *m*/*z* 394.1856 (calcd for $C_{24}H_{27}O_5$: 455.2064) due to the loss of the pyruvate unit and the acetic acid units producing fragment ions **3A** and **3B** (Fig. 1), respectively, and supported the structure of **3**.

The relative configuration of **3** was determined from the magnitude of coupling constants observed in the ^1H NMR spectrum. It was found that the stereochemistry of **1** and **3** was identical except for C-12. The H-12 showed a large ($J = 12.6$ Hz) coupling with H-13 suggesting that both of these protons were axial and the trienoic acid chain and carboxy group were equatorial in cyclohexyl chair conformation of the C-ring. To determine absolute configuration, **3** was reacted with (*R*)- and (*S*)-α-methoxy-α-trifluoromethyl-α-phenyl acetyl chlorides (MTPA chlorides). The reaction produced a complex mixture of products with no detectable product corresponding to Mosher esters was observed by LCMS. One of the major products observed showed an ion at *m*/*z* 429 (M+H) which was identified as a de-carboxy product **4**. Failure of the Mosher ester formation is likely due to the steric crowding at C-4. Acetylation of C-4 hydroxy group was accomplished for compound **1**.[15] The crystallization efforts have so far failed to produce suitable crystals for X-ray crystallographic analysis. Based on all the evidence, structure **3** was assigned for lucensimycin C.



3 (Lucensimycin C)



4

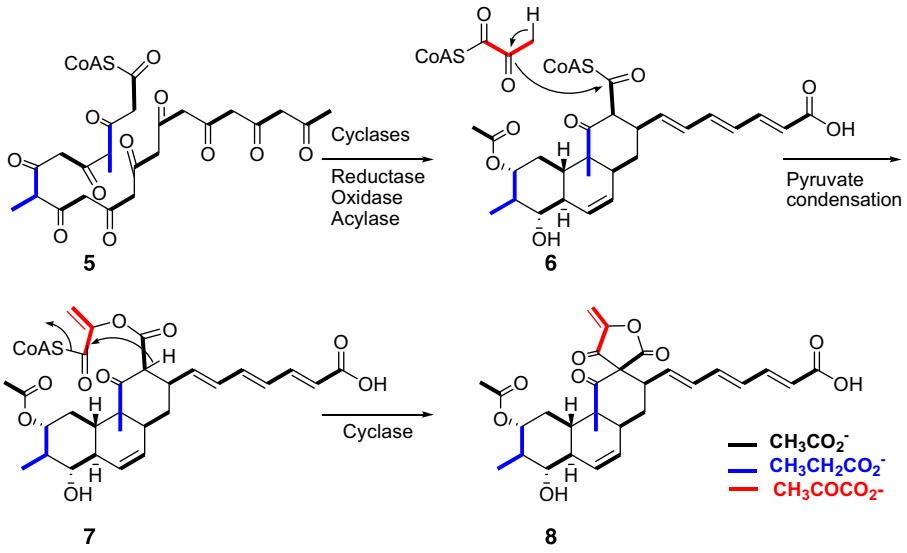


Fig. 2. Proposed biogenesis of Lucensimycins A (**1**) and C (**3**).

Biogenetically, lucensimycins appear to be derived from the cyclization of an undeca-polyketide originating from nine acetate and two propionate units with acetate as a starter unit followed by condensation of a pyruvate to produce lucensimycin C (**3**), which undergoes further cyclization to produce lucensimycin A (**1**) as illustrated in Figure 2.

Lucensimycin C was approximately 80-fold less active than lucensimycin A and showed 8 mm zone of clearance at 10 mg/mL (100 μg spotted) against *Staphylococcus aureus* seeded on Agar plate. Like lucensimycin A, it did not show any selectivity for *rpsD* strain *versus* other antisense strains and did not inhibit the growth of *S. aureus* in liquid assay at 250 μg/mL.[15]

In summary, we have described here the isolation and structure elucidation of lucensimycin C, which appears to be an ultimate biosynthetic intermediate of lucensimycin A. It showed significantly less antibiotic activity against *S. aureus* strain and was not pursued further. These compounds are distantly related to antibiotic and cytotoxic agent Delaminomycins A–C.[17]

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