Communications to the Editor

LAGUNAMYCIN, A NOVEL 5-LIPOXYGENASE INHIBITOR

II. STRUCTURAL STUDIES

Sir:

Lagunamycin, a new metabolite isolated from the culture filtrate of *Streptomyces* sp. AA0310 showed inhibitory activity against 5-lipoxygenases and antibacterial activity against Gram-positive bacteria[1]. The structure of lagunamycin has now been elucidated to be 6-diazo-4-[(*E*)-4,6-dimethyl-2-hepten-2-yl]-3-methyl-2,5,7,8-tetraoxoquinoline by a combination of chemical degradations and NMR studies.

The molecular formula of lagunamycin (**1**) was established as $C_{19}H_{21}N_3O_4$ based on elemental analysis[1] and high resolution FAB-MS ($(M+H)^+$ *m/z* calcd 356.1610, found 356.1611). The IR spectrum showed a characteristic absorption band at 2150 cm^{-1}, which suggested the presence of a diazo group.

The ^{13}C and ^1H NMR data are summarized in Table 1. All one-bond ^1H-^{13}C connectivities were determined by a ^{13}C-^1H COSY experiment. ^1H-^1H COSY, NOESY and long range ^{13}C-^1H COSY experiments indicated a partial structure of $C_{13}H_{21}NO$ containing an amide as depicted in Fig. 2. The geometry of the double bond (2′,3′) was established as "*E*" by measurement of $^3J_{CH}$ value (8.3 Hz) between C-1′ and 3′-H in a non-decoupled ^{13}C NMR spectrum.

The lower field ^{13}C NMR signals of **1** suggested a substituted pyridone (δ 116.3 s, 130.0 s, 138.6 s, 151.4 s and 161.3 s) and a 2-diazo-3-oxo-1,4-benzoquinone (δ 87.5 s, 168.8 s, 172.5 s and 173.6 s) nuclei by a comparison with the reported values of diazaquinomycin A[2] and 2-diazo-3-oxo-1,4-naphthoquinone[3], respectively. Similar stabilities of **1** and 2-diazo-3-oxo-1,4-naphthoquinone under acidic conditions indicated the presence of a diazo

Table 1. ^{13}C and ^1H NMR spectra of lagunamycin (in $CDCl_3$).

Number	^{13}C	^1H
1		9.60 (1H, s)
2	161.3 (s)	
3	130.0 (s)	
4	151.4 (s)	
4a	116.3 (s)	
5	173.6 (s)	
6	87.5 (s)	
7	168.8 (s)	
8	172.5 (s)	
8a	138.6 (s)	
9	14.0 (q)	2.18 (3H, s)
1′	16.8 (q)	1.90 (3H, d, J=1.3)
2′	137.4 (s)	
3′	135.0 (d)	4.86 (1H, dq, J=9.4, 1.3)
4′	30.4 (d)	2.68 (1H, m)
5′	46.6 (t)	1.19 (2H, m)
6′	25.9 (d)	1.61 (1H, m)
7′	22.4 (q)	0.93 (3H, d, J=6.4)
8′	23.2 (q)	1.93 (3H, d, J=6.4)
9′	20.4 (q)	1.01 (3H, d, J=6.6)

Fig. 1. Structure of lagunamycin.

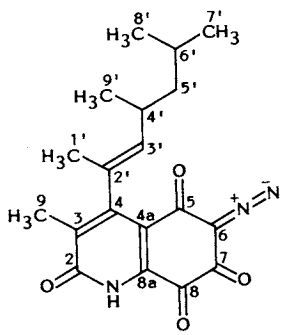

Fig. 2. A partial structure of lagunamycin as revealed by ^1H-^1H COSY, NOESY and ^1H-^{13}C long range COSY experiments.

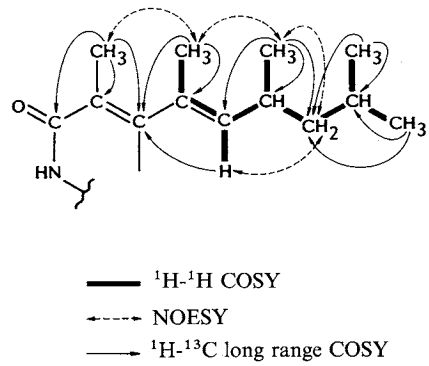

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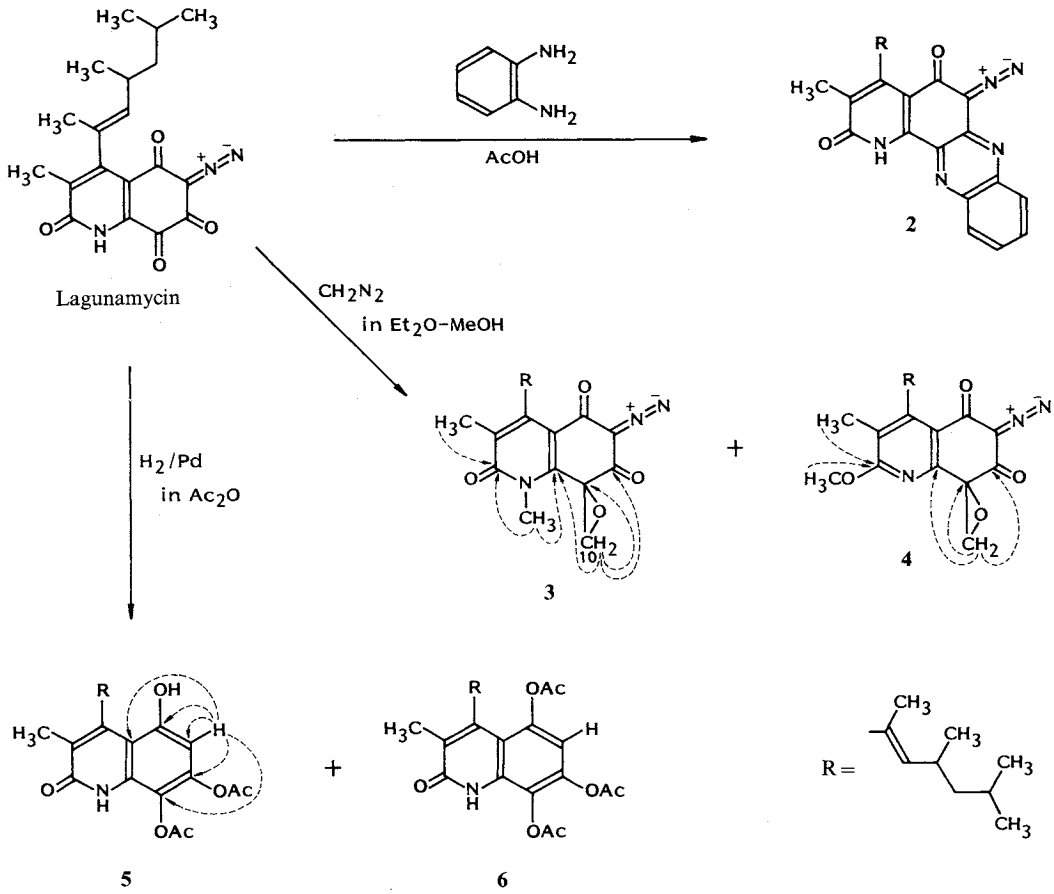
Table 2. ^{13}C NMR spectra of derivatives **3**, **4**, **5** and **6**.

Number	3	4	5	6	Number	3	4	5	6
2	163.3 (s)	164.9 (s)	163.0 (s)	162.8 (s)	4′	30.2 (d)	30.3 (d)	30.5 (d)	30.3 (d)
3	137.3 (s)	121.0 (s)	125.6 (s)	126.1 (s)	5′	46.8 (t)	46.8 (t)	46.2 (t)	46.4 (t)
4	150.8 (s)	155.4 (s)	147.7 (s)	148.3 (s)	6′	26.6 (d)	25.7 (d)	25.8 (d)	25.2 (d)
4a	116.4 (s)	120.7 (s)	106.4 (s)	112.2 (s)	7′	22.5 (q)	22.5 (q)	22.1 (q)	23.6 (q)
5	173.3 (s)	175.5 (s)	151.9 (s)	141.7 (s)	8′	23.1 (q)	23.2 (q)	23.1 (q)	24.0 (q)
6	83.8 (s)	86.5 (s)	104.4 (d)	112.1 (d)	9′	20.4 (q)	20.4 (q)	20.1 (q)	19.7 (q)
7	179.9 (s)	183.4 (s)	143.1 (s)	144.0 (s)	N-Me	33.0			
8	57.5 (s)	55.7 (s)	121.9 (s)	128.6 (s)	O-Me		54.2 (q)		
8a	145.3 (s)	151.1 (s)	131.9 (s)	131.2 (s)	Ac-CO			168.5 (s)	168.6 (s)
9	13.8 (q)	11.8 (q)	12.7 (q)	13.5 (q)				167.7 (s)	167.4 (s)
10	54.8 (t)	57.7 (t)							167.7 (s)
1′	17.1 (q)	17.0 (q)	17.3 (q)	17.1 (q)	Ac-Me			20.6 (q)	20.7 (q)
2′	131.7 (s)	131.2 (s)	133.3 (s)	131.9 (s)				20.7 (q)	21.2 (q)
3′	132.9 (d)	133.7 (d)	139.9 (d)	135.6 (d)					22.2 (q)

Fig. 3. Reaction products of lagunamycin and their long range ^{13}C-^{1}H COSY (⟶).



group in **1**. Combining these results, the structure of **1** was assigned as in Fig. 1. The following chemical degradation studies supported the assumption.

Reaction of **1** with *o*-phenylenediamine in acetic acid afforded a crystalline adduct **2** ($C_{25}H_{25}N_5O_2$) in a good yield, confirming the presence of an α-diketone functionality in **1**. Reaction of **1** with an excess of diazomethane in methanol and ether gave

two major products with the same molecular formula ($C_{21}H_{25}N_3O_4$). They were identified as *N*-methyl (**3**) and *O*-methyl (**4**) derivatives of **1**. Both of them also contained an epoxide group which was considered to be formed by reaction of diazomethane with the activated carbonyl group (C-8 ketone). In the ^{13}C NMR spectra of **3** and **4**, a carbonyl carbon of **1** was replaced by an oxygenated singlet carbon (δ 55~58), and in addition, a new triplet carbon (δ 54~58) was observed. The detailed analysis of **3** by long range ^{13}C-^{1}H COSY experiments revealed correlations between the amide methyl protons and C-8a, and epoxide methylene protons (10-H) and C-8a, and C-7 substantiating the pyridodiazoquinone structure of **1**.

Reductive acetylation of **1** with 10% palladium on carbon in acetic anhydride yielded a diacetyl (**5**) and a triacetyl (**6**) derivatives. These products did not shown the absorption of a diazo group in the IR spectra. Their ^{1}H and ^{13}C NMR spectra exhibited a new aromatic proton (δ 6.54 for **5** and 6.81 for **6**) and a corresponding doublet aromatic carbon (δ 104.4 for **5** and 112.2 for **6**). This information indicated that the diazo group of **1** had been replaced by a proton in the hydrogenation. The long range ^{13}C-^{1}H COSY experiment on **5** supported this observation. The new proton at δ 6.53 showed correlation to the four quaternary carbons at δ 106.4 (C-4a), 121.9 (C-8), 143.1 (C-7) and 151.9 (C-5), but no correlation to δ 131.9 (C-8a). These results evidenced that the diazo group was at C-6 position of **1**, and therefore the structure of **1** was determined as 6-diazo-4-[(*E*)-4,6-dimethyl-2-hepten-2-yl]-3-methyl-2,5,7,8-tetraoxoquinoline.

It is interesting to note that the des-diazo derivatives, **5** and **6** retained the 5-lipoxygenase inhibitory activity (IC_{50} 3.0 and 10 μg/ml, respectively) comparable to that of the parent antibiotic (IC_{50} 6.1 μg/ml).

Acknowledgments

We thank Prof. M. OHASHI of the University of Electrocommunication for his advice in the structural studies. Thanks are also due to Dr. T. TSUNO, Mr. T. MORIYAMA and Miss M. AOKI for their helpful assistance.

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(Received December 11, 1992)

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