Notes

NEW ANTITUMOR ANTIBIOTICS, DUOCARMYCINS B_1 AND B_2

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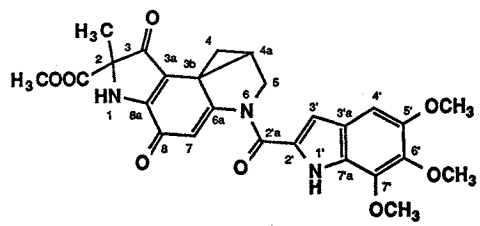
In the course of our screening program for new antitumor antibiotics, we have previously reported on new antibiotics, duocarmycins A (**1**),[1] C_1 (**2**) and C_2 (**3**)[2~4].

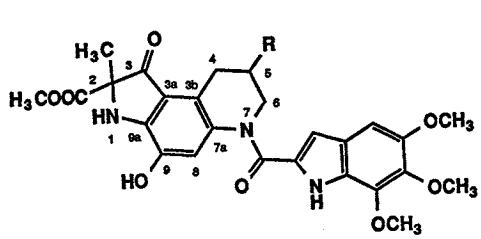
Medium modification has produced two new antitumor antibiotics duocarmycins B_1 (**4**) and B_2 (**5**) in the fermentation broth of *Streptomyces* sp. DO-89. In this paper, the production, isolation, physico-chemical properties and biological properties of duocarmycins B_1 (**4**) and B_2 (**5**) are reported.

Fermentation of *Streptomyces* sp. DO-89 was carried out at 28°C for 100 hours in a 200-liter fermenter with aeration at 15 liters/minute and agitation at 200 rpm. The production medium consisted of maltose 5%, dry yeast 1.5%, Ebios (Asahi Breweries, Limited) 2.5%, KBr 1%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.5% (pH 5.0). Production of antibiotics was monitored by HPLC analysis (YMC ODS AM312, MeOH - 0.05 M phosphate buffer (pH 4.0), 3:2). The fermentation broth (150 liters) was adjusted to pH 5.0 with hydrochloric acid and was filtered with the aid of diatomaceous earth. The mycelial cake was extracted with propanol. The propanol extract was diluted with water and was applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). The column was washed with water and with 80% aqueous methanol. Duocarmycin B_1 (**4**) was eluted with methanol followed by duocarmycin B_2 (**5**). The duocarmycin B_1 fraction was concentrated and chromatographed on a column of Diaion HP-20SS (Mitsubishi Chemical Industries Limited) with 85% aqueous methanol (pH 4.0). Active fractions were combined and extracted with ethyl acetate. The extract was concentrated and added with *n*-hexane to yield pure duocarmycin B_1 (**4**) as an yellow powder. Duocarmycin B_2 (**5**) fractions were concentrated

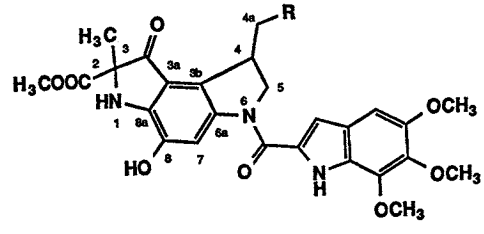
Fig. 1. Structures of duocarmycins.



1



2 R=Cl
4 R=Br



3 R=Cl
5 R=Br

Table 1. Physico-chemical properties of duocarmycins B_1 and B_2.

	B_1	B_2
Nature	Yellow powder	Orange crystal
MP (°C)	148~149	214~215
$[\alpha]_D^{20}$ (*c* 0.2, MeOH)	−113.5°	−57.5°
Elemental analysis		
Calcd:	C 53.07, H 4.45, N 7.14	C 53.07, H 4.45, N 7.14
Found:	C 53.20, H 4.66, N 6.46	C 53.20, H 4.49, N 7.18
MS (*m/z*, $(M+H)^+$)	588	588
Molecular formula	$C_{26}H_{26}N_3O_8Br$	$C_{26}H_{26}N_3O_8Br$
UV λ_{max} nm (ε)	208 (38,000), 329 (17,800), 410 (3,200)	208 (45,000), 248 (sh, 19,100), 298 (20,300), 337 (31,500), 434 (4,400)
Solubility		
Soluble:	MeOH, $CHCl_3$, EtOAc, DMSO	MeOH, $CHCl_3$, EtOAc, DMSO
Insoluble:	Hexane, water	Hexane, water

Table 2. ^{13}C NMR data for duocarmycins.

Carbon	C_1 (**2**)[3]	B_1 (**4**)	C_2 (**3**)[3]	B_2 (**5**)
C-2	70.1	71.1	71.2	71.2
C-3	197.8	196.8	196.6	196.6
C-3a	114.8	117.1	119.5	120.2
C-3b	114.7	116.6	115.6	115.6
C-4	32.9	33.9	42.3	42.0
C-4a			46.4	35.6
C-5	54.7	44.8	55.0	56.1
C-6	51.2	53.0		
C-6a			137.7	137.6
C-7			112.5	112.5
C-7a	128.4	128.9		
C-8	117.2	118.2	150.1	150.4
C-8a			144.2	144.2
C-9	152.2	151.7		
C-9a	141.2	141.6		
2-CH_3	20.2	21.8	22.0	22.0
2-$COOCH_3$	169.2	169.7	169.6	169.5
2-$COOCH_3$	52.6	53.4	53.4	53.4
C-2′	130.7	129.1	129.1	129.1
C-2′a	163.3	164.5	160.5	160.5
C-3′	106.6	108.3	107.9	107.9
C-3′a	122.7	123.1	123.5	123.5
C-4′	97.9	97.9	98.0	98.0
C-5′	149.1	150.2	150.4	150.1
C-6′	139.5	140.4	140.9	140.9
C-7′	139.0	138.9	138.7	138.7
C-7′a	125.4	126.1	126.0	126.0
5′-OCH_3	55.9	56.3	56.4	56.4
6′-OCH_3	60.9	61.5	61.5	61.5
7′-OCH_3	61.0	61.2	61.2	61.2

C_1 in DMSO-d_6. B_1, C_2 and B_2 in $CDCl_3$.

Table 3. Antitumor activities of duocarmycins against sarcoma 180.

Compound	Dose (mg/kg)	T/C* (%)
A (**1**)	0.0075	0.26
C_1 (**2**)	6	0.28
C_2 (**3**)	3	0.19
B_1 (**4**)	0.5	0.22
B_2 (**5**)	0.25	0.28
Mitomycin C	4	0.30

Single dose given iv on day-1 after tumor inoculation.

* T/C represents the ratio median tumor volume of the treated group divided by that of the control group.

and chromatographed over Diaion HP-20SS using 85% aqueous ethanol (pH 4.0). Active fractions were combined and crystallized from methanol to yield pure duocarmycin B_2 (**5**) as an orange crystal.

The physico-chemical properties of duocarmycins B_1 (**4**) and B_2 (**5**) are summarized in Table 1. The UV spectrum of duocarmycin B_1 (**4**) was very similar to that of duocarmycin C_1 (**2**). The molecular formula of duocarmycin B_1 (**4**) was determined as $C_{26}H_{26}N_3O_8Br$ by fast atom bombardment (FAB)-MS and microanalysis. ^1H and ^{13}C NMR spectra of duocarmycin B_1 (**4**) were quite similar to that of duocarmycin C_1 (**2**) except the signal of attributed to C-5 (δ 44.8). The molecular formula of duocarmycin B_2 (**5**) was determined as $C_{26}H_{26}N_3O_8Br$ by FAB-MS and microanalysis. UV spectrum, and ^1H and ^{13}C NMR spectra of duocarmycin B_2 (**5**) were also similar to that of duocarmycin C_2 (**3**) except the signal of attributed to C-4a (δ 35.6). These observations indicated that in-

stead of Cl atom in duocarmycin C_1 (**2**), Br atom is attached to C-5 in duocarmycin B_1 (**4**). Duocarmycin B_2 (**5**) is also substituted by Br atom instead of Cl atom in duocarmycin C_2 (**3**). These structures were also supported with next experiment. Treatment of duocarmycin A (**1**) with 1% KBr - acetone (1 : 1) gave duocarmycins B_1 (**4**) and B_2 (**5**) in the ratio of 1 : 4. This results suggest that duocarmycin A (**1**) is first produced by the microorganism, and bromide ion is added to duocarmycin A (**1**) forming duocarmycins B_1 (**4**) and B_2 (**5**). Treatment of duocarmycin B_2 (**5**) with base such as *iso*-Pr_2NEt or 1,5-diazabicyclo[5.4.0]undecene-5(DBU) gave easily duocarmycin A (**1**).

The LD_{50} value of duocarmycin B_1 (**4**) is 0.37 mg/kg (iv) in mice and that of duocarmycin B_2 (**5**) is 0.28 mg/kg (iv). Duocarmycins B_1 (**4**) and B_2 (**5**) are more potent than duocarmycins C_1 and C_2. The antitumor activity of duocarmycins against mouse sarcoma 180 is summarized in Table 3. Further detailed studies on antitumor spectra and toxicity of duocarmycins are in progress.

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