LACTOQUINOMYCIN, A NOVEL ANTICANCER ANTIBIOTIC

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ASSIGNMENT

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(Received for publication July 3, 1985)

Lactoquinomycin, a new antibiotic, $C_{24}H_{27}NO_8$, mp 151~159°C (dec), FAB-MS: *m/z* 458 (MH^+), is a basic substance, showing UV λ_{max}^{MeOH} (ε) 215 (37,600), 254 (10,700) and 432 nm (4,760), and IR $\nu_{max}^{CHCl_3}$ 1790 (γ-lactone), 1665 and 1650 (quinone) cm^{-1}. The structure of lactoquinomycin has been elucidated by ^1H NMR and ORD in comparison with those of kalafungin.

In the preceding paper[1], taxonomy of the lactoquinomycin-producing organism, and production, purification and some biological activities of the antibiotic have been described. We here report physico-chemical properties and structure assignment of lactoquinomycin. The detailed antitumor activity will be published elsewhere.

Physico-chemical Properties

Lactoquinomycin was obtained as orange crystalline powder, which melted at 151~159°C with decomposition. The antibiotic was transferred from water to ethyl acetate at alkaline pH and from ethyl acetate to water at acidic pH, suggesting that it is a basic substance. The water solution was orange at acidic pH and purple at alkaline pH.

The free basic form was soluble in ethyl ether, chloroform, ethyl acetate, acetone, methanol and water, but hardly soluble in *n*-hexane. It gave Rf values 0.27 (chloroform - ethanol, 1:1) and 0.30 (methanol) on TLC (Kiesel gel $60F_{254}$, Merck).

The antibiotic showed positive color reaction with magnesium acetate and Dragendorff reagents, but negative with ninhydrin, Elson-Morgan, and nitroprusside-acetaldehyde reagents.

The fast atom bombardment mass spectrum (FAB-MS) revealed the molecular ion peak at *m/z* 458 (MH^+). The UV and visible absorption spectra showed maxima at 215 (ε 37,600), 254 (10,700) and 432 nm (4,760) in methanol, 215 (ε 42,800), 253 (10,800) and 429 nm (4,980) in 0.01 N HCl - methanol, and 222 (ε 32,200, sh), 262 (8,680), 273 (8,640) and 558 nm (4,980) in 0.01 N NaOH - methanol (Fig. 1).

Lactoquinomycin had a specific rotation of $[\alpha]_D^{28}$ +316.9° (*c* 0.2, methanol). The antibiotic showed a positive Cotton effect (Fig. 2). The IR spectrum (chloroform) was consistent with the presence of 3 carbonyl groups, γ-lactone (1790 cm^{-1}), non-chelated quinone (1665 cm^{-1}) and chelated quinone (1650 cm^{-1}) (Fig. 3). ^{13}C NMR and ^1H NMR in $CDCl_3$, measured at 100 MHz and 400 MHz respectively, are presented in Tables 1 and 2.

The elemental analysis was as follows:

Anal Calcd for $C_{24}H_{27}NO_8$: C 63.01, H 5.95, N 3.06, O 27.98.
Found: C 61.18, H 6.21, N 3.20, O 27.70.

Fig. 1. UV absorption spectra of lactoquinomycin.

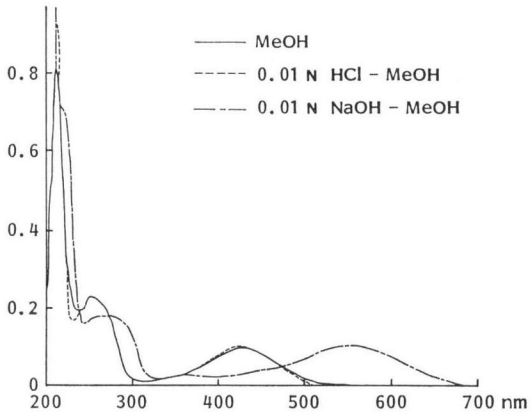


Fig. 2. ORD curve of lactoquinomycin.

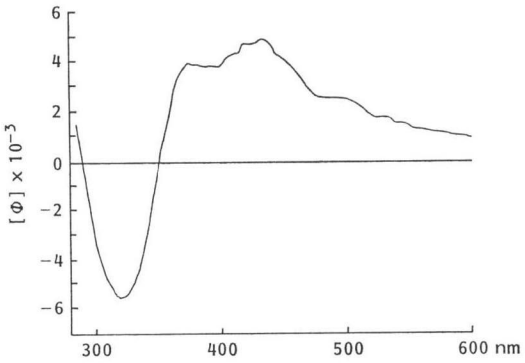


Fig. 3. IR spectrum of lactoquinomycin ($CHCl_3$).

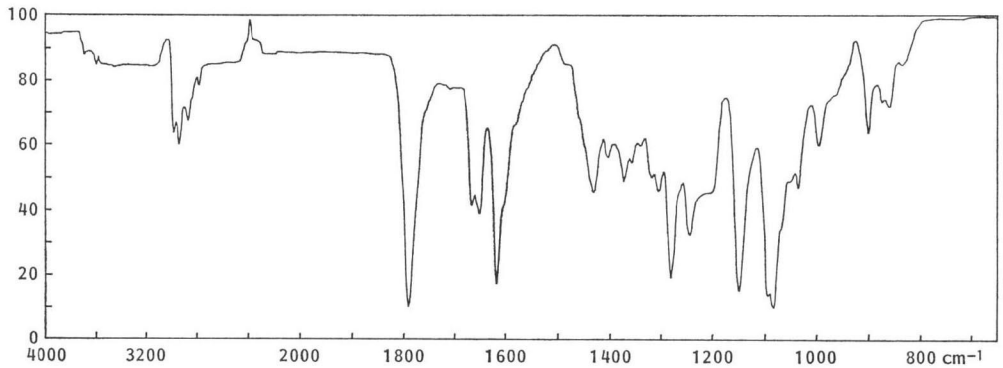


Table 1. 100 MHz ^{13}C NMR data of lactoquinomycin in $CDCl_3$.

Carbon	Chemical shift*	Multiplicity	Carbon	Chemical shift*	Multiplicity
1	66.3	d	10a	149.2	s
3	66.5	d	11	37.0	t
4	68.7	d	12	173.5	s
4a	134.9	s	1-CH_3	18.8	q
5	180.8	s	2′	72.2	d
5a	129.7	s	3′	28.2	t
6	119.6	d	4′	67.2	d
7	133.5	d	5′	71.5	d
8	138.6	s	6′	77.6	d
9	157.7	s	4′-$N(CH_3)_2$	40.3	q
9a	114.0	s	6′-CH_3	18.9	q
10	187.8	s			

* TMS (0 ppm) was used as an internal standard.

Structure Assignment

The results of elemental analysis and FAB mass spectrometry (MH^+ 458) gave the molecular formula of $C_{24}H_{27}NO_8$. The UV and IR spectra suggested that lactoquinomycin is a derivative of

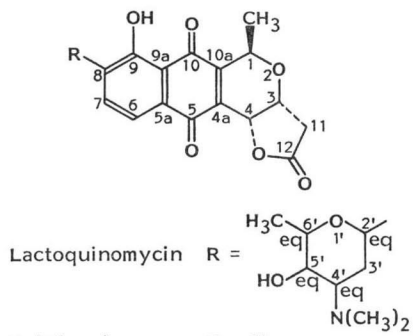
Table 2. 400 MHz ^{1}H NMR data of lactoquinomycin in $CDCl_3$ in comparison with kalafungin.

Proton	Lactoquinomycin*	Kalafungin[3]
1-H	5.08 q (7.0)**	5.05 q (7)
3-H	4.69 dd (5.1, 2.9)	4.69 dt (4.5, 3.0)
4-H	5.25 d (2.9)	5.20 d (3.0)
6-H	7.71 d (7.8)	7.00~7.78 m
7-H	7.91 d (7.8)	
8-H	—	
11-H_1	2.69 d (17.6)	2.57 d (18)
11-H_2	2.97 dd (17.6, 5.1)	3.02 dd (18, 4.5)
1-CH_3	1.57 d (7.0)	1.52 d (7)
9-OH	12.2 br s	11.80
2′-H	4.87 dd (10.9, 2.0)	
3′-H_{ax}	1.30 ddd (12.5, 12.4, 10.9)	
3′-H_{eq}	2.26 ddd (12.4, 3.8, 2.0)	
4′-H	2.78 ddd (12.5, 9.5, 3.8)	
5′-H	3.20 dd (9.5, 8.9)	
6′-H	3.53 dq (8.9, 6.2)	
4′-$N(CH_3)_2$	2.34 s	
5′-OH	3.4 br s	
6′-CH_3	1.43 d (6.2)	

* TMS (0 ppm) was used as an internal standard.

** δ_H, multiplicity, coupling constant.

Fig. 4. Structures of lactoquinomycin and kalafungin.



juglone (5-hydroxy-1,4-naphthoquinone). Considering the presence of γ-lactone, the structure of lactoquinomycin resembles that of kalafungin[2,3].

The comparison of ^{1}H NMR data of lactoquinomycin with those of kalafungin revealed that one of the three aromatic protons of kalafungin is substituted by a sugar moiety in lactoquinomycin (Table 2). Since an aromatic AB spin system (δ_H 7.71 and 7.91 ppm, J=7.8 Hz) was observed in the ^{1}H NMR spectrum of lactoquinomycin, the substituted position should be C-6 or C-8. The substitution of the sugar moiety at the C-8 position was elucidated as follows. The aromatic proton signals (δ_H 7.71 and 7.91 ppm) and carbon signals coupled with these protons (δ_c 119.6 and 133.5 ppm; confirmed by selective decoupling) imply that the C-8 is substituted[4]. Furthermore a long range coupling between 2′-H signal (δ_H 4.87 ppm) and phenolic carbon signal (C-9; δ_c 157.7 ppm) was observed, indicating that the C-8 position is substituted by the sugar moiety.

The ^{1}H NMR spectrum of lactoquinomycin indicates the presence of 2-substituted 4-dimethylamino-5-hydroxy-6-methyltetrahydropyran ring[10]. All the spin-couplings were confirmed by decoupling experiments. Lactoquinomycin is optically active, and the ORD curve (Fig. 2) suggests that the antibiotic has dihydropyran ring, whose stereochemistry is similar to kalafungin[3] but differs from nanaomycin D[5]. As a result, the relative stereochemistry of lactoquinomycin has been determined as shown in Fig. 4.

Discussion

Lactoquinomycin resembles luteomycin ($C_{23}H_{29}NO_9$)[6], No. 289 substance ($C_{26}H_{33}NO_{12}$)[7] and medermycin[8,9] in some physico-chemical characteristics. We carried out comparative studies with medermycin, because the molecular formula is identical and the chemical structure has been studied. The molecular formula of medermycin was first reported $C_{24}H_{29}NO_8$[8], and later revised to $C_{24}H_{27}NO_8$ by FD-MS[9]. Lactoquinomycin can be differentiated from medermycin by the physico-chemical properties and anticancer activity. Lactoquinomycin is rather stable but medermycin is labile. A significant antitumor activity is observed with the former[1] but not with the latter[8]. The MICs are different[1,8]. Since detailed studies on medermycin structure have not been published, the comparison of the two substances is difficult. However, lactoquinomycin might be an isomer of medermycin in the respect of sugar moiety-attaching position, although the precise relationship between the two substances could be determined by structural elucidation of medermycin.

Acknowledgments

The present works were partly supported by a grant-in-aid for cancer research from the Ministry of Education, Science and Culture, Japan. The authors express their deep thanks to Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo, for his kind advice and cooperation in the current experiments. We are greatly indebted to Prof. Y. SATO and Dr. T. ODA, Kyoritsu Pharmaceutical College, Tokyo, for ORD measurement, and Mr. K. FURIHATA of our Institute for skillful NMR spectrometry.

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