Communications to the Editor

DC92-B, A NEW ANTITUMOR ANTIBIOTIC FROM *ACTINOMADURA*

Sir:

We have isolated new anthraquinone antitumor antibiotics, DC92-B and related DC92-D, from a culture broth of *Actinomadura* sp. In this communication we report the production, isolation, physico-chemical properties and biological activities of DC92-B and DC92-D.

The producing organism was isolated from a soil collected in Machida-shi, Tokyo, Japan, and has been identified as *Actinomadura* sp. A stock culture maintained in a deep freezer (−70°C) was inoculated into seed medium consisting of glucose 10 g, soluble starch 10 g, yeast extract 5 g, Bacto-tryptone 5 g, beef extract 3 g and $CaCO_3$ 2 g per liter of tap water. A 5%-vegetative seed culture was inoculated into fermentation medium consisting of glucose 15 g, Pharmamedia 20 g, $MgSO_4 \cdot 7H_2O$ 0.5 g and KH_2PO_4 0.5 g per liter of tap water (pH 7.0 prior to sterilization). The antibiotics were detected by paper-disc assay against *Bacillus subtilis* on agar plate. The peak titers were usually reached after 4 days incubation at 28°C.

The culture broth (150 liters) was filtered and the filtrate was applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited), the column was washed with deionized water, 50% MeOH and then eluted with MeOH. MeOH eluate was evaporated and added with deionized water, adjusted to pH 10 and then extracted with EtOAc. The extract was concentrated to give a brown syrup, which was applied to a Sephadex LH-20 column and chromatographed with MeOH. The active fractions were combined and concentrated to dryness. The residue was chromatographed on an aminopropyl silane (NH_2) silica gel (J. T. BAKER, Chemical Co.) column with toluene - Me_2CO as eluents, and the active fractions were further purified with HPLC using a column packed with aminopropyl silane (NH_2) silica gel to yield 150 mg of DC92-B and 70 mg of DC92-D.

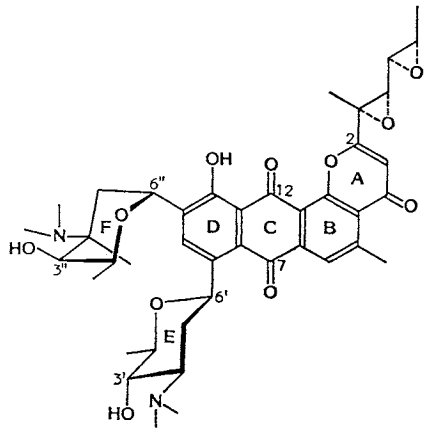
DC92-B and DC92-D, obtained as an orange powder, showed the properties as summarized in Table 1. The molecular formula of DC92-B was determined as $C_{42}H_{52}N_2O_{12}$ by secondary ion mass spectrum (SI-MS). The UV absorption maxima (244, 264 (sh), 424 nm in MeOH) and the IR spectrum of DC92-B are similar to those of anthraquinone type antibiotics[1~5]. The structure of DC92-B was assigned by NMR spectroscopic studies and was shown to be similar to that of hedamycin[6] except for the side chain at C-2 and for the ring F (Fig. 1). The molecular formula of DC92-D was determined as $C_{42}H_{50}N_2O_{12}$ by SI-MS. ^1H and ^{13}C NMR spectra of DC92-D are quite similar to that of DC92-B excepting that the ring E of DC92-D

Table 1. Physico-chemical properties of DC92-B and DC92-D.

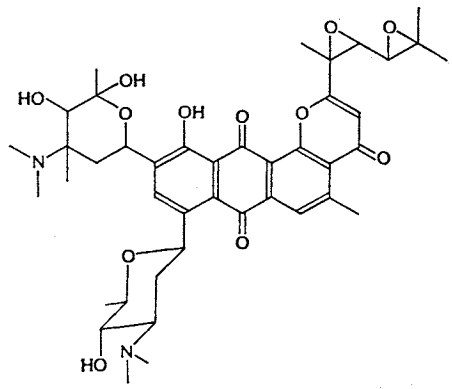
	DC92-B	DC92-D
Appearance	Orange powder	Orange powder
Molecular formula	$C_{42}H_{52}N_2O_{12}$	$C_{42}H_{50}N_2O_{12}$
MW	776	774
SI-MS (*m/z*)	777 $(M+1)^+$	775 $(M+1)^+$
UV λ_{max}^{MeOH} nm(ε)	244 (39,000), 264 (sh, 26,000), 424 (7,600)	243 (38,000), 264 (sh, 28,000), 384 (sh, 6,000), 424 (6,500)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	3450, 1657, 1632, 1587, 1465, 1442, 1422, 1380, 1308, 1254, 1076	3440, 1660, 1632, 1590, 1468, 1443, 1421, 1380, 1368, 1310, 1253, 1073
Rf value*	0.54	0.20
Solubility Soluble:	MeOH, EtOH, Me_2CO, DMSO, EtOAc, $CHCl_3$, toluene	MeOH, EtOH, Me_2CO, DMSO, EtOAc, $CHCl_3$, toluene
Insoluble:	H_2O, hexane	H_2O, hexane

* NH_2 Silica gel TLC (Merck, Art. No. 15647), solvent: toluene - Me_2CO (6 : 4).

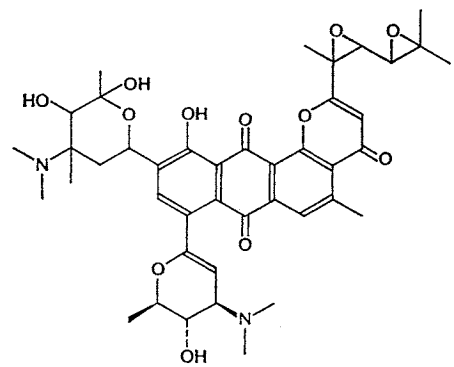
Fig. 1. The structures of hedamycin, DC92-B and DC92-D.



Hedamycin



DC92-B



DC92-D

has the enol structure (Fig. 1) which has been found in the ring E of photohedamycin[7]. Photohedamycin was reported as the photoproduct of hedamycin[8] and DC92-D is also obtained by treatment of DC92-B under the daylight. Details of structure determination will be reported elsewhere.

DC92-B and DC92-D are active mainly against Gram-positive bacteria as shown in Table 2. The LD_{50} value of DC92-B is 0.145 mg/kg (iv) in mice and that of DC92-D is 5.63 mg/kg (iv). DC92-B exhibits antitumor activity against murine lymphotic leukemia P388 *in vivo* showing 43% increase of life span (ILS) at a dose of 0.10 mg/kg by a single ip injection. DC92-B is also effective against murine sarcoma 180 *in vivo* showing a T/C 34% at a daily dose of 0.10 mg/kg (iv) for 5 days (Table 3). Further

Table 2. The antimicrobial spectrum of DC92-B and DC92-D (MIC, μg/ml).

Test organism	DC92-B	DC92-D
Staphylococcus aureus ATCC 6538P	0.04	1.5
Streptococcus faecium ATCC 10541	1.5	15
Bacillus subtilis #10707	0.15	3.0
Escherichia coli ATCC 26	10	>100
Klebsiella pneumoniae ATCC 10031	2.5	25
Shigella sonnei ATCC 9290	10	100
Salmonella typhi ATCC 9992	40	>100
Proteus vulgaris HX2 ATCC 6897	80	>100
Pseudomonas aeruginosa BinH#1	10	100
Candida albicans ATCC 10231	80	>100

Table 3. Antitumor activity of DC92-B.

(A) Against murine lymphotic leukemia P388 (ip-ip).

Compound	Dose (mg/kg)	Treatment schedule	ILS (%)
DC92-B	0.40	Once, day 1	−52
	0.20	Once, day 1	5
	0.10	Once, day 1	43
	0.050	Once, day 1	34
	0.025	Once, day 1	38
Mitomycin C	6.0	Once, day 1	65

(B) Against murine sarcoma 180 (sc-iv).

Compound	Dose (mg/kg)	Treatment schedule	T/C
DC92-B	0.20	Every day, days 1～5	Toxic
	0.10	Every day, days 1～5	0.34
	0.050	Every day, days 1～5	0.57
	0.025	Every day, days 1～5	0.51
Mitomycin C	6.0	Once, day 1	0.45

studies on antitumor activity and toxicity of DC92-B and DC92-D are in progress and will be reported in due course.

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