Communication to the Editor

DYNEMICIN A, A NOVEL ANTIBIOTIC WITH THE ANTHRAQUINONE AND 1,5-DIYN-3-ENE SUBUNIT

Sir:

The 1,5-diyn-3-ene-containing antibiotics represented by esperamicin[1,2] and calicheamicin[3] are receiving increasing attention because of their extremely potent antitumor activity and unusual structures. A unique mechanism of action involving phenyl diradical formation has been proposed for this family of antibiotics[4]. These antibiotics show extremely strong inhibition of growth of Gram-positive bacteria, especially the recombination-deficient mutants such as *Bacillus subtilis* M45 strain. During the course of our continuing search for new antitumor antibiotics using *B. subtilis* M45, dynemicin A, a novel violet-colored antibiotic was discovered in the fermentation broth of a new *Micromonospora* strain. The antibiotic exhibits very potent antibacterial activity, especially against Gram-positive bacteria, and prolongs the life span of mice inoculated with P388 leukemia. Structural studies revealed that dynemicin A is a unique hybrid of an anthraquinone and an 1,5-diyn-3-ene system. This communication describes the production, isolation, physicochemical properties, structure, and biological activities of dynemicin A.

The producing organism was isolated from a soil sample collected in Gujarat State, India and was identified as *Micromonospora chersina* sp. nov. No. M956-1. Antibiotic production was carried out in two 200-liter tank fermenters containing 120 liters each of a production medium (soluble starch 1.5%, glucose 0.5%, beet molasses 1%, fish meal 1% and $CaCO_3$ 0.5%, pH 7.0 before sterilization) at 28°C with agitation (250 rpm) and aeration (120 liters/minute). The antibiotic activity reached a maximum at 92 hours, as monitored by the paper-disc assay using *B. subtilis* PCI 219 as the test organism.

Dynemicin A (**1**) was isolated from the cultured broth by the following procedure. The whole broth (220 liters) was adjusted to pH 2.0 with 6 N HCl and extracted with BuOH (80 liters). The extract was concentrated *in vacuo* to an aqueous solution (1 liter) which deposited a dark brown precipitate. The solid collected by filtration was dissolved in MeOH (2 liters), combined with the aqueous filtrate and then loaded onto a column of Diaion HP-20 (10 i.d.×65 cm) previously equilibrated with 70% aqueous MeOH. After being washed with 80% MeOH, the activity was eluted from the column with 80% aqueous acetone. The residue (62 g) obtained upon concentration of the active eluate was rechromatogaphed on a column of Sephadex LH-20 (4 i.d.×40 cm) with MeOH elution.

Table 1. Physico-chemical properties of dynemicin A and triacetyldynemicin A.

	Dynemicin A	Triacetyldynemicin A
Nature	Violet amorphous powder	Orange rods
MP (°C, dec)	208~210	228~231
$[\alpha]_D^{24}$	+270° (*c* 0.01, DMF)	+1,300° (*c* 0.05, MeOH)
UV λ_{max}^{MeOH} nm (ε)	239 (24,900), 282 (sh), 569 (10,800), 599 (10,100)	244 (40,100), 313 (6,700), 482 (8,100)
Molecular formula	$C_{30}H_{19}NO_9$	$C_{36}H_{25}NO_{12}$
Microanalysis		Calcd for $C_{36}H_{25}NO_{12}\cdot H_2O$: C 63.43, H 3.99, N 2.06 — Found: C 63.20, H 3.75, N 2.16
SI-MS *m/z*, $(M+H)^+$	538	664
TLC[a] (Rf)	0.40	0.33

SI-MS: Secondary ion mass spectrum. [a] SiO_2; Xylene - methyl ethyl ketone - MeOH (5 : 5 : 1).

Fig. 1. IR spectrum of dynemicin A (KBr).

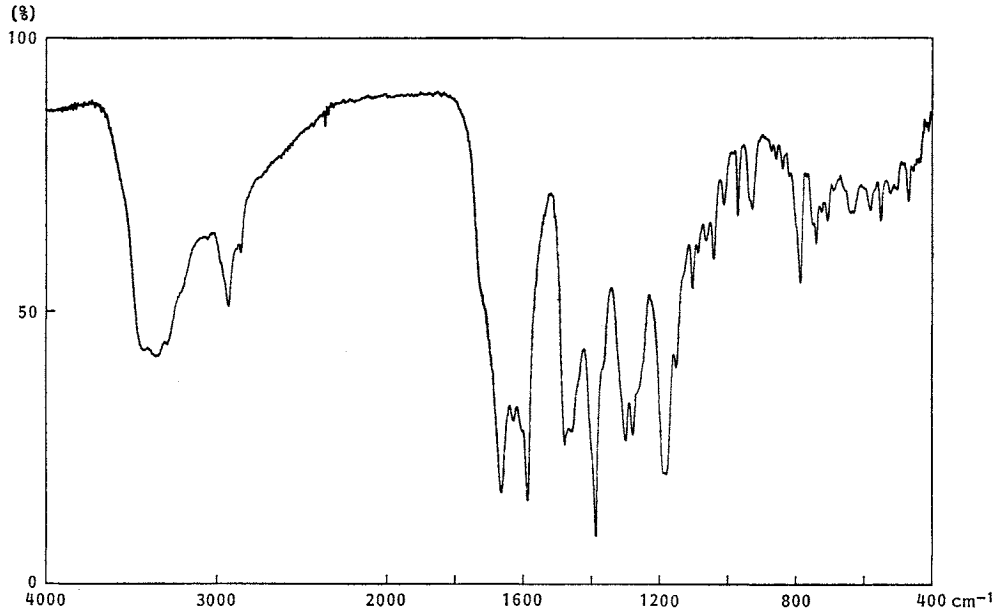


Table 2. ^{1}H NMR spectrum of triacetyldynemicin A (400 MHz in DMSO-d_6).

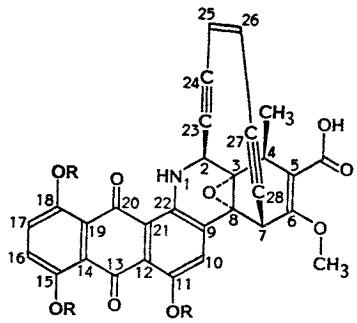
Proton No.	Triacetyldynemicin A
4-CH_3	1.25 (3H, d, J=7.3 Hz)
11-$OCOCH_3$, 15-$OCOCH_3$, 18-$OCOCH_3$	2.33 (3H, s), 2.36 (3H, s), 2.44 (3H, s)
4-H	3.55 (1H, q, J=7.3 Hz)
6-OCH_3	3.79 (3H, s)
7-H	4.78 (1H, s)
2-H	5.04 (1H, d, J=3.8 Hz)
25-H	6.05 (1H, d, J=1.3 Hz)
26-H	6.07 (1H, d, J=1.3 Hz)
16-H, 17-H	7.62 (2H, s, ×2)
10-H	8.03 (1H, s)
1-NH	9.41 (1H, d, J=3.8 Hz)
5-COOH	12.37 (1H, br)

Upon monitoring by TLC (see Table 1), the appropriate eluate was concentrated to yield a dark blue solid (56 mg). This was further purified by preparative TLC (SiO_2 and the same solvent as above TLC) followed by Sephadex LH-20 chromatography with MeOH elution to yield a homogeneous violet sample of **1** (5.7 mg).

1 is a violet amorphous solid soluble in DMSO, DMF and dioxane, slightly soluble in $CHCl_3$, EtOAc and MeOH and insoluble in H_2O and *n*-hexane. When treated with acetic anhydride in pyridine, **1** yielded a triacetyl derivative (**2**) with increased solubility. Their physico-chemical properties are summarized in Table 1. The IR spectrum of **1** (Fig. 1) contains a broad OH/NH absorption band at 3500~3200 cm^{-1} and carbonyl absorption bands at 1660 and 1630 cm^{-1}. The latter bands suggest a quinone group in the molecule. The IR spectrum of **2** exhibits a strong carbonyl band at 1770 cm^{-1} in addition to the bands observed for the spectrum of **1**. One methyl (δ 1.25), three acetyl methyls (2.33, 2.36 and 2.44), one OCH_3 (3.79), three methines (3.55, 4.78 and 5.04), two olefinic (6.05 and 6.07) and three aromatic protons (7.62×2 and 8.03) were observed in the ^{1}H NMR spectrum of **2** (Table 2). Corresponding carbon signals were found in the

^{13}C NMR. The ^{1}H NMR spectrum and the UV spectrum of **1** resemble those of ε-isorhodomycinone[5,6] suggesting a 1,4,6,9-tetrahydroxyanthraquinone or a related chromophore in the molecule. The remaining part of the molecule should have one CH_3, one OCH_3, three >CH, two –CH=, two >C=, six quaternary carbons and one carboxyl carbon. Among the quaternary carbons, four carbons appeared at δ 88.8, 89.6, 97.3 and 99.4 strongly suggesting a conjugated diyne system from spectral comparison with esperamicin. The complete structure was elucidated by X-ray crystallography of crystalline **2**† (Fig. 2).

Fig. 2. The structures of dynemicin A and triacetyldynemicin A.



Dynemicin A R=H
Triacetyldynemicin A R=$COCH_3$

Dynemicin A (**1**) and its triacetate (**2**) showed extremely strong activity against Gram-positive bacteria as shown in Table 3. Gram-negative bacteria, anaerobic bacteria and fungi are considerably less sensitive to both compounds. On the whole, **2** is two to eight times more potent than **1** against the organisms tested. **1** exhibited significant *in vivo* activity against *Staphylococcus aureus* Smith infection in mice with the PD_{50} being 0.13 mg/kg by ip administration. No toxic signs were observed in the mice after administration of 5 mg/kg (ip) of **1**. Both compounds showed marked cytotoxic activity against B16 melanoma, Moser human carcinoma, HCT-116 human carcinoma and the normal and vincristin-resistant P388 leukemia cells with IC_{50} of 0.004~0.005 μg/ml. In *in vivo* tests, **1** and **2** produced significant prolongation of life span of mice inoculated with P388 leukemia and B16 melanoma (Table 4). The active dose ranges were rather broad though the T/C values were maintained at not very high levels.

It is apparent that dynemicin A is a new member of the esperamicin/calicheamicin family of antibiotics in terms of possessing the unique 1,5-diyn-3-ene unit. However, it is distinctly different from the preceding antibiotics in possessing the violet-colored chromophore of a substituted anthraquinone. It should also be noted that unlike the esperamicin antibiotics, **1**

Table 3. Antibacterial spectra of dynemicin A and triacetyldynemicin A.

Organism	MIC (μg/ml)	
	Dynemicin A	Triacetyldynemicin A
Staphylococcus aureus FDA 209P	0.000013	0.0000063
S. aureus Smith	0.000025	0.0000063
S. epidermidis D153	0.0000063	0.0000031
Micrococcus luteus PCI 1001	0.0008	0.0002
Bacillus subtilis PCI 219	0.0000063	0.0000031
Escherichia coli NIHJ	0.05	0.0063
Klebsiella pneumoniae D11	0.0063	0.0008
Pseudomonas aeruginosa A9930	0.025	0.0063
Proteus vulgaris A9436	0.0063	0.0031
Clostridium difficile A21675	0.0063	0.0031
Bacteroides fragilis A22693	0.2	0.1
Candida albicans IAM 4888	12.5	0.4
Cryptococcus neoformans D49	12.5	0.8
Aspergillus fumigatus IAM 2530	6.3	0.1
Trichophyton mentagrophytes D155	12.5	0.4

† Full details of the structure determination will be forthcoming; M. KONISHI, H. OHKUMA, T. OKI, H. KAWAGUCHI and J. CLARDY.

Table 4. Antitumor activity of dynemicin A.

	P388 leukemia		B16 melanoma	
	Dose qd 1→3, ip (mg/kg/day)	T/C (%)	Dose qd 1→9, ip (mg/kg/day)	T/C (%)
Dynemicin A	1.0	135[a]	1.0	159[a]
	0.5	130	0.5	137
	0.25	135	0.25	137
	0.13	130	0.13	122
	0.063	130	0.063	141
	0.031	130	0.031	122
Mitomycin C	3.0	200	2.0	222
	1.0	135	1.0	152
	0.3	140	0.5	133
	0.1	115	0.25	111

[a] T/C≧125 means significant antitumor effect.

exhibited significant *in vivo* antibacterial activity and low toxicity. The anthraquinone chromophore moiety of dynemicin A is presumed to play an important role in its biological activity and further studies on the subject will be pursued.

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