DIANEMYCIN, AN ANTIBIOTIC OF THE GROUP AFFECTING ION TRANSPORT

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Dianemycin is an antibiotic previously reported to affect cation transport and to be effective in treating coccidiosis in chickens. It is isolated from a fermentation broth of *Streptomyces hygroscopicus*. The compound was found to be primarily active against gram-positive organisms. The chemical properties of dianemycin indicate a close relationship to monensin and other acidic cation complexing compounds.

Several years ago a report by LARDY and co-workers[1] indicated that a new antibiotic, named dianemycin, affected cation transport in mitochondria. More recently GORMAN *et al.*[2] related the general chemical properties of dianemycin to those of a group of closely related acidic compounds which included monensin, nigericin and X-206 (Ro-2-2879). SHUMARD and CALLENDER[3] have shown that these compounds are quite active against coccidial infections in chickens. Because of current interest in cation transporting substances, we shall describe in this paper the fermentation and isolation as well as the antimicrobial properties of dianemycin.

Dianemycin was first detected as a product of a strain of *Streptomyces hygroscopicus* found in a soil sample collected in Korea. This strain of *S. hygroscopicus* has been assigned the culture number NRRL 3444. The compound was detected while screening fermentation broths for antibacterial activity. Extraction of both mycelia and filtered fermentation broth followed by chromatography led to the isolation of a crystalline acid. This acid readily formed crystalline salts with alkali metals, and these salts

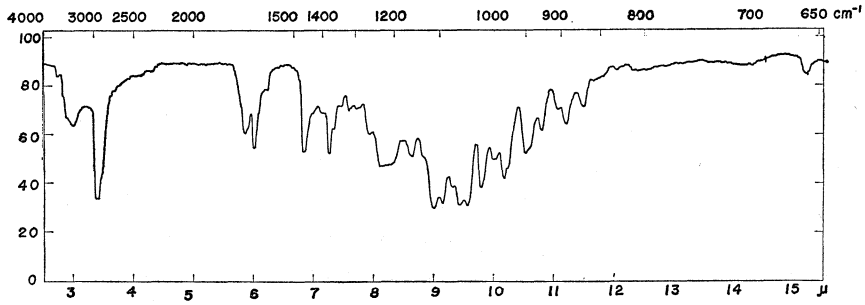
Table 1. Antimicrobial spectrum of dianemycin

Organism	Minimum inhibitory concentration (mcg/ml)	Organism	Minimum inhibitory concentration (mcg/ml)
Staphylococcus aureus 3055	1.56	*Trichophyton mentagrophytes*	100
Bacillus subtilis	3.12	*Pseudomonas solanacearum*	50
Mycobacterium avium	0.78	*Alternaria solani*	12.5
Streptococcus faecalis	1.56	*Botrytis cinerea*	6.25
Lactobacillus casei	0.78	*Ceratostomella ulmi*	50
Leuconostoc citrovorum	6.25	*Colletotrichum pisi*	50
Vibrio coli Iowa No. 10	12.5	*Helminthosporium sativum*	12.5
Mycoplasma gallisepticum	25	*Penicillium expansum*	50
Mycoplasma, N strain	50	*Spicaria divaricata*	50
Saccharomyces pastorianus	50		

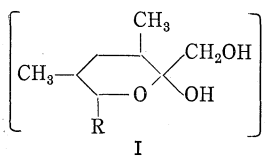
exhibit similar solubility characteristics to the acidic form. As with related members of this series[2], both acid and salts are only slightly soluble in water but are soluble in most organic solvents. Dianemycin is highly inhibitory to various gram-positive bacteria and has moderate antifungal activity (Table 1).

A study of the infrared spectrum (Fig. 1) showed an α, β-unsaturated carbonyl group which was confirmed by the ultraviolet maximum at 232 mμ. In contrast, the other members of this group show only the absorption due to the unsaturation of the carboxylic acid moiety. The highest mass peak in the mass spectrum of dianemycin is 888 mass units, indicating it to be considerably larger than either monensin[2] or nigericin[4], the two members of this group whose structures have been determined. A molecular weight value of 958 was obtained by titration and 950 from single crystal X-ray analysis. These values and microanalysis indicated an empirical formula of about $C_{51}H_{88}O_{16}$.

Fig. 1. Infrared spectrum of dianemycin ($CHCl_3$)



Dianemycin reacted readily with one equivalent of sodium metaperiodate liberating formaldehyde. This reaction also occurs with nigericin and monensin, and has been shown to result from cleavage of the terminal 1, 2-glycol present in each at the non-carboxylate end of the molecule (I). Thus this portion of dianemycin appears to be functionally equivalent to the aforementioned antibiotics.



I

Thin-layer chromatography on silica in ethyl acetate[2] served to differentiate dianemycin from other related antibiotics.

Experimental

Fermentation Studies

Stock cultures of *Streptomyces hygroscopicus* (WAKSMAN and HENRICI) were maintained at 4°C following growth on asparagine-starch agar slants for 5~7 days at 30°C.

Vegetative inoculum was grown in a medium of the following composition: 5 g, beef extract (Difco); 5 g, N-Z amine (Sheffield); 5 g, peptone; 5 g, NaCl; 10 g, glucose; and 1,000 ml of cold tap water. Inoculated medium, 100 ml per 500 ml Erlenmeyer flasks, was incubated 48 hours at 30°C on a reciprocal shaker at 108 strokes per minute. The resulting culture was used to provide a 5 % inoculum for the fermentation flasks. The fermentation medium was composed of: 10 g, glucose; 20 g, edible molasses; 5 g, peptone; 2 g, $CaCO_3$; and 1,000 ml of cold tap water. The incubation was carried out on a reciprocal shaker operating at 108 strokes per minute for 48 hours at 30°C. *Sarcina lutea* was used as the test organism in an agar diffusion assay.

Isolation and Purification

At the conclusion of the fermentation three percent of diatomaceous filter aid was added, and the broth was filtered at harvest pH. The mycelial cake was extracted with methanol; the methanol extract was concentrated *in vacuo* to an aqueous phase which was combined with the filtrate. This solution was adjusted to pH 3 with 6 N HCl and was extracted twice with one-third volume of chloroform. The chloroform extract was dried with Na_2SO_4 and was filtered through a carbon (Pittsburgh 12×40) column packed in chloroform. The active fractions were combined, and the solvent was removed. The residue was dissolved in diethyl ether, diluted with hexane, and the inactive precipitate which formed was removed. The filtrate was concentrated to an oil which crystallized upon standing in an acetone-water (2:1) mixture. This crystalline product was the carboxylic acid form of dianemycin (m. p. 72~74°C).

Preparation of Dianemycin Sodium Salt

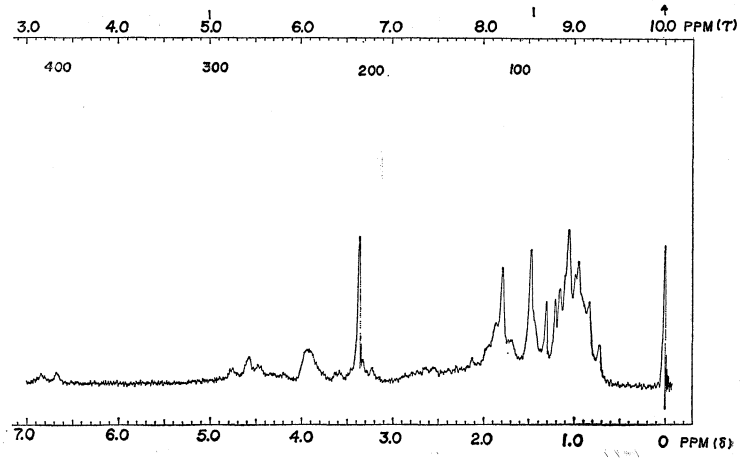
Dianemycin was dissolved in acetone (100 mg/ml), a half volume of water was added, and the solution was titrated with 1 N NaOH to pH 9~9.5. The solution was allowed to stand for crystallization to occur. The resulting crystals melted at 210~212°C and were shown to be the sodium salt by the infrared absorption band at 6.45 μ and sodium analysis (atomic absorption spectroscopy).

Physical-Chemical Properties

Dianemycin is a white crystalline acidic compound insoluble in water but readily soluble in most organic solvents. The melting point varies with the solvent of crystallization: 72~74°C (acetone-water), 156~157°C (ethanol-water). The optical rotation is $[\alpha]_D^{25}$ +39.9° (*c* 2, methanol), pK'a=6.6 (66 % DMF), and the compound exhibits an ultraviolet absorption maximum at 232 mμ, A_m=1600/100 molecular weight units. The elemental analysis of C 63.68, H 9.47, and O 27.37 percent indicated an empirical formula of $C_{51}H_{88}O_{16}$, (956) based on molecular weights of 958 determined by titration and 950 by X-ray analysis. The infrared spectrum in $CHCl_3$ is shown in Fig. 1. The nuclear magnetic resonance spectrum (Fig. 2) showed the presence of CH_3O-, $-\overset{H}{\underset{|}{C}}-\overset{H}{\underset{|}{C}}=\overset{|}{C}-\overset{O}{\overset{||}{C}}-$, $CH_3\overset{|}{\underset{|}{C}}-O-$, and many CH_3-C-.

The sodium salt of dianemycin is very similar to the acid in solubility properties, being insoluble in water and soluble in organic solvents. It has a melting point of 210~212°C, $[\alpha]_D^{25}$ +37.1 (*c* 1, methanol).

Fig. 2. NMR spectrum of dianemycin ($CHCl_3$) with TMS standard



Periodate Oxidation

The products of the reaction between dianemycin and sodium metaperiodate in *t*-butanol were distilled into a dimedone solution. The crystals (m. p. 190~193°C) which formed were collected and shown to be identical with an authentic sample of the dimedone derivative of formaldehyde. The gummy residue remaining after the distillation had the following properties : $\gamma_{max}^{CHCl_3}$ 1712, 1653 cm^{-1} ; λ_{max}^{EtOH} 231 mμ, indicating retention of the chromophore. One mole of periodate was consumed per mole of dianemycin in the above reaction.

Chromatography

The Rf values of dianemycin on paper chromatography were 0.28 using a water-ethanol-acetic acid (70 : 24 : 6) system and 0.77 using a 10 % aqueous propanol system. *Bacillus subtilis* was used as the bioautograph organism. The Rf value on silica gel thin-layer chromatography was 0.25 with ethyl acetate as the developing solvent. Vanillin-H_2SO_4 was used as the detector spray.

Biological Properties

The antimicrobial spectrum is shown in Table 1. The antibiotic inhibits the gram-positive organisms, mycoplasma, mycobacterium, and fungi, but no activity is seen against gram-negative organisms. Dianemycin has exhibited activity against coccidia, controlling an infection of *Eimeria tenella* in chicks at a dosage of 20~40 g/ton of diet[3]. The compound did not control an experimental staphylococcal infection in mice. The LD_{50} of dianemycin is about 40 mg/kg subcutaneous in mice.

Dianemycin possesses insecticidal activity. At a concentration of 1,000 ppm in a spray, it is lethal to milkweed bugs and horseflies.

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