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Lactonamycin, a New Antimicrobial Antibiotic Produced by *Streptomyces rishiriensis* MJ773-88K4

II. Structure Determination

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The absolute structure of a new antibiotic lactonamycin is described. The NMR studies deduced one of four possible structures for the aglycon attached by a rhodinose through glycosidic bond. The stereochemistry of the sugar obtained by an acid hydrolysis was determined to be L-form by measuring optical rotation. The stereochemistry of the aglycon was determined by X-ray crystallographic analysis.

Lactonamycin **1** has been isolated from a culture broth of *Streptomyces rishiriensis* MJ773-88K4[1]. The antibiotic showed potent antimicrobial activities against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-registant *Enterococcus* (VRE). The taxonomy of the producing strain, fermentation, isolation, physico-chemical properties and antimicrobial activities were described in the previous paper[2]. This paper deals with structure determination of **1**.

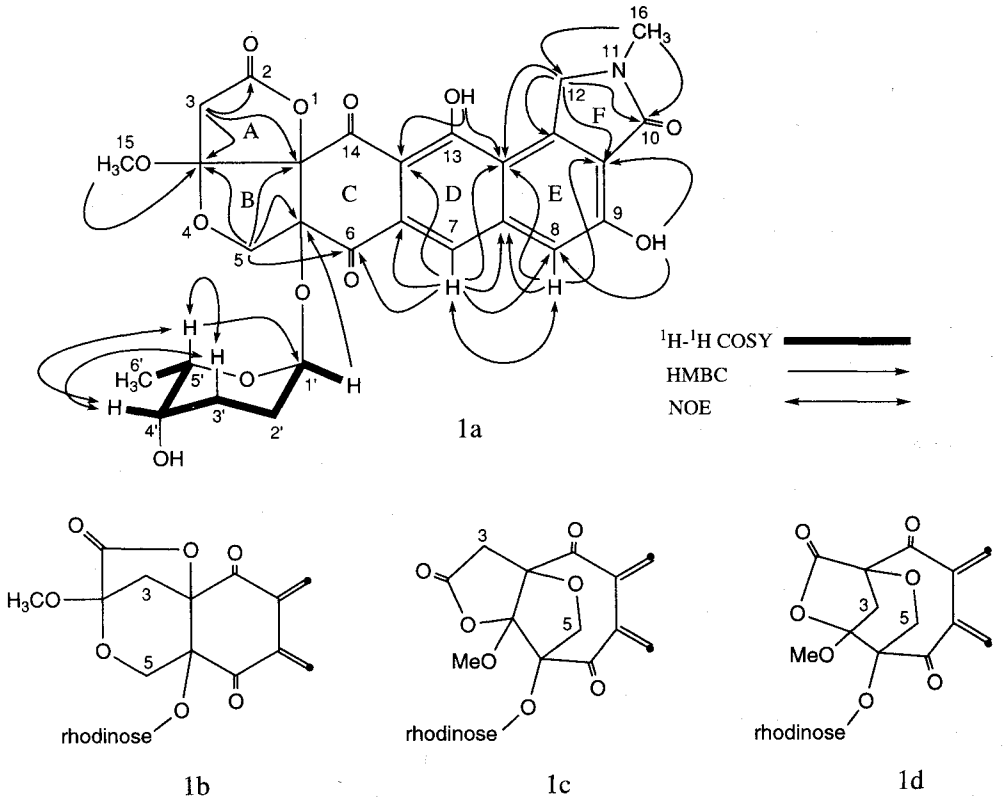
Results and Discussion

Spectroscopic Studies

The molecular formula of **1** was established as $C_{28}H_{27}NO_{12}$ by HRFAB-MS measurement [*m/z*, found 570.1613, calcd. 570.1612 for $C_{28}H_{28}NO_{12}(M+H)^+$]. The ^{13}C and ^1H NMR data are listed in Table 1. The ^{13}C NMR and ^1H NMR spectra displayed 28 carbon signals and 26 proton signals, respectively. The proton-carbon connectivities were determined by the heteronuclear multiple quantum coherence (HMQC) experiment. Interpretation of the ^{13}C NMR signals were as follows: three methyls, five methylenes, five methines and fifteen quaternary carbons including four carbonyl carbons. The DEPT experiment also supported above results. The proton-proton connectivities were determined by double-quantum filtered COSY (DQF-COSY). The results established the proton sequence of the sugar moiety 1′-H (δ 4.880) to 6′-H_3 (δ 0.93). The small coupling constant of 1′-H and 4′-H (δ 3.54), and the presence of the NOEs among axial 3′-H (δ 1.95), 4′-H and 5′-H (δ 3.87) clarified the relative stereochemistry. As shown in Fig. 1a, this sugar was determined to be 2,3,6-trideoxy-*threo*-hexopyranoside (rhodinose).

No other proton-proton connectivity was observed, that is, all protons in the aglycon moiety were isolated. Only the heteronuclear multiple-bond correlation (HMBC) experiment gave the information on this part,

Fig. 1. Observed correlations in the ^1H-^1H COSY, HMBC and NOE experiments of **1**.



and the results were shown in Fig. 1a. The long-range couplings of two aromatic protons 7-H (δ 8.03) and 8-H (δ 7.32), and two phenolic protons 9-OH (δ 9.40) and 13-OH (δ 13.70) established a 1,7-dihydroxynaphthalene ring.

16-H_3 which have a characteristic chemical shift (δ_H 3.30, δ_C 29.1) should be attached to a nitrogen atom. The long-range couplings from this methyl proton to the carbonyl carbon C-10 (δ 169.0) and C-12 (δ 55.0) suggested the partial structure, $-C^{12}H_2-N(C^{16}H_3)-(C^{10}=O)-$. The ring connectivity between ring D–E and ring F was established by the long-range coupling from 12-H_2 (δ 4.98) to C-12b (δ 116.7). Attachment of a ketone carbonyl carbon C-6 (δ 190.4) to C-6a (δ 130.8) was deduced from the correlation between 7-H and C-6. Another ketone carbonyl carbon C-14 (δ 192.6) was placed adjacent to C-13a (δ 109.4) to form a hydrogen bonding with the 13-OH because of its lower chemical shift, δ 13.70.

The remaining parts so far were one methylene (C-3 at δ 37.2) and one oxygenated methylene (C-5 at δ 74.3), $-C^2OO-$, two oxygenated quaternary carbons (C-5a at δ 86.1 and C-14a at δ 90.3) and $-O-C^{3a}R_1R_2-OMe^{15}$ deduced from the correlation between 15-H_3 (δ 3.17) and C-3a (δ 112.9) in the HMBC spectrum.

The long-range couplings from 5-H_2 (δ 4.32 and 4.88) to C-3a, C-5a, C-6 and C-14a, from 3-H_2 (δ 2.92 and 3.04) to C-2 (δ 171.1), C-3a and C-14a offered four possible structures related to the left part of the aglycon (ring A–B–C), as described in Fig. 1a, 1b, 1c and 1d. The proton chemical shift of 13-OH (δ 13.70), which represented typical hydrogen bonding of the rigid 6–6 fused ring system, was agreeable for the structures described in Fig. 1a or 1b. The structure in Fig. 1a was more plausible than one in Fig. 1b, considering the relatively low-field chemical shift of 3-H_2 caused by adjacent carbonyl group. The connection between rhodinose and the aglycon was not assured by HMBC experiment measured in $CDCl_3$ due to signal overlapping between 5-H_2 and 1′-H at δ ~4.88, but when measured in acetone-d_6, these proton signals were well separated. The HMBC correlation between 1′-H (δ 4.78) and C-5a

Table 1. The ^{13}C and ^{1}H NMR data of **1** in $CDCl_3$.

position	δ_C [a]	δ_H [b]	J value (Hz)
2	171.1 s		
3	37.2 t	2.92 d	16.4
		3.04 d	16.4
3a	112.9 s		
5	74.3 t	4.32 d	9.0
		4.88 d	9.0
5a	86.1 s		
6	190.4 s		
6a	130.8 s		
7	120.5 d	8.03 s	
7a	141.8 s		
8	112.8 d	7.32 s	
9	157.4 s		
9a	120.9 s		
10	169.0 s		
12	55.0 t	4.98 s	
12a	142.8 s		
12b	116.7 s		
13	164.0 s		
13a	109.4 s		
14	192.6 s		
14a	90.3 s		
15	52.7 q	3.17 s	
16	29.1 q	3.30 s	
1'	96.1 q	4.88 br	
2'	23.1 t	1.52 br d	13.2
		1.86 tt	3.6, 13.2
3'	25.1 t	1.66 dq	3.6, 13.2
		1.95 ddt	1.7, 3.6, 13.2
4'	67.0 d	3.54 br s	
5'	67.5 d	3.87 q	6.0
6'	16.5 q	0.93 d	6.0
9-OH		9.40 s	
13-OH		13.70 s	

4'-OH was not observed.
[a] 125 MHz, chemical sifts in ppm, multiplicity.
[b] 500 MHz, chemical sifts in ppm, multiplicity.

(δ 68.7) in acetone-d_6 was thus observable, indicating that rhodinose was attached to C-5a through glycosidic bond. The proposed planer structure of **1** was shown in Fig. 1a.

Absolute Stereochemistry

X-Ray crystallographic analysis was also carried out to ascertain above-described presumptive structure. Yellowish brown prism crystals were grown from *n*-hexane-dichloromethane-methanol solution and one of them was suitable for X-ray analysis. A perspective view of the molecule is depicted in Fig. 2a.

The absolute structure of **1** was determined by degradation study. Treatment of **1** with 1 N HCl (1 ml) in tetrahydrofuran (2.5 ml) at room temperature for 18 hours gave an aglycon (lactonamycinon) in quantative yield and rhodinose in 74% yield. The optical rotation value of rhodinose obtained by degradation of **1** was $[\alpha]_D^{21}$ $-7.7°$ (*c* 0.3, MeOH), while the reported value of L-rhodinose was $[\alpha]_D^{20}$ $-11\pm1.6°$ (*c* 0.3, MeOH)[3]. Therefore, the rhodinose of lactonamycin was determined to be the L-form. Thus, the absolute configuration of **1** was determined to be (3a*S*,5a*S*,14a*S*)-3,3a,5,5a,6,11,12,14-octahydro-5a-{[(5*S*,6*S*)-5-hydroxy-6-methyltetrahydropyran-2-yl]oxy}-3a-methoxy-11-methyl-2,6,10,14-tetraoxofuro[2″,3″,4′,5′]-furo[4′,3′,6,7]naphtho[2,3-e]isoindole-9,13-diol, as described in Fig. 2b.

Only plant constituent viburnolides[3] have been reported to possess hexahydrofurano[3,2-b]furan-2-one structure attached by an ornamented oxygen atom at position 3a like lactonamycin, but the other substituents in viburnolides are completely different from those of lactonamycin. Furthermore, naphtho[e]isoindole ring in lactonamycin is also novel among the natural products. From these view points, we emphasize the structural novelty of lactonamycin.

Experimental

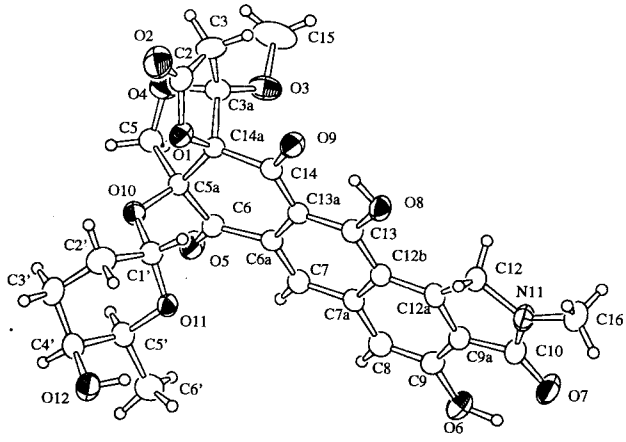
General

The ^{13}C and ^{1}H NMR spectra were recorded on a JEOL JNM-A500 spectrometer. Chemical shifts are given in ppm from TMS as an internal standard. FAB-MS and HRFAB-MS were obtained on a JEOL JMS-SX102 mass spectrometer. Optical rotation was taken by a Perkin-Elmer 241 polarimeter using a micro-cell (light path 10 cm).

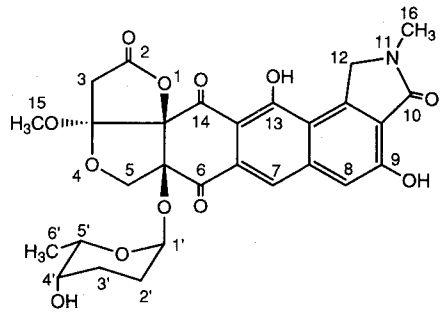
Acid Hydrolysis of Lactonamycin

Lactonamycin (64.9 mg, 0.112 mmol) in tetrahydrofuran (2.5 ml) was added 1 N HCl (1 ml). After the reaction mixture had been stirred at room temperature for 18 hours, THF was removed by evaporation. The resultant aqueous solution was diluted with water (20 ml) and extracted with ethyl acetate (20 ml, 10 ml). The aqueous layer containing the rhodinose was reserved for the further purification. The ethyl acetate layer containing the aglycon was washed with water (20 ml), dried

Fig. 2. A perspective view of the molecule structure of **1**.



2a



2b

over Na_2SO_4, and evaporated to dryness to give an lactonamicinon (50.7 mg, quantitative yield) as a yellow powder. The aqueous phase above-mentioned was neutralized with silver carbonate, then the solid precipitated (AgCl) was filtered off. The filtrate was evaporated, then dried *in vacuo*. The residue was dissolved in $CHCl_3$ (3 ml) and insoluble material was filtered off again. After evaporation of $CHCl_3$, α,β-anomeric mixture of rhodinose (9.7 mg, 74% yield) was obtained as a colorless syrup. The ^1H NMR spectrum and Rf value were in good accordance with those of the published data[4].: $[\alpha]_D^{21}$ $-7.7°$ (*c* 0.3, MeOH).

X-Ray Crystallography for **1**

A pale yellowish brown prism crystal of $0.10 \times 0.20 \times 0.30$ mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation. Crystal data are as follows: Empirical formula; $C_{28}H_{27}NO_{12}$. Formula weight; 569.52. Crystal system; monoclinic. Space group; C2. Lattice parameters; $a=35.493(5)$ Å, $b=9.850(2)$ Å, $c=15.368(2)$ Å, $\beta=102.52(1)°$, $V=5244(1)$ Å^3. Z value; 8. D_{calc}; 1.442 g/cm^3. μ(CuKα); 9.72 cm^{-1}. The reflection data were collected at a temperature of $21 \pm 1°$C using the ω–2θ scan technique to a maximum 2θ value of 120.1°. Of the 8323 reflections which were collected, 7795 were unique. The data were corrected for Lorentz and polarization effects. The structure was solved by a direct methods (SIR92)[5] and expanded using Fourier techniques[6]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 7035 observed reflections ($I > 3.00\sigma(I)$) and 738 variable parameters and converged with unweighed and weighted agreement factors of $R=0.049$ and $Rw=0.076$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.63 and $-0.22 e^-/Å^3$, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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