ANTIBIOTIC AC6H, A NEW COMPONENT OF TETROCARCIN GROUP ANTIBIOTICS

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We wish to describe here isolation and structural elucidation of a new antibiotics, AC6H, from the culture broth of *Micromonospora carbonaceae* subsp. *carbonaceae* K55-AC6, which was isolated from a soil sample collected at Kawanishi-city, Hyogo Prefecture, Japan.

The strain was inoculated to a seed medium (glucose 1.0%, soybean meal 1.0%, meat extract 0.5% and $CaCO_3$ 0.4%) in a 500-ml Erlenmeyer flask and incubated on a rotary shaker at 27°C for 3 days. The seed culture (2 ml) was transferred to 500-ml Erlenmeyer flasks containing 100 ml of a production medium, consisting of lactose 1.0%, soybean meal 1.0%, meat extract 0.5%, $CaCO_3$ 0.4% and Allophosite 1.0%.

After 5 days of cultivation, the culture filtrate (5 liters) was passed through a column of Diaion HP-20, which was washed with 60% aq. MeOH and developed with MeOH (2 liters). The active eluate

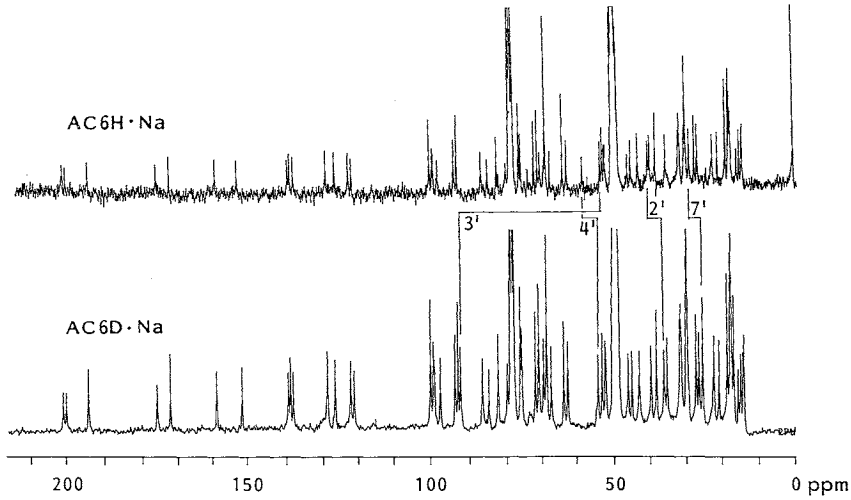
Table 1. Chemical shift assignments of AC6H·Na and AC6D·Na.

Carbon atom	AC6H·Na Chemical shift δ (ppm)	AC6D·Na Chemical shift δ (ppm)	Carbon atom	AC6H·Na Chemical shift δ (ppm)	AC6D·Na Chemical shift δ (ppm)
1	175.1	175.1	1′	97.3	96.8
2	98.5	98.5	2′	39.7	35.8
3	201.0	200.9	3′	53.1	91.5
4	52.0	52.0	4′	58.1	54.0
5	39.2	39.1	5′	69.8	69.1
6	31.4	31.4	6′	17.3	17.0
7	42.3	42.3	7′	28.6	25.4
8	35.0	35.0	8′	158.4	158.4
9	75.3	75.3	9′	52.5	52.8
10	44.5	44.4	*	171.2	171.2
11	128.1	128.0	*	99.6	99.6
12	125.7	125.8	*	98.8	98.8
13	51.7	51.8	*	92.8	92.8
14	138.1	138.1	*	92.1	92.1
15	121.9	121.7	*	85.6	85.6
16	31.2	31.2	*	81.4	81.4
17	78.9	78.9	*	74.9	75.0
18	138.6	138.7	*	71.4	71.4
19	121.1	120.7	*	70.5	70.5
20	45.4	45.5	**	68.2	68.2
21	70.5	70.5	*	67.0	67.0
22	152.2	151.1	*	63.5	63.5
23	137.0	137.3	*	62.3	62.3
24	29.7	29.7	*	37.7	37.8
25	83.9	83.9	*	31.7	31.6
26	200.2	200.1	**	29.7	29.7
27	15.1	15.1	*	27.2	27.3
28	22.4	22.4	*	26.5	26.5
29	14.3	14.3	*	21.0	21.0
30	14.5	14.6	*	18.8	18.8
31	15.8	15.8	*	18.0	18.0
32	194.3	194.4	*	17.8	17.8
			*	17.6	17.6

Asterisks (*·**) were correlated with the carbons belonging to amicetose and digitoxose moieties and were not fully assigned yet.

Asterisks (**) should the presence of two carbon signals.

Fig. 1. ^{13}C NMR spectra of AC6H·Na and AC6D·Na (270 MHz, $CDCl_3$-CD_3OD).



was concentrated to a small volume (350 ml) *in vacuo*, and it was extracted with chloroform (100 ml × 3) after dilution with water (150 ml). All chloroform extracts were combined and dried *in vacuo* to afford a crude AC6 complex, that was further purified by silica gel chromatography using $CHCl_3$-MeOH-H_2O (85:15:1.5 v/v) as a solvent system to give two active fractions, designated as crude AC6D and AC6H fractions. The AC6H fraction was concentrated and applied to preparative silica gel TLC using the same solvent system and the band of Rf 0.29 corresponding to AC6H·Na was extracted with MeOH to give a pale yellow powder (29.7 mg). It showed a single HPLC peak at 18.4 minutes by a Senshu Pak ODS-3251-H column (8 × 250 mm, solvent MeOH-H_2O (7:3), flow rate 1.5 ml/minute) monitored with UV absorption at 254 nm. The AC6D fraction gave a component of AC6D·Na (84 mg, mp 236~241°C) by the same procedures. AC6D·Na was treated with dil. HCl to afford a free AC6D compound. The spectral data and the physico-chemical properties of AC6D (UV, IR, NMR and mass) are in good agreement with the data published for tetrocarcin A[1~5] and antlermicin A[6].

Antibiotic AC6H, slightly yellow amorphous powder of mp 239~241.5°C (dec), has a molecular formula of $C_{67}H_{98}O_{22}N_2$ (HRFAB-MS: Calcd: *m/z* 1283.6689, Found *m/z* 1283.6680 $(M+H)^+$), $[\alpha]_D$ (MeOH) −82.64, absorption peaks at λ_{max}^{MeOH} nm (ε) 235 (19,660), 266 (13,130), 278 (9970 sh) in UV region and ν_{max} (KBr) 3400, 2900, 1730, 1710, 1640, 1620, 1530, 1240, 1120, 1050 cm^{-1} in IR region. ^{13}C NMR spectrum of AC6H was closely related with that of tetrocarcin A (AC6D) as shown in Table 1. Precise comparison of these spectra (Fig. 1) revealed the same chemical shifts for 59 out of 63 carbons of AC6H and tetrocarcin A (AC6D), and the remaining 4 carbons distributed around C17~3' of tetrocarcin A. The quarternary C–NO_2 signal (δ 91.5 ppm) of tetrocarcin A was replaced by the quarternary carbon (δ 53.1) of AC6H. AC6H has a molecular weight of 1283, 30 mass units less than that of tetrocarcin A.

Mild acid hydrolysis of AC6H with 0.2 N hydrogen chloride in acetone at 60°C for 1 hour gave a tetronolide derivative, which had the same difference in molecular weight with the hydrolysate of tetrocarcin A, tetronitrosyl tetronolide, and strong acid hydrolysis of AC6H at 80°C for 2 hours gave the same aglycone as tetrocarcin A and an additional sugar component, designated as A-sugar. The structure of A-sugar was determined by the analyses of mass and NMR spectra. The EI mass spectrum of TMS-A-sugar revealed no molecular ions, but the ions at *m/z* 115, 130, 158, 176, 214 and 231 were diagnostic for the structure of the sugar compound. The FAB-MS spectrum of A-sugar gave a $(M+Na)^+$ ion at *m/z* 241. ^{13}C NMR data were in agreement with the data reported by ALAN K. MALLAMS[7] for 2,3,4,6-tetradeoxy-4-(methoxy-carbonylamino)-3-*C*-methyl-3-amino-D-xylo-hexopyranosyl structure, which was prepared by the reduction of tetronitrose, a sugar moiety of tetrocarcin A, with Raney nickel. These results clearly suggested that the structural difference of AC6H with AC6D existed at the nitrosugar moiety of tetrocarcin A, and AC6H had a C–NH_2

Fig. 2. Structures of AC6H and AC6D.

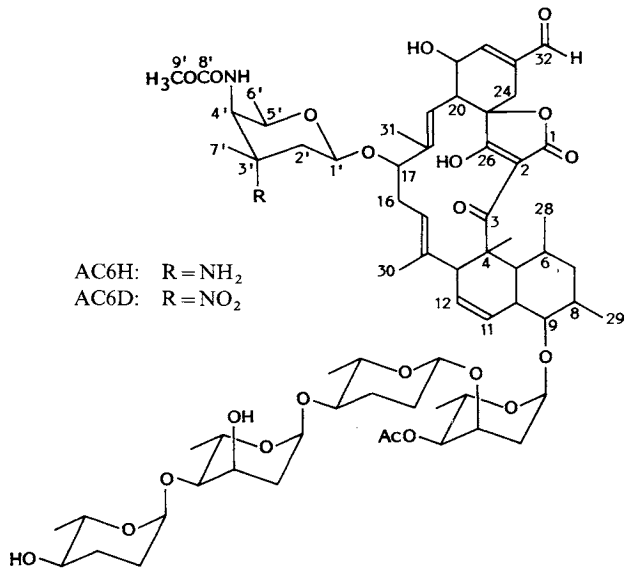


Table 2. Antitumor activity of AC6D and AC6H on P388 leukemia.

Sample	Dose (mg/kg)	Survival days	T/C (%)
AC6H	100 day 1, 5, 9	Toxic	
	50 day 1, 5, 9	11.8	118
	25 day 1, 5, 9	11.0	110
AC6D	100 day 1, 5, 9	15.0	150
Adriamycin	5 day 1	>15	>150
Control		10.0	100

group instead of the C–NO_2 group of tetrocarcin A (AC6D) as shown by the ninhydrin positive character (Fig. 2).

Antitumor activity of AC6H was examined using P388 leukemia cells (CDF1 mouse, 6 weeks) to show slight elongation of mean survival days (Table 2). Cytotoxicity of AC6H against P388 leukemia and B16 melanoma cells were 6.25 and 25 μg/ml respectively, whereas those of AC6D (tetrocarcin A) were 0.39 and 0.78 μg/ml.

In conclusion, we have elucidated the structure of a new antitumor antibiotic, AC6H by spectroscopic comparison with tetrocarcin A and chemical derivation from tetrocarcin A.

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