Microbial and Chemical Conversion of Antibiotic K-41.

I. Isolation and Identification of Conversion Product

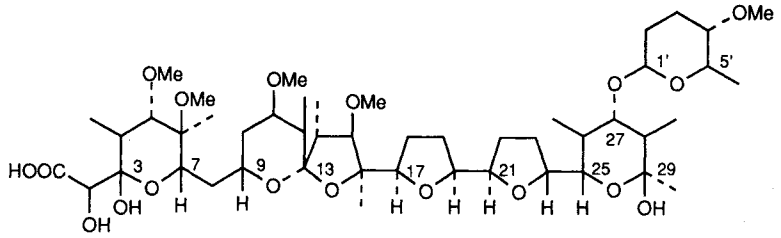
Sir:

Antibiotic K-41[1,2] (**1**), a carboxylic polyether antibiotic, was isolated from *Streptomyces hygroscopicus* K-41 and exhibited antibacterial activity against Gram-positive bacteria, anticoccidal activity and delayed toxicity for poultry *in vivo*. In order to improve the antibiotic features, we attempted microbial and chemical conversion of **1**, and isolated a bioconversion product 27C6 (**2**) as a new derivative (Fig. 1). We wish to describe the isolation of organism, the determination of the structure of **2**, and of its antibiotic activity.

The organism was isolated from a soil sample collected at this campus in Minato-ku, Tokyo, Japan. Based on its phenotypic characteristics, the strain KP-27C6 is considered to be a strain of *Leclercia adecarboxylata*. Strain KP-27C6 was cultured in a medium containing of peptone 1.0% and sodium chloride 0.5% (adjusted to pH 7.4 before sterilization) at 27°C for 2 days on a rotary shaker. After agitation for 48 hours, **1** was added to the medium as a methanolic solution (1000 μg/ml). The cultivation was continued for 5 days monitoring the antibiotic conversion on a silica gel TLC plate (MERCK: Kieselgel 60; Solvent EtOAc). Fermentation broth was separated by centrifugation (7500 rpm × 20 minutes), and the broth supernatant was extracted with $CHCl_3$: MeOH (3 : 1). The organic layer was concentrated to dryness *in vacuo* to afford a crude product, that was further purified by a silica gel column chromatography (MERCK: Kieselgel 60), using $CHCl_3$-MeOH gradient as a solvent system. The bioconversion product, designated as **2**, was recovered from the fractions and crystallized from Hexane : EtOAc (3 : 1). Recovery rate was *ca.* 23%.

Physico-chemical properties are given in Table 1. Compound **2** was soluble in methanol, acetone, ethyl acetate and chloroform but insoluble in water. The UV spectrum showed an absorption peak at λ_{max} 232 nm, suggesting the presence of an α,β-unsaturated ketone system in the structure. The IR spectrum showed absorption at 3400 ~ 3100 and 1670 cm^{-1} corresponding to hydroxyl and α,β-unsaturated ketone, respectively. Elemental analysis (Found: C 59.72%, H 8.17%, Na 2.80%; Calcd.: C 59.83%, H 8.21%, Na 2.79%) and HR-FAB-MS data (Found 823.4424, Calcd. 823.4392) were consistent with $C_{41}H_{67}O_{15}Na$ for the sodium salt indicating the loss of $C_7H_{14}O_3$ moiety from the original antibiotic. In the positive FAB-MS, diagnostic cationized molecules *m/z* 823 $(M+Na)^+$ and 845 $(M+2Na-H)^+$ were detected for **2**-Na. Furthermore, **2**-Na gave a base peak at *m/z* 761, 62 daltons less than the molecular ion, which is characteristic for polyether antibiotics having a β-hemiketal carboxylic acid group {$(M+Na-CO_2-$

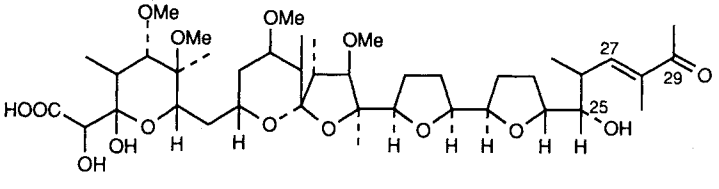
Fig. 1. Structure of Antibiotic K-41 (**1**) and compound 27C6 (**2**).



Antibiotic K-41 (**1**)

1N-NaOH / 50% MeOH
20% Cs_2CO_3

Leclercia adecarboxylata Isolate 27C6



Compound 27C6 (**2**)

Table 1. Physico-chemical properties of K-41 (**1**) and 27C6 (**2**).

	2	**1**
MP	185~187°C (dec.)	196~198°C
$[\alpha]_D$ (MeOH) 25°C	−2.53° (*c* 1.424)	+1.9° (*c* 1.017)
Mol. Formula	$C_{41}H_{67}O_{15}Na$	$C_{48}H_{81}O_{18}Na$
Mol. weight (SI-MS)	823 $(M+1)^+$	969 $(M+1)^+$
Mol. weight (HR-FAB-MS) (Calcd.)	823.4424 $(M+1)^+$ 823.4392	
UV λ_{max} (MeOH)	232 nm	End absorption
IR (KBr) ν_{max} (cm^{-1})	3400 br., 1670, 1610, 1460, 1370, 1180, 1100, 1050, 990, 940	3200 br., 2800, 1620, 1160, 1070, 980, 950
TLC Rf (EtOAc)	0.36	0.81

Table 2. ^{13}C- and ^1H-NMR chemical shifts for the Na-salts of K-41 (**1**) and 27C6 (**2**) (500MHz, $CDCl_3$).

Carbon	**2**		**1**	
	^{13}C, σ ppm	^1H, σ ppm	^{13}C, σ ppm	^1H, σ ppm
1	180.0		178.9	
2	71.3	3.97	71.9	3.90
3	99.2		99.0	
4	38.6	2.19	38.7	2.15
5	85.8	3.32	85.6	3.35
6	78.1		78.3	
7	67.5	3.80	66.7	3.82
8	31.8	1.58	32.4	1.54
9	61.7	3.85	61.4	3.96
10	31.0	1.23, 2.18	31.1	1.15, 2.09
11	79.8	3.44	79.8	3.37
12	37.0	1.85	36.9	1.80
13	107.4		106.9	
14	46.9	2.15	46.2	2.13
15	94.5	3.52	94.7	3.56
16	84.6		83.3	
17	82.9	3.83	83.7	3.76
18	25.9	1.86, 1.98	25.6	1.79, 1.94
19	23.1	1.80	23.1	1.77
20	79.3	3.94	79.4	4.53
21	78.7	4.57	79.3	3.95
22	29.6	1.38, 2.03	29.2	1.43, 1.98
23	24.5	1.74, 2.08	24.2	1.83, 2.15
24	83.3	4.12	80.8	4.38
25	74.5	4.07	74.4	3.91
26	36.4	2.63	39.1	1.28
27	145.4	6.67	82.9	3.37
28	136.8		47.0	1.48
29	200.3		98.4	
4-Me	12.1	1.07	12.1	1.08
6-Me	10.8	1.16	11.0	1.16
12-Me	12.8	1.03	12.6	0.98
14-Me	11.8	1.07	11.5	1.02
16-Me	28.4	1.61	28.5	1.61
26-Me	17.6	1.09	13.4	1.02
28-Me	11.2	1.76	12.7	1.04
29-Me	25.5	2.32	26.9	1.28
5-OMe	61.1	3.56	61.0	3.56
6-OMe	50.9	3.37	50.8	3.37
11-OMe	58.6	3.47	59.3	3.47
15-OMe	60.1	3.41	60.2	3.42
1′			102.7	4.46
2′			30.5	1.46, 1.95
3′			27.3	1.30, 2.21
4′			80.4	2.79
4′-OMe			56.8	3.35
5′			74.5	3.28
5′-Me			18.3	1.22

$H_2O)^+\}$[3]. A comparison of the ^{13}C- and ^1H-NMR chemical shifts (JEOL JMN α-500 MHz, $CDCl_3$), obtained for **2** and **1**, is shown in Table 2. The ^{13}C-NMR spectrum of **2** gave 41 carbon signals, which were assigned to $12\times CH_3$, $6\times CH_2$, $16\times CH$ and $7\times C$ by an INEPT experiment. Further distinctive changes in the ^{13}C-NMR spectrum of **2** were observed at the signals from C27 to C29 and their connectivity was conformed with HMBC spectra as C26 (36.4 ppm)–C27 (145.4 ppm)–C28 (136.8 ppm)–C29 (200.3 ppm)–C30 (29–Me, 25.5 ppm) and together with the assignment of $-^{27}CH=^{28}C-^{29}C(=O)-^{30}CH_3$ system. Other chemical shifts were mostly superimposable with those of **1** except for the removal of seven signals, belonging to the amicetose moiety in **1**. From these date, we estimated the hydrolytic elimination of amicetose moiety followed by dehydration in **1** and the chemical structure of **2** as shown in Fig 1. Compound **2**, having a reactive α,β-unsaturated ketone, was expected to be useful for further modification of antibiotic structure, however, the recovery rate was not so high enough, and the chemical derivation was followed to alter the conversion yield.

In the preliminary run, stirring of **1** in 1 N-hydrochloric acid or alkaline solution, we observed a spot, showing the same mobility with **2** on a TLC plate. Considering the solubility of **1**, **1** (10 g) was dissolved in 1 N-NaOH/50%MeOH (600 ml) and stirred for 4 hours at room temperature. After neutralization, the solvent was evaporated to half volume and the reaction product was extracted with EtOAc, concentrated *in vacuo* and developed with silica gel column chromatography using Hexane-EtOAc (Stepwise gradient). Target fractions were collected and the pure **2** was obtained as white powder (yield 38%) after evaporation of the solvent. As an additional method, **1** was dissolved in methanol, followed by addition of 20% secium carbonate in methanol solution (pH 9.0) and was allowed to stir for 7 hours at room temperature. The reaction mixture was concentrated, purified on a silica gel column developing with $CHCl_3$-MeOH stepwise gradient to afford **2** in a yield of 65%.

The antimicrobial activity against bacteria and *Eimeria tenella* are given Table 3 and 4. Biological activity of **1** was not observed in **2**. However, **2** has a reactive moiety in structure, that chemical conversion *via* **2** are next interests.

Table 3. Antimicrobial activities K-41 (**1**) and 27C6 (**2**).

Strain		MIC values (μg/ml)	
		2	**1**
E. coli	JC-2	>25	>25
S. typhimurium	13311	>25	>25
S. aureus	209P	>25	1.56
M. gallisepticum	S-6	>50	3.13
M. hyopneumoniae	ST-11	12.5	3.13
T. hyodysenteriae	YD-6	>25	0.2
M. scrofulaceum	19075	50	0.2
B. bronchiseptica	ABU-1	>25	>25

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Table 4. Growth depress effect of *Eimeria tenella* (*in vitro*).

Concentration (μg/ml)	**2**		**1**	
	Toxic (−~+++)	Inhibition (%)	Toxic (−~+++)	Inhibition (%)
10.0	+	100	+++	
2.5	−	67.7	+++	
0.625	−	0.8	+	100
0.156	−	<0	−	99.7
0.039	−	<0	−	68.5
0.0098	−	<0	−	31.8
0.0024	−	<0	−	<0
0.0006	−	<0	−	<0

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