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Structural elucidation of lemonomycin, a potent antibiotic from *Streptomyces candidus*

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Abstract

The structure of the benzoquinone antibiotic, lemonomycin (**1**), has been characterized using spectroscopic methods. The pyrrolidine substructure is new to this class, and the sugar moiety is encountered for the first time.

Lemonomycin, a potent broad-spectrum antibiotic, was isolated from the fermentation broth of *Streptomyces candidus* (LL-AP191) as reported in a previous paper.[1] The MIC's of this compound were recorded as 0.2 and 0.05 μg/ml, respectively, for susceptible strains of *Staphylococcus aureus* and *Bacillus subtilis*, and measured in a recent test to be 0.4 and 0.2 μg/ml for methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus faecium*. Although the production, isolation, and antibacterial activity were described in the original paper, the structure of lemonomycin has not been disclosed. As part of a continuous program to discover new agents to overcome increasing resistance in antimicrobial chemotherapy,[2] we reexamined lemonomycin and characterized its structure as **1** by using spectroscopic methods (Fig. 1).

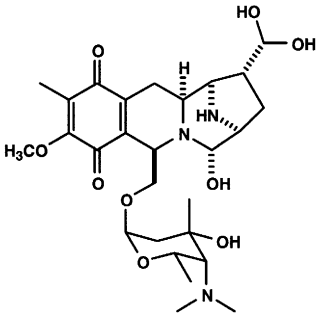

Fig. 1. Structure of lemonomycin (**1**)

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Lemonomycin hydrochloride (100 mg), isolated several years ago,[1] was purified by reverse phase HPLC (C18 column, 8–30% acetonitrile in water (0.01% trifluoroacetic acid)) to afford the lemonomycin trifluoroacetate (**1**, 52 mg).[3] The molecular formula of **1** was determined by high resolution electrospray mass spectrometry to be $C_{27}H_{41}N_3O_9$. Although the compound was readily dissolved in DMSO and methanol, D_2O was found to be the solvent that gave the best NMR spectral data, and was therefore used for this study (Table 1).

Table 1
NMR spectral data of lemonomycin (**1**)

	chemical shift (D_2O)[a]		^1H-^{13}C HMBC correlation
No.	^1H (500 MHz, mult, J in Hz)	^{13}C (75 MHz, mult)	(J = 6 Hz)
1	4.30 (m)	54.4 (CH)	C-8, C-9, C-10, C-17
3	3.39 (br d, 9.0)	52.4 (CH)	C-1, C-4, C-10, C-11, C-15
4	2.76 (br d, 17.5)	26.5 (CH_2)	C-5, C-9, C-10
	2.13 (m)		C-3, C-9, C-10, C-11
5		190.2 (C)	
6		133.3 (C)	
6-CH_3	1.96 (3H, s)	11.0 (CH_3)	C-5, C-6, C-7
7		158.3 (C)	
7-OCH_3	3.91 (3H, s)	64.1 (CH_3)	C-7
8		184.6 (C)	
9		140.7 (C)	
10		144.5 (C)	
11	4.03 (m)	62.9 (CH)	C-3, C-4, C-13, C-14, C-15, C-16
13	4.10 (br s)	63.4 (CH)	C-11, C-15, C-17
14	2.09 (m)	28.6 (CH_2)	C-11, C-15, C-16, C-17
	2.01 (m)		C-13, C-15, C-16, C-17
15	2.64 (ddd, 9.2, 4.5, 4.5)	43.4 (CH)	C-3, C-13, C-14, C-16
16	5.18 (d, 4.5)	92.9 (CH)	C-11, C-14, C-15
17	4.88 (d, 2.9)	81.4 (CH)	C-1, C-3
18	3.77 (br d, 10)	71.4 (CH_2)	C-9, C-1'
	3.64 (br d, 10)		C-1, C-9, C-1'
1'	5.08 (br d, 4.4)	99.9 (CH)	C-18, C-3', C-5'
2'	2.05 (m)	40.7 (CH_2)	C-1', C-3', C-4', 3'-CH_3
	1.92 (m)		C-1', C-3', C-4', 3'-CH_3
3'		69.7 (C)	
3'-CH_3	1.33 (3H, s)	31.4 (CH_3)	C-2', C-3', C-4'
4'	3.13 (br s)	72.7 (CH)	C-2', C-3', 3'-CH_3, 4'-$N(CH_3)_2$
4'-NCH_3	3.08 (3H, s)	49.7 (CH_3)	C-4', 4'-NCH_3 (44.4)
4'-NCH_3	3.07 (3H, s)	44.4 (CH_3)	C-4', 4'-NCH_3 (49.7)
5'	3.98 (br q, 7.2)	64.9 (CH)	C-1', C-4', C-6'
6'	1.46 (3H, d, 7.2)	20.0 (CH_3)	C-4', C-5'

[a] 1-D NMR signals were assigned by ^1H-^1H COSY, TOCSY, and ^1H-^{13}C HMQC experiments.

The ^{13}C NMR spectrum displayed signals for six methyls, four methylenes, 10 methines and seven quaternary carbons. The initial analysis of the NMR spectral data indicated that the molecule consisted of a benzoquinone-containing aglycon and an amino sugar moiety (Fig. 2). Detailed analysis of NMR spectral data, in particular, the ^1H–^1H COSY and ^1H–^{13}C HMBC data, revealed the structure for the aglycon moiety (**A**). The resonances at δ 190.2, 184.6, and four other olefinic carbon signals were characteristic for a quinone moiety. The 2- or 3-bond ^1H–^{13}C correlations in the HMBC spectrum from the methoxyl protons at 3.91 to olefinic carbon C-7 at δ 158.3, and from the allylic methyl at 1.96

to C-5 at 190.2, C-6 at 133.3, and C-7 were indicative of a methoxyl and a methyl substituents on a *p*-benzoquinone moiety. Two methine proton signals at δ 4.30 and 3.40 were assigned to H-1 and H-3 in the tetrahydropyridine ring. The H-1 showed strong correlations in the HMBC spectrum to C-8 at δ 184.6, C-9 at 140.7, and C-10 at 144.5, whereas H-3 correlates to C-1 at 54.4 and C-10. These correlations, in conjunction with the COSY data, determined the 1-hydroxymethyl-6-methyl-7-methoxy-1,2,3,4-tetrahydro-isoquinoline-5,8-dione substructure.

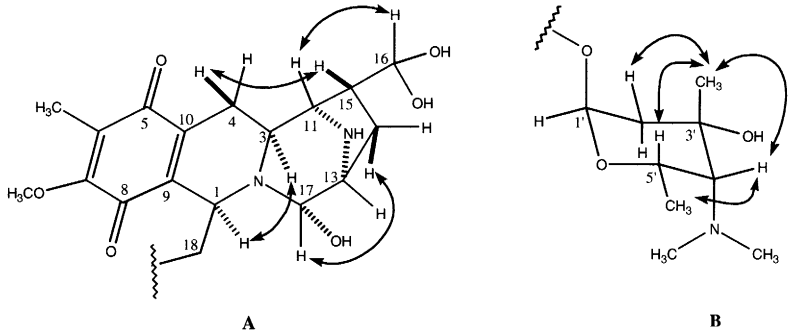

Fig. 2. Substructures for aglycon (**A**) and amino sugar (**B**) moieties, and selected NOEs (indicated by arrows) which determined the stereochemistry

In the ^{1}H–^{1}H COSY spectrum, the correlations in the spin system from H-11 at δ 4.03 to H-15 at 2.64, to H_2-14 at 2.09 and 2.01, to H-13 at 4.10, and to H-17 at 4.88 were clearly observed. Although the ^{1}H–^{1}H coupling between H-3 at δ 3.39 and H-11 was weak, the observation of the 2- and 3-bond ^{1}H–^{13}C correlations from H-3 to C-11 at 62.9, and C-15 at 43.4 required that C-11 be adjacent to C-3. The 3-bond ^{1}H–^{13}C correlations from H-17 at δ 4.88 to C-1 at 54.5 and C-3 at 52.4 indicated that C-17 was attached to N-2, and the mutual ^{1}H–^{13}C correlations from H-11 to C-13 and H-13 to C-11 required that these two centers flank N-12. The respective chemical shift data for H-17 at δ 4.88 and C-17 at δ 81.4 suggested that there was a hydroxyl group at this center. In addition, the chemical shift data of H-16 at 5.18 and C-16 at 92.9 could be attributed to the presence of an aldehyde hydrate, which was connected to C-15 by the coupling between H-15 and H-16, together with the relevant ^{1}H–^{13}C correlations (Table 1).

The remaining proton and carbon signals could be assigned to an amino sugar moiety (**B**). The anomeric proton H-1′ at δ 5.08 was coupled to the methylene protons H_2-2′ at 2.05 and 1.92 in the COSY spectrum and correlated to C-3′ at 69.7 and C-5′ at 64.9 in the HMBC spectrum. Furthermore, the methyl singlet 3′-CH_3 at δ 1.33 correlated to C-2′ at 40.7, C-3′ at 69.7, and C-4′ at 72.7, whereas the methyl doublet H_3-6′ at 1.46 correlated to C-4′ and C-5′. The NMR data also identified a dimethylamino group, which was connected to C-4′ by HMBC cross peaks from N(CH_3)$_2$ proton signals centered at δ 3.07 to C-4′ at 72.7 and N(CH_3)$_2$ carbon signals at 49.7 and 44.4. The evidence for the attachment of the sugar moiety to the aglycon at C-18 was found in a 3-bond correlation from H-1′ at δ 5.08 to C-18 at 71.4 in the HMBC spectrum.

The relative stereochemistry of **1** was determined on the basis of ^{1}H–^{1}H coupling constants and NOE data observed from the ROESY experiment (Fig. 2). For the aglycon, the NOE between H-1 at δ 4.30 and H-3 at 3.39 indicated that these two protons were both axial and oriented on the same side of the tetrahydropyridine ring, depicted as down.[4] The NOE between the H-4$_{ax}$ at δ 2.13 and H-15 at 2.64 defined much of the stereochemistry on the aglycon involving chiral centers, C-3, C-11, C-13, and C-15. Furthermore, the NOE between H-17 at δ 4.88 and H-14 at 2.09 required that the hydroxyl group on C-17 should be down. For the amino sugar moiety, the anomeric proton H-1′ at δ 5.08 was indicated

to have an equatorial orientation by its small coupling constants. The stereochemistry of the remaining chiral centers was defined by the NOEs between CH_3-3′ at δ 1.33 and H-5′ at 3.98 and between CH_3-3′ and H-4′ at 3.13, along with the small coupling constant between H-4′ and H-5′.

As shown in Fig. 3, compound **1** was treated with acidic isopropanol to give an acetal (**2**), which was subsequently converted with NaCN to the cyano derivative **3**.[5] In addition to the antibiotic activity, **1** and **3** also showed in vitro activity against a human colon tumor cell line (HCT116) and their respective IC_{50}'s were measured as 0.36 and 0.26 μg/ml.

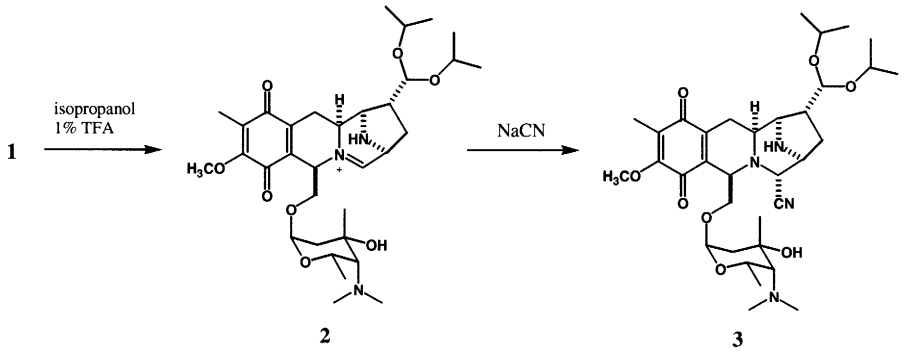

Fig. 3. Derivatization of lemonomycin (**1**)

The 6-methyl-7-methoxy-1,2,3,4-tetrahydroisoquinoline-5,8-dione substructure is precedented in several anticancer agents, such as saframycins[6] and renieramycins,[7] and has been shown to derive from tyrosine biosynthetically.[8] The pyrrolidine portion, however, is new to this class of antibiotics and the unit consisting of C-17, C-13, C-14, C-15, and C-16 is possibly derived from glutamic acid. In addition, the 2,6-dideoxo-4-amino sugar is rare in nature and to our knowledge, the sugar moiety in lemonomycin, featuring a 3-hydroxy-3-methyl substitution pattern, is encountered for the first time.

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3. $[\alpha]_D$=−128° (c 0.23, H_2O); HR-ESI-MS *m/z* 552.2910 (MH^+, 10%, $C_{27}H_{42}N_3O_9$ requires: 552.2915), 276.6494 (MH_2^{2+}, 100%); UV λ_{max} (MeOH, HP1100 diode array detector) 272, 362 nm; IR ν_{max} (KBr) cm^{-1} 3403, 2942, 1655, 1600, 1465, 1451, 1381, 1347, 1234.
4. The absolute stereochemistry of lemonomycin has not been determined experimentally, but the configuration of the aglycon was depicted according to the absolute stereochemistry of the isoquinoline antibiotics, saframycins (Ref. 6).
5. A solution of lemonomycin (**1**, 25 mg) in isopropanol (1 ml), DMSO (0.5 ml), and trifluoroacetic acid (TFA, 15 ul) was stirred at rt for 16 h. The reaction mixture was then chromatographed by HPLC on a C18 column (5 μm, 30×250 mm), using a gradient solvent of 8–30% MeCN in water (0.05% formic acid) over 17 min (20 ml/min). The UV peak at 15 min was collected (25 ml), which contained compound **2**. ESI-MS *m/z* 618.2 (MH^+), UV λ_{max} (0.01% TFA in MeCN/H_2O, diode array detector) 272, 362 nm. To this solution, NaCN (30 mg) was added, and the resulting mixture was stirred for 15 h. The solution was then evaporated in vacuo, and the residue was separated by HPLC (C18 column, 15–30% MeCN in water (0.05% formic

acid)) to afford **3** (10.2 mg, 40% overall yield). ESI-MS *m/z* 645.4 (MH^+); UV λ_{max} (0.01% TFA in MeCN/H_2O, diode array detector) 272, 362 nm; ^1H NMR (CD_3OD) δ 4.88 (br d, 4.5 Hz, H-1′), 4.52 (d, 3.8 Hz, H-16), 4.04 (d, 2.5 Hz, H-17), 3.99 (3H, OCH_3), 3.91 (m, H-5′), 3.89 (m, H-1), 3.78 (2H, m, CH's on isopropoxy's), 3.72 (dd, 10.2, 3.6 Hz, H-18), 3.71 (m, H-13), 3.53 (dd, 10.2, 1.5 Hz, H-18), 3.29 (m, H-11), 2.73 (br d, 10.5 Hz, H-3), 2.67 [6H, s, $N(CH_3)_2$], 2.66 (m, H-4), 2.36 (m, H-15), 2.20 (d, 1.8 Hz, H-4′), 2.02 (dd, 17.0, 2 Hz, H-4), 1.92 (3H, s, CH_3-6), 1.85 (m, H-2′), 1.78 (2H, m, H_2-14), 1.67 (br d, 14.4 Hz, H-2′), 1.30 (3H, d, 6.9 Hz, H_3-6′), 1.17 (3H, s, CH_3-3′), 1.11-1.18 (12H, m, CH_3's on isopropoxy's).

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