LACTOQUINOMYCIN B, A NOVEL ANTIBIOTIC

TAKAYOSHI OKABE†, KUNIKO NOMOTO and NOBUO TANAKA*

Institute of Applied Microbiology, University of Tokyo,
Tokyo 113, Japan

(Received for publication October 3, 1985)

Streptomyces tanashiensis IM8442T was found to produce lactoquinomycin B, a novel antibiotic, together with lactoquinomycin A. Lactoquinomycin B was purified, and the physico-chemical and biological characteristics were studied. Lactoquinomycin B, $C_{24}H_{27}NO_9$, mp 149~152°C (dec), FD-MS *m/z* 473 (M^+), is a basic substance, showing UV λ_{max}^{MeOH} (ε) 239 (15,100), 287 (3,450) and 369 nm (5,300), and IR $\nu_{max}^{CHCl_3}$ 1790 (γ-lactone), and 1700 and 1650 (quinone) cm^{-1}. The structure of lactoquinomycin B was elucidated by ^1H NMR and ^{13}C NMR in comparison with those of lactoquinomycin A, indicating that B is a 4a,10a-epoxide derivative of A. Lactoquinomycin B displayed inhibitory activity against bacteria, particularly Gram-positive organisms, and cytotoxicity against human and murine tumor cell lines. LD_{50} for mice was *ca.* 40 mg/kg by iv route.

During the course of our screening for new antitumor antibiotics, using drug-resistant neoplastic cells, *Streptomyces tanashiensis* IM8442T was found to produce a novel antibiotic, which inhibits growth of drug-resistant cell sublines of L5178Y murine lymphoblastoma more significantly than that of the parental cell line. The antibiotic was named lactoquinomycin according to the chemical structure. The production, isolation, characterization and structure assignment were described in the previous papers[1,2]. We have recently found that the organism produces another novel antibiotic together with lactoquinomycin. The latter is a major component and the former a minor one. The name of lactoquinomycin A is used for the major antibiotic, previously reported, in this publication. The new agent is an epoxide derivative of lactoquinomycin A and designated lactoquinomycin B. The present report describes the isolation, physico-chemical properties, structure assignment and some biological activities of lactoquinomycin B.

Production and Isolation

The antibiotics in the culture fluid (2 liters), produced by the procedure previously reported[1], were extracted with EtOAc (2 liters×2) at pH 7.5, transferred to water layer (10 mM HCl, 400 ml), and again extracted with EtOAc (400 ml×2). Remaining water was removed by Na_2SO_4 from the EtOAc layer, which was then concentrated to 40 ml *in vacuo*. A crude powder was obtained by addition of *n*-hexane to the concentrated solution. The powder was dissolved in 10 mM sodium acetate buffer, pH 5.5, and adsorbed on CM-Toyopearl 650M column. Lactoquinomycins B and A were separated by elution with linear gradient of 100~200 mM NaCl in 10 mM sodium acetate buffer, pH 5.5. Fractions containing each antibiotic were collected, adjusted to pH 7.5, and transferred to EtOAc, which was dried over Na_2SO_4 and concentrated *in vacuo*. Lactoquinomycins A (*ca.* 120 mg) and B (*ca.* 10 mg) were obtained by addition of *n*-hexane (Fig. 1).

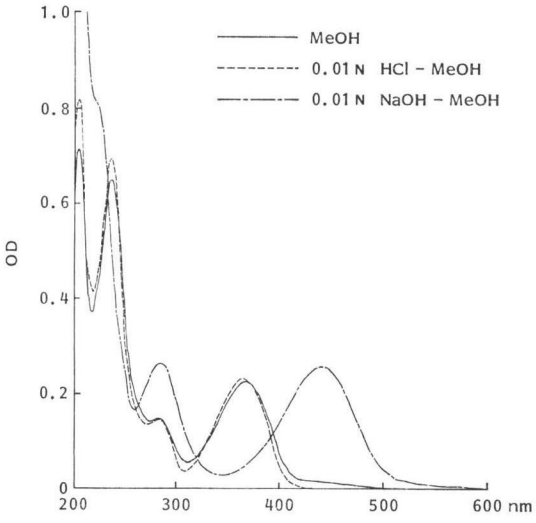
Physico-chemical Characteristics

Lactoquinomycin B was obtained as light-yellow crystalline powder, which melted at 149~152°C

† Present address: Exploratory Research Laboratories, Banyu Pharmaceutical Co., Ltd., 2-9-3 Shimo-Meguro, Meguro-ku, Tokyo 153, Japan.

Fig. 1. Isolation scheme for lactoquinomycins A and B.

Fig. 2. UV spectra of lactoquinomycin B.

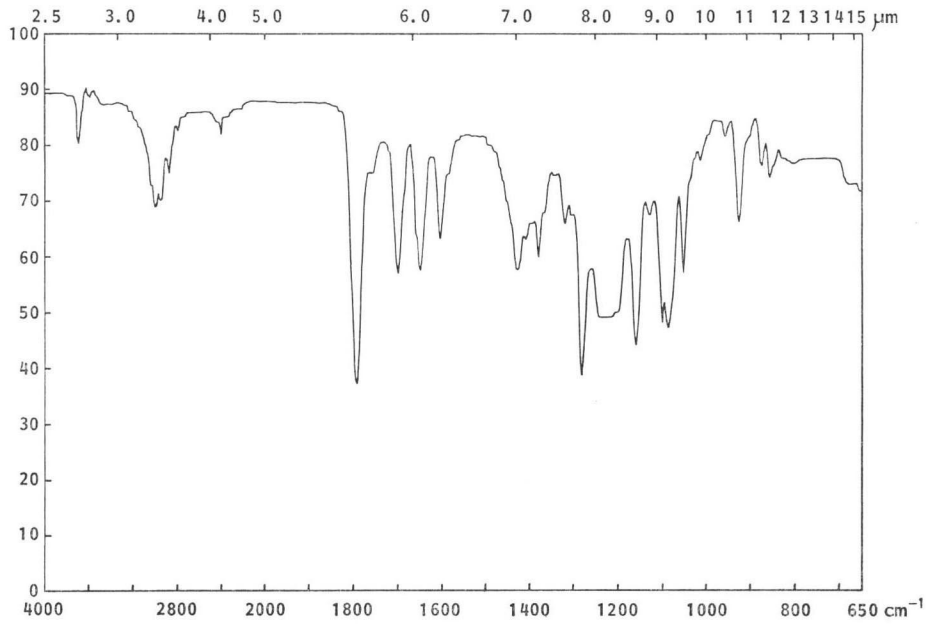


with decomposition. The antibiotic was transferred from water to EtOAc at alkaline pH and from EtOAc to water at acidic pH, suggesting that it is a basic substance. The water solution was pale yellow at acidic pH and orange yellow at alkaline pH.

The free basic form was soluble in ethyl ether, $CHCl_3$, EtOAc, Me_2CO, MeOH and water, but hardly soluble in *n*-hexane. It gave Rf values of 0.28 ($CHCl_3$ - EtOH, 1: 1) and 0.30 (MeOH) on TLC (Kiesel gel $60F_{254}$, Merck).

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

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



The FD-MS revealed the molecular ion peak at *m/z* 473 (M^+). The UV and visible absorption spectra showed maxima at 239 (ε 15,100), 287 (3,450) and 369 nm (5,300) in MeOH, 240 (ε 16,800), 285 (3,550) and 366 nm (5,650) in 0.01 N HCl - MeOH, and 223 (sh, ε 19,800), 287 (6,430) and 442 nm (6,240) in 0.01 N NaOH - MeOH (Fig. 2).

Lactoquinomycin B had a specific rotation of $[\alpha]_D^{29}$ +145.5° (*c* 0.15, MeOH). The IR spectrum ($CHCl_3$) was consistent with the presence of 3 carbonyl groups, γ-lactone (1790 cm^{-1}), non-chelated quinone (1700 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 $C_{24}H_{27}NO_9$: C 60.88, H 5.75, N 2.96, O 30.41.
Found: C 58.91, H 5.81, N 2.77, O 29.63.

Structure Assignment

The physico-chemical properties of lactoquinomycin B are similar to those of lactoquinomycin A, suggesting that the structure of B resembles, but somewhat differs from, that of A. The results of elemental analysis and FD-MS (M^+ 473) of B give the molecular formula of $C_{24}H_{27}NO_9$, indicating that B possesses one additional oxygen atom in comparison with that of A ($C_{24}H_{27}NO_8$).

The UV spectra of B resemble those of frenolicin[3], which contains 5-hydroxy-1,4-naphthoquinone 2,3-epoxide as a chromophore (Fig. 2). In the IR spectrum of B, the absorption due to the non-chelated carbonyl group (1700 cm^{-1}) is shifted to a higher wave number than that of A (1665 cm^{-1}) (Fig. 3). The ^{13}C NMR spectrum of B shows large upfield shifts of 60.0 and 64.4 ppm for C-4a and C-10a, compared with the corresponding signals of A (134.9 and 149.2 ppm) (Table 1). Comparing the ^1H NMR spectrum of B with that of A, the 1-H signal of B is shifted to upfield from that of A (from 5.08 to 4.81 ppm), and the 4-H signal shows a downfield shift (from 5.25 to 5.44 ppm) (Table 2).

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

Carbon	Lactoquinomycin A	Lactoquinomycin B	Carbon	Lactoquinomycin A	Lactoquinomycin B
1	66.3*(d)**	64.2 (d)	10a	149.2 (s)	64.4 (s)***
3	66.5 (d)	64.8 (d)	11	37.0 (t)	35.6 (t)
4	68.7 (d)	69.2 (d)	12	173.5 (s)	173.3 (s)
4a	134.9 (s)	60.0 (s)***	1-CH_3	18.8 (q)	15.2 (q)
5	180.8 (s)	187.6 (s)	2′	72.2 (d)	72.4 (d)
5a	129.7 (s)	129.7 (s)	3′	28.2 (t)	28.1 (t)
6	119.6 (d)	120.0 (d)	4′	67.2 (d)	67.2 (d)
7	133.5 (d)	134.2 (d)	5′	71.5 (d)	71.5 (d)
8	138.6 (s)	139.1 (s)	6′	77.6 (d)	77.6 (d)
9	157.7 (s)	157.8 (s)	4′-$N(CH_3)_2$	40.3 (q)	40.3 (q)
9a	114.0 (s)	113.5 (s)	6′-CH_3	18.9 (q)	19.0 (q)
10	187.8 (s)	193.9 (s)			

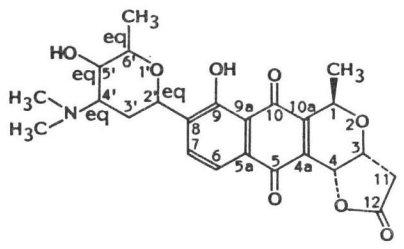
* δ_C relative to TMS. ** Multiplicity. *** The assignments may be interchanged.

Table 2. 400 MHz ^1H NMR data of lactoquinomycin B in comparison with lactoquinomycin A in $CDCl_3$.

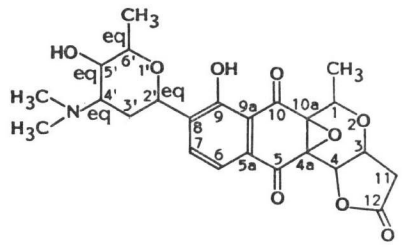
Proton	Lactoquinomycin A	Lactoquinomycin B
1-H	5.08 q (7.0)*	4.81 q (7.3)
3-H	4.69 dd (5.1, 2.9)	4.56 dd (6.6, 4.5)
4-H	5.25 d (2.9)	5.44 d (4.5)
6-H	7.71 d (7.8)	7.61 d (7.8)
7-H	7.91 d (7.8)	7.92 d (7.8)
11-H_1	2.69 d (17.6)	2.59 d (18.3)
11-H_2	2.97 dd (17.6, 5.1)	2.87 dd (18.3, 6.6)
1-CH_3	1.57 d (7.0)	1.59 d (7.3)
9-OH	12.2 br s	11.6 br s
2′-H	4.87 dd (10.9, 2.0)	4.82 dd (10.5, 2.3)
3′-H_{ax}	1.30 ddd (12.5, 12.4, 10.9)	1.25 ddd (12.5, 12.3, 10.5)
3′-H_{eq}	2.26 ddd (12.4, 3.8, 2.0)	2.22 ddd (12.5, 3.6, 2.3)
4′-H	2.78 ddd (12.5, 9.5, 3.8)	2.70 ddd (12.3, 9.6, 3.6)
5′-H	3.20 dd (9.5, 8.9)	3.15 dd (9.6, 9.1)
6′-H	3.53 dq (8.9, 6.2)	3.50 dq (9.1, 6.5)
4′-$N(CH_3)_2$	2.34 s	2.29 s
5′-OH	3.4 br s	2.8 br s
6′-CH_3	1.43 d (6.2)	1.40 d (6.5)

* δ_H relative to TMS, multiplicity, coupling constant.

Fig. 4. The structure of lactoquinomycins A and B.



Lactoquinomycin A



Lactoquinomycin B

Based on the above data, the additional oxygen atom should be placed between C-4a and C-10a, implying that B is a 4a,10a-epoxide derivative of A. Therefore, the structure of lactoquinomycin B has been determined as illustrated in Fig. 4, although the stereochemistry is not yet elucidated.

Table 3. Antimicrobial activity of lactoquinomycin B.

Organism	MIC (μg/ml)	Organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	5.0	*Aspergillus niger* JIS 1-1	>200
Bacillus subtilis PCI 219	10	*A. oryzae* IAM 2630	>200
B. cereus T	20	*Botrytis cinerea* IAM 5126	>200
Corynebacterium xerosis	25	*Mortierella ramannicinus* IAM 6128	>200
Mycobacterium smegmatis R-15	200	*Penicillium chrysogenum* IAM 7326	>200
M. phlei IAM 12064	100	*Candida albicans*	>200
Escherichia coli B	50	*C. utilis* Y 21-6	>200
E. coli K-12	>200	*Cryptococcus neoformans* IAM 122533	>200
Pseudomonas aeruginosa IFO 3455	>200	*Saccharomyces cerevisiae* Y23-9	>200
P. fluorescens H 3	>200		
Salmonella enteritidis 11	>200		
Shigella sonnei	200		
Proteus vulgaris	>200		

Bacteria: Müller-Hinton agar medium (Difco) at 37°C.
Fungi: Yeast extract 0.2% - sucrose 1% agar medium (pH 6.0) at 27°C.

Table 4. Cytotoxicity of lactoquinomycin B.

Cell line	IC_{50}* (μg/ml)
K562 human leukemia	0.16
P388 murine leukemia	0.12
L1210 murine leukemia	0.20
L5178Y murine lymphoma	
parental	0.43
adriamycin-resistant	0.21
aclarubicin-resistant	0.43
bleomycin-resistant	0.19

* The viable cell number was determined by the trypan blue dye exclusion method.

The media used were RPMI 1640 with 10% fetal calf serum for L1210 and K562 cells, RPMI 1640 with 10% fetal calf serum and 5 μM 2-hydroxyethyldisulfide for P388 cells, and RPMI 1640 with 10% horse serum for L5178Y cells. The cells of 2×10^4/ml were incubated with lactoquinomycin B at 37°C for 72 hours in an atmosphere of 5% CO_2 and 95% air.

Antimicrobial Activity

Lactoquinomycin B showed inhibitory activity against bacteria, particularly Gram-positive organisms, but no significant activity against fungi. The MIC is presented in Table 3.

Cytotoxicity

Lactoquinomycin B displayed cytotoxicity for cell lines of K562 human myeloid leukemia, L1210 and P388 murine leukemia, and L5178Y murine lymphoblastoma in culture (Table 4). The 50% inhibitory concentrations, observed by the trypan blue dye exclusion method, were in a range of 0.12~0.43 μg/ml. The antibiotic was more effective against adriamycin- and bleomycin-resistant cell sublines of L5178Y lymphoma than the parental cell line.

Acute Toxicity

The LD_{50} of lactoquinomycin B for male *dd*Y mice was *ca.* 40 mg/kg by iv injection.

Acknowledgment

The authors express deep thanks to Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo, for his kind advice and cooperation in the present study.

References

1) TANAKA, N.; T. OKABE, F. ISONO, M. KASHIWAGI, K. NOMOTO, M. TAKAHASHI, A. SHIMAZU & T. NISHIMURA: Lactoquinomycin, a novel anticancer antibiotic. I. Taxonomy, isolation and biological activity. J. Antibiotics 38: 1327~1332, 1985
2) OKABE, T.; K. NOMOTO, H. FUNABASHI, S. OKUDA, H. SUZUKI & N. TANAKA: Lactoquinomycin, a novel anticancer antibiotic. II. Physico-chemical properties and structure assignment. J. Antibiotics 38: 1333~1336, 1985
3) ELLESTAD, G. A.; M. P. KUNSTMANN, H. A. WHALEY & E. L. PATTERSON: The structure of frenolicin. J. Am. Chem. Soc. 90: 1325~1332, 1968