LYDICAMYCIN, A NEW ANTIBIOTIC OF A NOVEL SKELETAL TYPE

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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The structure of a new antibiotic designated lydicamycin was elucidated as shown in Fig. 1 by NMR spectral analysis including a variety of 2D techniques. Lydicamycin possesses a novel skeleton contanining tetramic acid and amidinopyrrolidine moieties.

In the preceding paper[1], we have described the fermentation, isolation and biological activity of a new antibiotic, lydicamycin, as well as the taxonomy of the producing organism, *Streptomyces lydicus* 2249-S3. This paper describes the physico-chemical properties and structure elucidation of lydicamycin (Fig. 1).

Physico-chemical Properties

Lydicamycin was obtained as a colorless powder revealing its physico-chemical properties as summarized in Table 1. The IR and ^1H NMR spectra of lydicamycin are shown in Figs. 2 and 3, respectively. The molecular formula of lydicamycin was established as $C_{47}H_{74}N_4O_{10}$ by using HRFAB-MS and elemental analysis. The UV spectra and positive ferric chloride reaction for lydicamycin suggested the presence of an α-acyltetramic acid chromophore[2].

Table 1. Physico-chemical properties of lydicamycin.

Appearance	Colorless powder
MP	161~166°C
Molecular formula	$C_{47}H_{74}N_4O_{10}$
FAB-MS (*m/z*)	Calcd: 855.5483,
	Found: 855.5541 $(M+H)^+$
Elemental analysis	Calcd:
	C 66.02, H 8.72, N 6.55, O 18.71
	Found:
	C 64.32, H 8.37, N 6.36, O 20.00
$[\alpha]_D^{18}$	+75.1° (*c* 1, MeOH)
UV λ_{max} nm (ε)	
MeOH	207 (19,600), 245 (9,900),
	282 (10,000)
0.01 N HCl-MeOH	207 (18,900), 250 (sh, 6,600),
	282 (9,900)
0.01 N NaOH-MeOH	208 (19,900), 245 (9,700),
	281 (9,600)
IR ν_{max} (KBr) cm^{-1}	3370, 2970, 2940, 2880, 1655,
	1610, 1580, 1560, 1450, 1420,
	1375, 1315, 1230, 1065, 1010,
	975, 920, 880

Structure Elucidation

The HRFAB-MS revealed a fragment ion peak

Fig. 1. Structure of lydicamycin (relative stereochemistry).

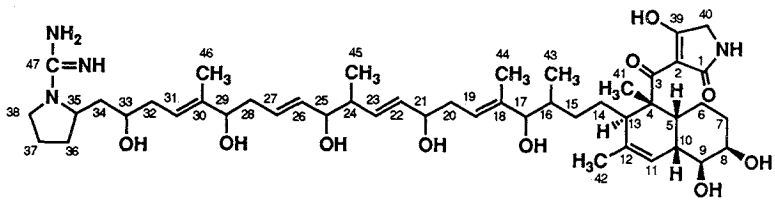


Fig. 2. IR spectrum of lydicamycin (KBr).

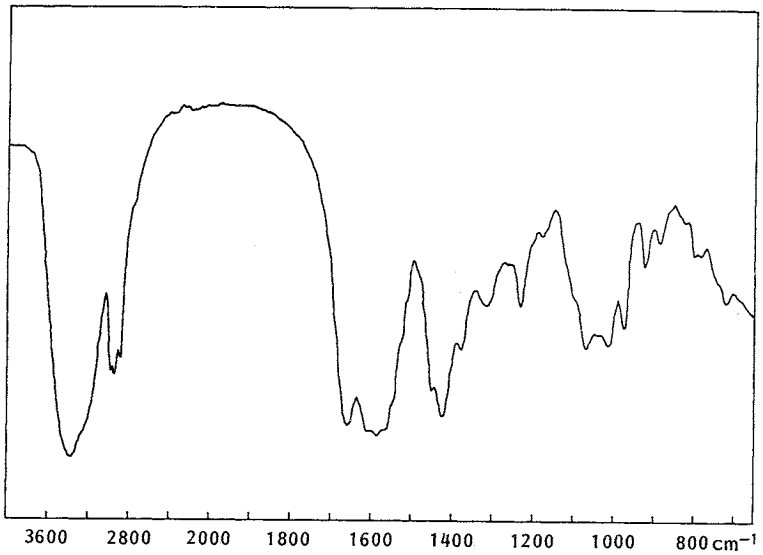
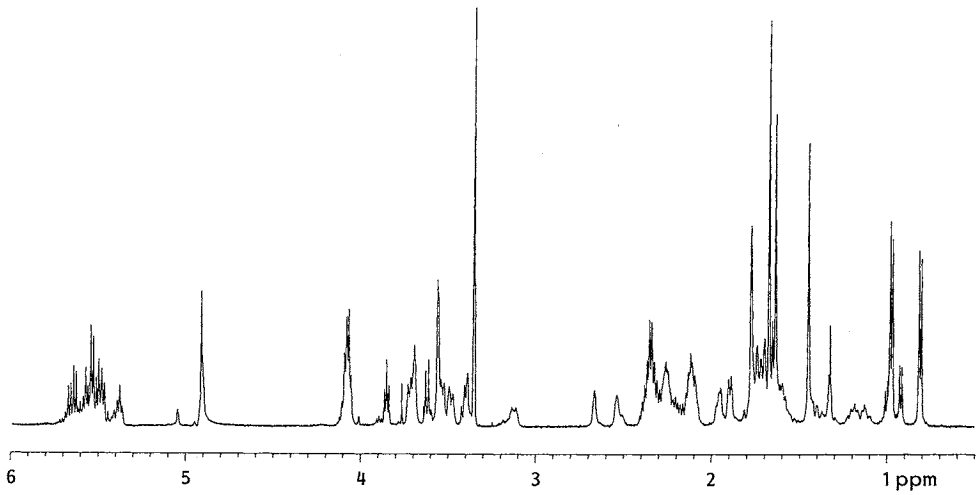


Fig. 3. ^{1}H NMR spectrum of lydicamycin (500 MHz, in MeOH-d_4).



(*m/z* 728.5247, Calcd for $C_{42}H_{70}N_3O_7$: 728.5241), which was derived by elimination of $C_5H_4NO_3$, thereby showing that the structure of lydicamycin terminated with an α-carbonyltetramic acid moiety. All 47 carbons were visible in the ^{13}C NMR spectrum (Table 2), and DEPT experiments established the presence of 62 carbon bound protons (6 methyls, 12 methylenes and 20 methines).

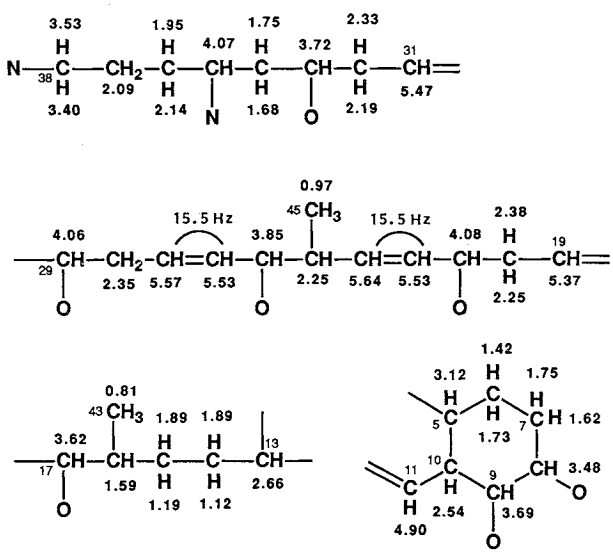
^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments elucidated all one bond ^{1}H-^{13}C connectivities and the partial structures as shown in Fig. 4. Overlapping vicinal proton signals such as 6-H (δ 1.73 and 1.42): 7-H (δ 1.75 and 1.62) and 14-H (δ 1.89 and 1.12): 15-H (δ 1.89 and 1.19) were analyzed by the aid of a homonuclear Harmann-Hahn (HOHAHA)[3] experiment. The connection of the partial structures in lydicamycin thus established was determined by a heteronuclear multiple-bond correlation (HMBC)[4] experiment, which revealed ^{1}H-^{13}C long-range couplings between four tertiary methyl protons (41-H, 42-H, 44-H, 46-H) and

Table 2. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR spectral data for lydicamycin in methanol-d_4.

No.	δ_C (m)[a]	δ_H	No.	δ_C (m)[a]	δ_H
1	181.0 (s)		25	77.6 (d)	3.85
2	103.0 (s)		26	134.3 (d)	5.53
3	204.0 (s)		27	129.8 (d)	5.57
4	54.5 (s)		28	39.2 (t)	2.35
5	33.6 (d)	3.12	29	78.3 (d)	4.06
6	23.4 (t)	1.73, 1.42	30	140.4 (s)	
7	29.6 (t)	1.75, 1.62	31	123.0 (d)	5.47
8	71.1 (d)	3.48	32	37.4 (t)	2.33, 2.19
9	75.5 (d)	3.69	33	70.2 (d)	3.72
10	43.7 (d)	2.54	34	41.7 (t)	1.73, 1.68
11	120.2 (d)	4.90	35	57.6 (d)	4.07
12	141.1 (s)		36	32.2 (t)	2.14, 1.95
13	45.0 (d)	2.66	37	24.1 (t)	2.09
14	29.9 (t)	1,89, 1.12	38	48.2 (t)	3.53, 3.40
15	37.9 (t)	1.89, 1.19	39	192.3 (s)	
16	37.9 (d)	1.59	40	50.7 (t)	3.56
17	83.7 (d)	3.62	41	17.9 (q)	1.45
18	139.7 (s)		42	23.4 (q)	1.77
19	124.0 (d)	5.37	43	17.1 (q)	0.81
20	36.7 (t)	2.38, 2.25	44	12.0 (q)	1.63
21	73.7 (d)	4.08	45	16.5 (q)	0.97
22	134.3 (d)	5.53	46	12.0 (q)	1.67
23	135.0 (d)	5.64	47	155.8 (s)	
24	44.0 (d)	2.25			

[a] Multiplicity.

Fig. 4. Partial structures obtained from a ^{1}H-^{1}H COSY experiment for lydicamycin.



following their relevant carbons: C-3, C-4, C-5, C-13; C-11, C-12, C-13; C-17, C-18, C-19; and C-29, C-30, C-31, as shown in Fig. 5. These results established the partial structure representing C-3 to C-38.

With regard to the remaining part, ^{1}H-^{13}C long range couplings were observed between isolated N-CH_2 protons (40-H, δ 3.56) and two quaternary carbons (C-1, δ 181.0; C-39, δ 192.3). As shown in

Fig. 6, these units, C-2 (δ 103.0) and C-3 (δ 204.0) were elucidated to form the α-actyltetramic acid moiety in order to exaplain the characteristic UV absorption and FAB-MS fragment of lydicamycin.

The only remaining carbon (C-47, δ 155.8) could be assigned to either urea, urethane or guanidine residue, based on its ^{13}C chemical shift. A fragment ion peak (m/z 113.0917, Calcd for $C_5H_{11}N_3$: 113.0953) in the HRFAB-MS indicated clearly the presence of a guanidine group. Non-equivalent chemical shifts for the methylene protons (38-H, δ 3.53 and 3.40), but not for the ones at the next position (37-H, δ 2.09), suggested that C-38 had to be closely located to an asymmetric carbon to form a five-membered ring. These results revealed that an *N*-amidinopyrrolidine moiety consisted of C-35 to C-38 and C-47 as shown in Fig. 6.

The geometrical configurations of lydicamycin were elucidated to be 18*E*, 22*E*, 26*E* and 30*E* by upfield chemical shifts for C-44 (δ 12.0) and C-46 (δ 12.0), and coupling constants of olefinic protons ($J_{22\text{-}23}=15.5$ Hz, $J_{26\text{-}27}=15.5$ Hz).

The planar structure of lydicamycin thus obtained was confirmed by high resolution FAB-MS, which revealed fragment peaks by cleavages at the α-positions of the pyrrolidine ring, secondary alcohols, and α-carbonyltetramic acid moiety, as summarized in Fig. 7.

The relative stereochemistry for the bicyclic portion substituted with the α-carbonyltetramic acid moiety was analyzed by decoupling and NOESY experiments. Large coupling constants between 5-H and 6-H_{ax} (12.0 Hz), 6-H_{ax} and 7-H_{ax} (12.0 Hz), and 7-H_{ax} and 8-H (11.0 Hz) indicated a chair conformation for the six-membered ring representing C-5 to C-10 and axial bonds for 5-H and 8-H. Additionally, small coupling constants less

Fig. 5. Partial structures around *C*-methyl groups in lydicamycin as revealed by HMBC.

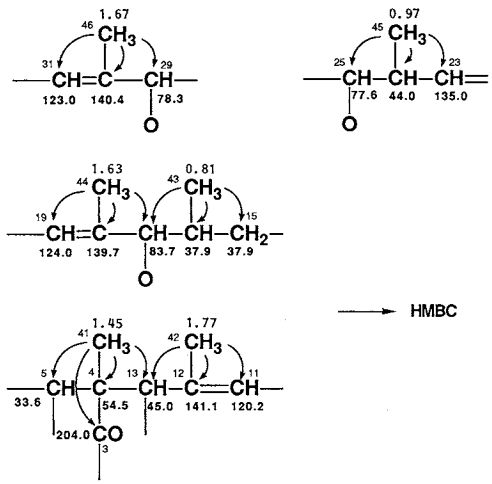


Fig. 6. NMR data for *N*-amidinopyrrolidine and α-acyltetramic acid moieties of lydicamycin.

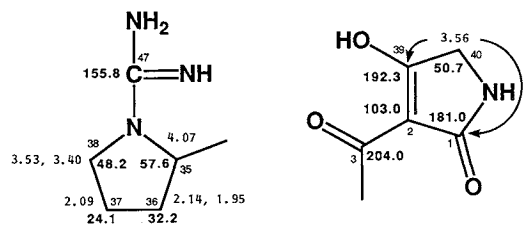
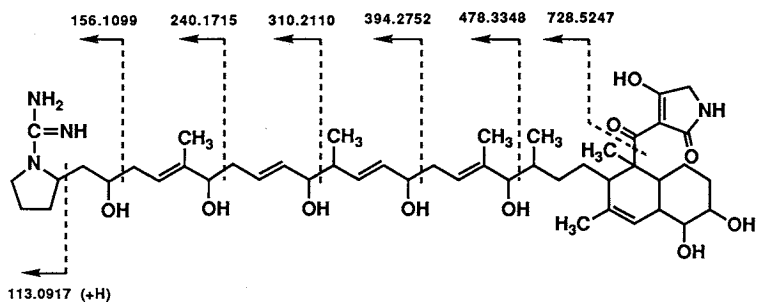
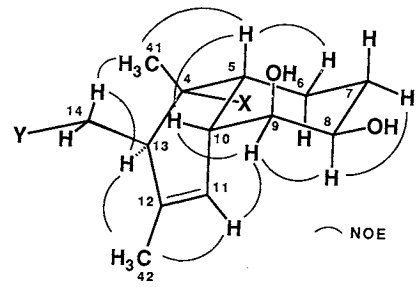


Fig. 7. HRFAB-MS fragmentation analysis for lydicamycin.



than 3 Hz between 5-H and 10-H, 8-H and 9-H, and 9-H and 10-H revealed equatorial bonds for 9-H and 10-H and a *cis* junction for the bicyclic system, which were confirmed by NOEs as summarized in Fig. 8.

Fig. 8. NOESY data summary for the bicyclic portion of lydicamycin.



NOEs observed between 5-H and 41-H, 9-H and 11-H, 13-H and 42-H, 13-H and 14-H (δ 1.89), and 14-H (δ 1.89) and 41-H established a pseudo-boat conformation for the cyclohexene ring in the bicyclic portion and the stereochemistries at C-4 and C-13 as shown in Fig. 8. The relative stereochemistry thus proved gave an anti conformation between the two bulky substituents, α-carbonyltetramic acid and long side chain. Similar bicyclic systems substituted with a tetronic acid moiety were reported on macrocyclic antibiotics, chlorothricin[5] and kijanimicin[6], which were considered to be biosynthetically related to lydicamycin, but their ring junctions were *trans* in both cases.

Lydicamycin represents a new class of antibiotics which appears to be predominantly polyketide-derived with the aminopyrrolidine moiety presumably originating from an amino acid. Further studies on the stereochemistry and biosynthesis are in progress.

Experimental

Specific rotation was obtained with a Jasco DIP-140 spectropolarimeter at 589.6 nm and 18°C. Mass spectra were measured on a Jeol HX-110 spectrometer in the FAB mode using glycerol matrix. UV spectra were recorded using a Shimadzu UV-300 spectrophotometer. NMR spectra were obtained on a Jeol JNM-GSX500 spectrometer with ^1H NMR at 500 MHz and ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard.

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