VOL. 53 NO. 10, OCT. 2000 THE JOURNAL OF ANTIBIOTICS pp. 1154 — 1157

Microbial and Chemical Conversion of Antibiotic K41. II. Preparation of K41-DA1, -DA2 and -DA3, Deamicetosyl Derivatives of Antibiotic K41

MIDORI HOSHI and TOYOSHIGE ENDO

Department of Pharmaceutical Sciences, Kyoritsu College of Pharmacy
1-5-30 Shiba-koen, Minato-ku, Tokyo 105-8512, Japan

(Received for publication June 30, 2000)

Antibiotic derivative K41-DA1, -DA2 and -DA3 (**2**~**4**), deamicetosyl derivatives of antibiotic K41 (**1**), were prepared by acidic degradation of K41 and following hydrogenation reaction. K41-DA2 (**3**) showed comparable antimicrobial activities to K41 *in vitro* but not *in vivo*.

Antibiotic K-41 (**1**)[1,2], a carboxylic polyether antibiotic, was isolated from the culture broth of *Streptomyces hygroscopicus* K41 and exhibited antibacterial activity against Gram-positive bacteria and anticoccidal activity, together with delayed toxicity for poultry *in vivo*. We attempted structural conversion of **1** to improve the antibiotic features through microbial and chemical procedures, and isolated compound 27C6[3] as a conversion product of *Leclercia adecarboxylata* Strain KP-27C6. Recently, compound 27C6 was also isolated as a metabolite of marine bacterium, *Streptomyces* sp. CNH-248.[4]

In this paper, we described an acidic elimination of amicetose moiety from K41 molecule and preparation of K41-DA1 (**2**), followed by hydrogenation reaction of **2** and preparation of K41-DA2 (**3**) and K41-DA3 (**4**) (Fig. 1.).

Chemistry

Removal of Amicetose Moiety

Acidic degradaion of K41 (**1**) was carried out as follows, expecting the elimination of amicetose moiety. **1** was dissolved in AcCN : H_2O=95 : 5 (400 ml) and stirred with *p*-toluenesulfonic acid (16 mmol) for 3 hours at room temperature.[5] After neutralization with $NaHCO_3$, the reaction mixture was extracted with CH_2Cl_2 and purified with silica gel column chromatography using hexane-EtOAc as a solvent. The product, obtained as white powder (yield 62%) and designated as K41-DA1 (**2**), had the molecular formula of $C_{41}H_{67}O_{15}Na$ and, together with its spectral data, suggested the removal of sugar moiety and the formation of a double bond at $-C_{27}=C_{28}-$ in the molecule. Detailed NMR spectral data were listed in Table 2 and other physico-chemical data were shown in Table 1.

Hydrogenation of K41-DA1 (2)

Next attempt was focused on the reduction of the double bond of **2** to evaluate the effect of sugar moiety on the biological activity. **2** (7.2 mmol) was dissolved in EtOH and was added with Pd-C (16%). After agitation in H_2 atomosphere for 4 hours at room temperature, reaction mixture was filtrated and concentrated *in vacuo* and developed on a silica gel column with hexane-EtOAc (stepwise gradient) . Analysis of the fractions suggested the formation of two major products, and each product was collected and designated as K41-DA2 (**3**) and K41-DA3 (**4**), respectively.

3 (yield 56%) had the molecular formula of $C_{41}H_{69}O_{15}Na$ and the spectral data showed the reduction of double bond.

4 (yield 29%) had the molecular formula of $C_{41}H_{67}O_{14}Na$, suggesting the retention of double bond, and the spectral data showed the migration of double bond to $-C_{28}=C_{29}-$ position.

From the NMR spectral data, these compounds had the same carbon number of 41, and their chemical shifts were mostly superimposable with those of **1** except for the eight signals of C_{25}~C_{29} on F-ring according to the modification of their structures.

This paper is dedicated to Sir E. P. ABRAHAM.

Fig. 1. Structure of antibiotic K41(**1**), K41-DA1 (**2**), K41-DA2 (**3**) and K41-DA3 (**4**).

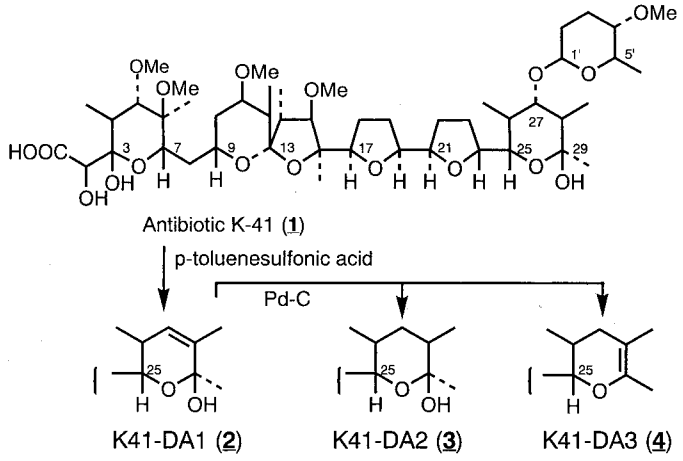

Table 1. Physico-chemical properties of K41 (**1**), K41-DA1 (**2**), K41-DA2 (**3**) and K41-DA3 (**4**).

	2	**3**	**4**	**1**
M. P.	206-208 °C (dec.)	192-195 °C (dec.)	168-172 °C (dec.)	196-198 °C
$[\alpha]_D$ (MeOH) 25 °C	-15.7 (c 0.100)	+11.7 (c 0.033)	-0.19 (c 0.733)	+1.9 (c1.017)
Mol. Formula	$C_{41}H_{67}O_{15}Na$	$C_{41}H_{69}O_{15}Na$	$C_{41}H_{67}O_{14}Na$	$C_{48}H_{81}O_{18}Na$
Mol. Weight (SIMS)	823 $(M+1)^+$	825 $(M+1)^+$	807 $(M+1)^+$	969 $(M+1)^+$
Mol. Weight (HR-FBMS)	823.4427 $(M+1)^+$	825.4585 $(M+1)^+$		
(Calcd.)	823.4403	825.4557		
	C H Na O	C H Na O	C H Na O	
Elemental Analysis Found	59.51 8.01 3.14 29.37	59.55 8.27 3.32 28.86	58.11 8.35 2.77 30.77	
Calcd.	59.84 8.21 2.79 29.16	59.69 8.43 2.79 29.09	58.41 8.49 2.73 30.37	
	(Dif)	(Dif)	(Dif)	
UV λ max (MeOH)	End absorption	End absorption	End absorption	End absorption
IR ν max (cm^{-1})	3400 br., 2900, 1620, 1100, 980, 950, 780	3400 br., 2890, 1620, 1460, 1360, 1190, 1160, 990, 950	3200 br., 2800, 1620, 1460, 1380, 1100, 990, 950	3200 br., 2800, 1620, 1160, 1070, 980, 950
TLC Rf. (EtOAc)	0.67	0.80	0.47	0.81

Biological Activity

The antimicrobial activity against bacteria and *Eimeria tenella* are given in Table 3 and 4. Biological activity, typical for polyether antibiotics, was not observed in **2**. But **3** and **4** have antimicrobial activity at the same level of **1**. Moreover, **3** showed the inhibitory activity against growth depress effect of *Eimeria tenella in vitro*, however, this was not observed *in vivo*.

Considering the structural differences between **1** and **3**, some oxygenic function, such as $-OCH_3$ group or a sugar moiety, must be essential for the expression of anticoccidal activity.

Further interest on their ion selectivity is a next target.

Experimental

General

^1H and ^{13}C NMR spectra were recorded with JEOL α-500 spectrometer in $CDCl_3$ solution and mass spectra were measured with JEOL JMS-DX303 spectrometer. Other reagents are commercially available.

Acid Degradation

To a solution of K41 (**1**, 14 mmol) in acetonitrile : H_2O=95 : 5 (400 ml), *p*-toluenesulfonic acid (16 mmol) was added and stirred at room temperature for 3 hours.[5] After

Table 2. ^{13}C-NMR chemical shifts for the Na-salts of K41 (**1**), K41-DA1 (**2**), K41-DA2 (**3**) and K41-DA3 (**4**) (500 MHz, $CDCl_3$).

Carbon	**2** ^{13}C, σ ppm	**3** ^{13}C, σ ppm	**4** ^{13}C, σ ppm	**1** ^{13}C, σ ppm
1	178.5	178.7	178.3	178.9
2	71.6	71.7	71.1	71.9
3	99.0	99.0	99.2	99.0
4	38.7	38.7	38.7	38.7
5	85.3	85.5	85.8	85.6
6	78.3	78.3	78.4	78.3
7	66.7	66.7	66.7	66.7
8	32.4	32.4	32.2	32.4
9	61.4	61.4	61.5	61.4
10	31.2	31.1	30.9	31.1
11	79.8	79.7	79.3	79.8
12	36.8	36.9	37.0	36.9
13	107.0	106.9	107.0	106.9
14	46.3	46.2	46.2	46.2
15	94.5	94.7	94.7	94.7
16	83.4	83.4	83.5	83.3
17	83.6	83.7	83.6	83.7
18	25.5	25.6	25.9	25.6
19	23.0	23.1	23.8	23.1
20	79.6	79.3	79.7	79.4
21	79.5	79.3	79.4	79.3
22	29.1	29.2	28.6	29.2
23	24.8	24.2	24.4	24.2
24	81.2	80.9	81.0	80.8
25	73.0	75.3	79.9	74.4
26	31.5	32.8	29.1	39.1
27	126.5	36.7	35.9	82.9
28	136.7	39.5	100.3	47.0
29	94.6	96.9	144.2	98.4
4-Me	12.2	12.1	12.2	12.1
6-Me	11.1	11.0	11.0	11.0
12-Me	12.7	12.6	12.7	12.6
14-Me	11.6	11.6	11.5	11.5
16-Me	28.5	28.4	28.7	28.5
26-Me	17.4	17.4	17.5	13.4
28-Me	18.8	16.8	17.6	12.7
29-Me	26.2	26.7	15.9	26.9
5-OMe	61.0	61.1	61.1	61.0
6-OMe	50.8	50.8	50.8	50.8
11-OMe	59.5	59.1	58.1	59.3
15-OMe	60.2	60.2	60.3	60.2
1'				102.7
2'				30.5
3'				27.3
4'				80.4
4'-OMe				56.8
5'				74.5
5'-Me				18.3

neutralization with $NaHCO_3$, the solvent was evaporated and extracted with CH_2Cl_2. Organic layer was washed with saturated $NaHCO_3$ and NaCl and concentrated *in vacuo*. Resulting mass was developed on a silica gel column with stepwise gradient of hexane-EtOAc system. Target fractions were collected, and the pure Compound K41-DA1 (**2**) was obtained as white powder (yield 62%) after evaporation of the solvent.

Hydrogenation of K41-DA1

K41-DA1 (**2**, 7.2 mmol) was dissolved in EtOH and stirred in H_2 atomosphere for 4 hours together with Pd-C (16%). Reaction mixture was filtrated and concentrated *in vacuo* and developed on silica gel column with hexane-

Table 3. Antimicrobial activities K41 (**1**), K41-DA1 (**2**), K41-DA2 (**3**) and K41-DA3 (**4**).

Strain		MIC values (μg/ml)			
		2	**3**	**4**	**1**
E. coli	JC-2	25<	25<	25<	25<
S. typhimurium	13311	25<	25<	25<	25<
S. aureus	209P	12.5	1.56	1.56	1.56
M. gallisepticum	S-6	3.13	12.5	3.13	0.39
M. hyopneumoniae	ST-11	12.5	6.25	3.13	3.13
T. hyodysenteriae	YD-2	3.13	0.39	0.78	0.39
H. pleuropneumoniae	2M-1	25<	25<	25<	25<

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

Concentration (μg / ml)	**2**		**3**		**4**		**1**	
	Toxic (-～+++)	Inhibition (%)	Toxic (-～+++)	Inhibition (%)	Toxic (-～+++)	Inhibition (%)	Toxic (-～+++)	Inhibition (%)
10.0	++	100	+++		+++		+++	
1.0	-	99.8	++	100	++～+++	100	++	100
0.1	-	69.8	-	98.0	-	68.8	-	99.7
0.01	-	<0	-	17.1	-	43.1	-	70.2
0.001	-	<0	-	<0	-	<0	-	<0

EtOAc (stepwise gradient). Target fractions were collected and the pure K41-DA2 (**3**, yield 56%) and K41-DA3 (**4**, 29%) were obtained after evaporation of the solvent.

Analysis of ^{13}C NMR Spectra

Total carbons and functional groups were analysed as follows by INEPT experiment.

	$-CH_3$	$-CH_2-$	>CH–	>C<	Total
K41-DA1 (**2**)	12	6	16	7	41
K41-DA2 (**3**)	12	7	16	6	41
K41-DA3 (**4**)	12	7	15	7	41

Acknowledgments

Authors are grateful to Mr. T. SAITO and Miss M. FUJITA for their technical assistance, Dr. Y. NAKAGAWA and Dr. H. KAWAGUCHI of Aburahi Laboratories Shionogi Pharmaceutical Co. Ltd., for mass spectra and biological activities, and Dr. HAYAKAWA of Institute of Molecular and Cellular Biology, Tokyo University for elemental analysis.

References

1) TSUJI, N.; K. NAGASHIMA, M. KOBAYASHI, Y. WAKISAKA, Y. KAWAMURA, S. KOZUKI & M. MAYAMA: Two new antibiotics, A-218 and K-41. Isolation and characterization. J. Antibiotics 29: 10～14, 1976
2) SHIRO, M.; H. NAKAI, K. NAGASHIMA & N. TSUJI: X-ray determination of the structure of the polyether antibiotic K-41. J. C. S. Chem. Comm.: 682～683, 1978
3) HOSHI, M.; K. W. SHIMOTOHONO, T. ENDO, K. FURIHATA & H. SETO: Microbial and chemical conversion of antibiotic K-41. I. Isolation and identification of conversion product. J. Antibiotics 50: 631～634, 1997
4) CHENG, X. C.; P. R. JENSEN & W. FENICAL: Arenaric acid, a new pentacyclic polyether produced by a marine bacteria (Actinomycetales). J. Nat. Product. 62: 605～607, 1999
5) A. C. GOUDIE & N. D. WALSHE (Pfizer): Polycyclic ether antibiotic. U.S. 4,804,680, Feb. 14, 1989