NOVEL ANTITUMOR ANTIBIOTICS, TETROCARCINS

Sir:

We isolated novel antitumor antibiotics designated as tetrocarcins from a broth culture of *Micromonospora chalcea* KY11091 isolated from a soil sample collected in Sendai-shi, Miyagi, Japan. The seed flasks were inoculated with stock cultures maintained in a deep freezer (−70°C) and grown for 48~72 hours at 28°C. The seed medium consisted of 4 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 1.5 g KH_2PO_4, 5 g $(NH_4)_2SO_4$, 20 g sucrose, 10 g fructose, 10 g glucose, 5 g corn steep liquor and 20 g $CaCO_3$ per liter of tap water. A 5% vegetative seed was used to inoculate into the fermentation medium which consisted of 40 g soluble starch, 8 g yeast extract, 0.09 g $MgSO_4 \cdot 7H_2O$, 0.15 g KH_2PO_4, 0.21 g K_2HPO_4 and 30 mg $ZnSO_4 \cdot 7H_2O$ per liter of tap water. The pH of the medium was adjusted to 7 prior to sterilization. The fermentation was carried out at 30°C for 72~96 hours in a 30-liter jar fermentor with aeration and agitation.

The culture filtrate was absorbed on nonporous resin HP-20 and eluted with acetone. The eluate was passed through a column of charcoal and concentrated to dryness. The residue was subjected to silica gel chromatography with $CHCl_3$-MeOH to obtain tetrocarcin complex that was further purified by silica gel chromatography with C_6H_6 - Me_2CO (2: 1) to obtain tetrocarcins A, B and C.

We described the isolation and physico-chemical characteristics of tetrocarcin A (formerly called DC-11) in 1979[1,2,3]. The spectral data of UV and IR of tetrocarcin A shown in Table 1 are in good agreement with the recently published data for antlermicin A[4]. A sample of antlermicin A which was kindly supplied by Dr. ISONO (The Institute of Physical and Chemical Research, Japan) showed identical Rf values with those of tetrocarcin A in various thin-layer chromatographies (Table 2). The PMR spectrum of tetrocarcin A indicated the presence of a formyl (δ=9.58) and an acetyl (δ=2.09) group as seen in antlermicin A. The CMR spectrum of tetrocarcin A in $CDCl_3$ (Fig. 1) resembled closely to that of antlermicin A, although the latter was measured in acetone-^{12}C, d_6.[4] These data confirm that antlermicin A is identical with tetrocarcin A. As shown in Tables 1 and 2, tetrocarcins B and C were clearly distinct from tetrocarcin A, although they were closely related to one another. Hydrolysis of tetrocarcin A by mild acid gave sugars A and B and a component f-1. The mass spectrum of methyl glycoside of sugar A showed the molecular ion at *m/e* 146.

Table 1. Physico-chemical characteristics of tetrocarcins.

	Tetrocarcin A	Tetrocarcin B	Tetrocarcin C
m.p.	198~202°C	192~196°C	187~190°C
Mol. Form.	$C_{67}H_{96}N_2O_{24}$	$C_{61}H_{86}N_2O_{22}$	$C_{69}H_{98}N_2O_{25}$*
Anal.	C H N	C H N	C H N
obs.	60.4 7.5 2.1	60.8 7.4 2.3	59.3 7.0 1.9
calcd.	61.3 7.4 2.1	61.1 7.2 2.3	61.1 7.3 2.1
$[\alpha]_D$	$[\alpha]_D^{21}$ −74.3° (*c* 1.0, Me_2CO)	$[\alpha]_D^{19}$ −55.8° (*c* 1.0, Me_2CO)	$[\alpha]_D^{22}$ −62.5° (*c* 1.0, $CHCl_3$)
IR (KBr) cm^{-1}	3440, 2930, 1760, 1732, 1688, 1633, 1540, 1231, 1120, 1050	3440, 2930, 1762, 1733, 1688, 1631, 1540, 1233, 1119, 1050	3440, 2930, 1733, 1687, 1630, 1540, 1225, 1119, 1050
UV: λ_{max} nm(ε) in 90% MeOH	232sh (17900), 268 (10400), 278sh (8960)	234sh (18200), 268 (10700), 278sh (9260)	232sh (18200), 268 (10500), 278sh (9200)

* Although tetrocarcin C contains 2 moles each of amicetose and digitoxose and two acetyl groups, its CMR spectrum is different at several points from those of other tetrocarcins. The molecular formula, therefore, has not been confirmed.

Fig. 1. CMR spectrum of tetrocarcin A in $CDCl_3$ at 25.1 MHz.

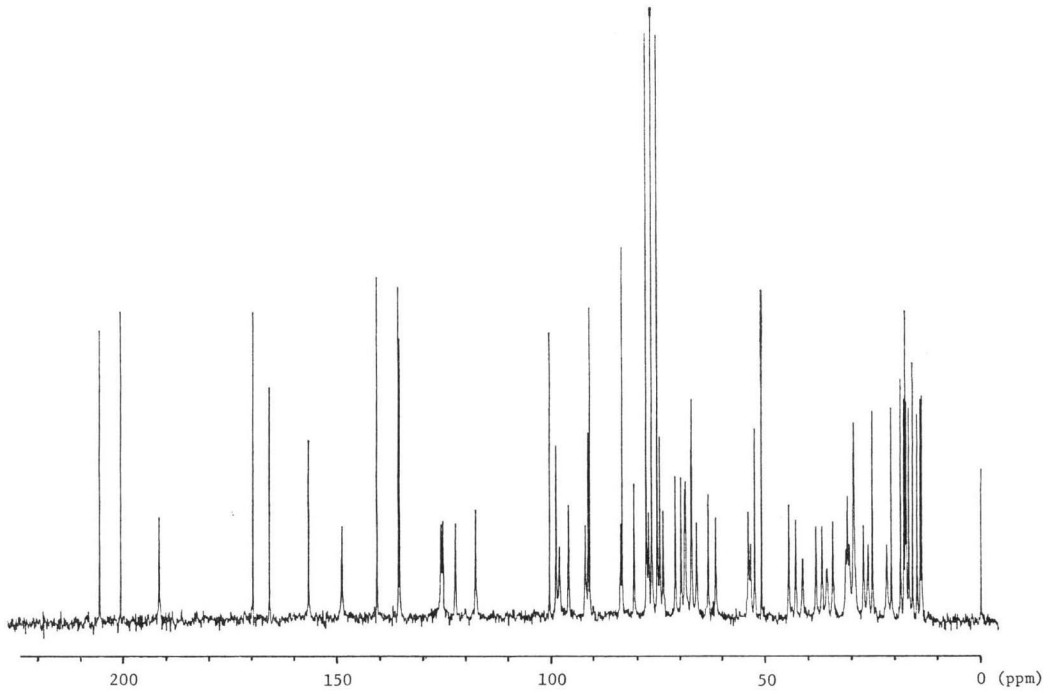

Table 2. Rf values of tetrocarcins A, B, C and antlermicin A.

Solvent	Tetrocarcin			Antlermicin A
	A	B	C	
$CHCl_3$-MeOH (90: 10 v/v)[a]	0.57	0.52	0.56	0.57
C_6H_6-Me_2CO (20: 30 v/v)[a]	0.54	0.47	0.49	0.54
AcOEt-AcOH (20: 1 v/v)[a]	0.42	0.47	0.40	0.42
$CHCl_3$-dioxane (95: 5 v/v)[b]	0.60	0.40	0.57	0.60

Silica gel plates were used in a) and silanized silica gel plates were used in b). Spots were detected by conc. H_2SO_4, exposure to UV or bioautography using *B. subtilis* as an indicator.

The sugar A was identified with L-amicetose by the comparison of its PMR spectrum, $[\alpha]_D$ ($-168.7°$ in $CHCl_3$) of its α-methyl pyranoside and m.p. (152~154°C) of its 2,4-DNP derivative with those reported in the literature ($[\alpha]_D -139°$ in $CHCl_3$, m.p. 154~156°C)[5]. The sugar B was identified with L-digitoxose by the comparison of its PMR spectrum, $[\alpha]_D$ ($-37.3°$ in MeOH) and m.p. (110~112°C) with those in the literature ($[\alpha]_D -38°$ in MeOH[6], $[\alpha]_D +36.5°$ in MeOH and m.p. 105~108°C for D-enantiomer[7]). Further hydrolysis of the component f-1 gave two components f-2 and f-3. The molecular formulae of the two components f-2 and f-3 were determined to be $C_{32}H_{40}O_8$ and $C_9H_{16}N_2O_6$, respectively, by their mass and CMR spectra. Their structures will be published in the near future[8].

Mild acid hydrolyses of tetrocarcins B and C gave the same components to those of A. Gas chromatographic analyses showed that tetrocarcin A had 2 moles each of L-amicetose and L-digitoxose and tetrocarcin B had 1 mole of L-amicetose and 2 moles of L-digitoxose. On the basis of these findings it is concluded that tetrocarcin A is composed of 2 moles of L-amicetose, 2 moles of L-digitoxose, 1 mole of acetic acid and the components f-2 and f-3, and its molecular formula, thus, is deduced to be $C_{67}H_{96}N_2O_{24}$. The molecular formula of tetrocarcin B is also determined to be $C_{61}H_{86}N_2O_{22}$ by the similar manner. Since tetrocarcins contain tetronic acid moiety[8] and have strong affinity to metal ions, it is very dif-

Table 3. Antibacterial activity of tetrocarcins by the agar dilution method.

Test organisms	MIC (mcg/ml)		
	A	B	C
Staphylococcus aureus ATCC 6538P	20	20	30
Bacillus subtilis No. 10707	0.1	0.1	0.1
Klebsiella pneumoniae ATCC 10031	>100	>100	>100
Escherichia coli ATCC 26	>100	>100	>100
Shigella sonnei ATCC 9290	>100	>100	>100

The medium consisted of 3 g tryptone, 3 g meat extract, 1 g yeast extract, 1 g glucose and 16 g agar per liter of tap water. The pH of the medium was adjusted to 7 prior to sterilization.

Table 4. Antitumor activity of tetrocarcins A and B against mouse sarcoma 180 and mouse leukemia P388.

	Dose (mg/kg)	T/C	
		Sarcoma 180	P388
Tetrocarcin A	25	0.78	1.20
	50	0.52	1.40
	75	0.42	1.50
Tetrocarcin B	12.5	0.70	1.20
	25	0.50	1.39
	50	0.37	1.50

Drugs were injected intraperitoneally after 24 hours of tumor implantation. T/C represents the ratio of the median tumor volume (sarcoma 180) or the median survival time (P388) of the treated group divided by that of the control group.

ficult to obtain metal-free samples. In the case of tetrocarcins A and C, this may be the reason why the elementary analyses do not agree well with the calculated values.

Tetrocarcins A, B and C showed antibacterial and antitumor activity (Tables 3 and 4). The LD_{50} values of tetrocarcins in mice were 60~80 mg/kg of body weight by intraperitoneal injection. Tetrocarcins caused little reduction in the number of leucocytes in mice during treatments.

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