A New Channel-forming Antibiotic from *Streptomyces coelicolor* A3(2) Which Requires Calcium for its Activity

By J. H. LAKEY, ¹ E. J. A. LEA, ¹* B. A. M. RUDD, ²† H. M. WRIGHT ² AND D. A. HOPWOOD ²

¹ School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.
² John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

(Received 28 April 1983; revised 22 July 1983)

A recently discovered antibiotic (CDA; calcium-dependent antibiotic) of *Streptomyces coelicolor* A3(2) was found to be effective against a wide range of Gram-positive bacteria only in the presence of calcium ions. Producer and non-producer strains were identified and several media tested for their ability to support antibiotic production. The action of calcium was not simulated by any of the other cations tested. The antibiotic was found to induce discrete conductance fluctuations in planar lipid bilayer consistent with a channel-forming action. The electrical potential difference caused by a concentration difference of various salts across the CDA-containing bilayer, showed the channel to be cation-selective but of a size that discriminated against tetramethyl ammonium and choline ions. The data indicate that the antibiotic activity of CDA is due to its action as a calcium-dependent ionophore.

**INTRODUCTION**

*Streptomyces coelicolor* A3(2) produces three antibiotics of known structure: methylenomycin (Wright & Hopwood, 1976a), actinorhodin (Wright & Hopwood, 1976b) and undecylprodigiosin (Rudd & Hopwood, 1980; S-W. Lee, C-J. Chang & H. C. Floss, personal communication). Recently a fourth antibiotic activity was discovered (Rudd, 1978); the antibiotic was called CDA (for calcium-dependent antibiotic) because it kills bacteria only in the presence of calcium ions. Here we describe some biological and biophysical properties of CDA. It appears to have a novel action as a channel former in artificial membranes: the channels conduct monovalent cations, but only in the presence of calcium.

**METHODS**

Bacterial strains. Micro-organisms tested for resistance or sensitivity to CDA are given in Table 1. *Streptomyces coelicolor* A3(2) derivative 2377 (hisAl ursAl strAl act-3 SCP1 SCP2) was used as the routine source of CDA.

Media. Minimal medium (MM) and complete medium (CM) were described by Hopwood (1967). Nutrient agar (NA) and nutrient broth (NB) were from Oxoid; soft nutrient agar was NA diluted with an equal volume of NB. TY was described by Beringer (1974) and R2 by Okanishi et al. (1974). When required, NA was supplemented with calcium by adding a solution of Ca(NO₃)₂. TSB was Difco Trypticase Soya Broth.

Preparation of solutions of CDA. Most experiments were performed with an unpurified solution prepared as follows. Plates of NA (33 ml per plate) were spread with spores of the *S. coelicolor* A3(2) strain 2377 which is unable to produce two of the three previously characterized antibiotics of *S. coelicolor* A3(2) – methylenomycin and actinorhodin. (The third antibiotic, undecylprodigiosin is not extracted into water.) After incubation at 30 °C for 4–7 d, the plates were frozen at −20 °C and thawed. The material was centrifuged at 12000 g for 30 min and

† Present address: Glaxo Group Research, Greenford, Middlesex UB6 0HE, U.K.

**Abbreviations**: CM, complete medium; MM, minimal medium; NA, nutrient agar; NB, nutrient broth; TSB, Trypticase Soya Broth.

0022-1287/83/0001-1219 $02.00 © 1983 SGM
the supernatant decanted. This procedure allowed the recovery of 45–65% of the volume of the original agar medium as a clear brown, alkaline (pH 9) solution containing CDA. When a more concentrated preparation was required, the liquid was evaporated to dryness on a Buchi rotary evaporator and the solid deposit was re-dissolved in water. The solution appeared to retain its CDA activity on storage at 4 °C for several months.

Tests of microbiological activity of CDA. Spores or cell suspensions of test organisms were embedded in 3 ml soft agar poured over base layers containing 20–30 ml NA supplemented with 8 mM-Ca(NO₃)₂, and 13 mm diameter antibiotic assay discs impregnated with 0.1 ml CDA solutions were placed on the agar surface. Inhibition zones around the test discs indicated CDA activity (usually control plates containing no added Ca(NO₃)₂ were included to avoid any possible confusion by other inhibitory substances in the test liquid; these normally showed no inhibition zones). Sometimes the diameters of inhibition zones were enhanced by placing the inoculated plates at 4 °C for a few hours before incubating at 30 °C. The test organism for routine assay of CDA was a strain of Bacillus mycoides.

Measurements of membrane conductivity. Bilayer lipid membranes were formed by the brush technique of Mueller et al. (1963) on the ends of a set of five polyethylene tubes from solutions of purified egg-lecithin and cholesterol in n-decane containing 0.25% (w/v) lecithin and 0.125% (w/v) cholesterol. The membranes were formed in the salt solutions specified in the results section. (All solutions were unbuffered and contained double glass-distilled water.) When CDA was used, it was present on both sides of the membrane in equal concentrations. The membranes were successively on each membrane of the set 5 min after formation. The whole apparatus was mounted in a glass thermostat bath on a specially designed magnetic stirrer. Experiments were carried out at 24 °C unless otherwise specified. Measurements of single membrane channels were carried out as previously described (Lea & Collins, 1979).

Conductivity at a fixed CDA and salt concentration was measured at increasing temperatures. At the end of the experiment the apparatus was cooled and membrane conductances were measured again. The phase transition of egg lecithin occurs at about 0 °C, at least 5 °C below the range used.

Measurements of ion selectivity. For each of a number of salts XCl, the ratio \( \alpha = P_x/P_C \) (the ratio of cation to anion permeabilities) was calculated from the transmembrane potential difference (p.d.) resulting from different concentrations of salts on the two sides of the membrane. Using an electrometer (EIL Vibron Model 33B), p.d. was measured between a chlorided silver wire inside each tube and a common reference wire placed in a solution identical to that inside the tubes and connected to the outer solution via an agar bridge containing 0-5% agar in 3M-KCl. The transmembrane p.d. could thus be read directly, avoiding liquid junction potentials. In all these experiments the conductance due to CDA was at least 10 times that of the control conductance before addition of CDA.

RESULTS

Discovery and general properties of CDA

It was discovered by chance (Rudd, 1978) that derivatives of S. coelicolor A3(2) produce an antibiotic which inhibits Bacillus subtilis, but only if calcium is present in the test medium. This antibiotic was produced by strains unable to synthesize any of the three antibiotics previously identified from S. coelicolor A3(2) – methylenomycin (Kirby et al., 1975; Wright & Hopwood, 1976a), actinorhodin (Wright & Hopwood, 1976b; Rudd & Hopwood, 1979) or undecylprodigiosin (the 'red' antibiotic; Rudd & Hopwood, 1980; S-W. Lee, C-J. Chang & H. G. Floss, personal communication). The new material was called CDA (for calcium-dependent antibiotic). It was found to be a small molecule (able to cross dialysis membrane) and to be rather heat-stable: boiling for at least 10 min did not reduce the activity of impure solutions of CDA (Fig. 1), and even autoclaving at 121 °C for 15 min did not destroy it completely.

Producers of CDA and conditions for production

Several streptomyces closely related to S. coelicolor A3(2) were tested for the ability to produce CDA. Two such strains – S. violaceoruber K673 (Kutzner & Waksman, 1959 and S. coelicolor 199 (Bradley, 1960) – were found to be producers; three others – S. coelicolor F204 (Hopwood, 1959), S. coelicolor ISS (Sermonti & Spada-Sermonti, 1955) and S. lividans 66 (Lomovskaya et al., 1972) – were non-producers, as was S. parvulus ATCC 12434. The failure to detect CDA production by S. lividans 66 and S. coelicolor ISS was not due to its active extracellular destruction since mixed cultures of S. coelicolor A3(2) strain 2377 with either of these strains produced CDA at normal levels.
Several agar media were tested for their ability to support production of CDA by *S. coelicolor* A3(2) strain 2377. Production was most marked on TY agar or NA, less on R2 agar, less still on CM agar and undetectable on MM agar. Oxoid nutrient agar was chosen as the usual medium for production and (with calcium addition) for assay of CDA; it was preferable to Difco nutrient agar, which evidently contained a significant calcium concentration since a low level of CDA activity was observed on this medium in the absence of added calcium. Only very slight production was found in 250 ml shake flask cultures of strain 2377 in liquid NB medium, but significant activity was observed in TSB.

The possible effect of calcium on the production of CDA by strain 2377 was tested by preparing samples of CDA solution from NA plates with or without the addition of 8 mM Ca(NO$_3$)$_2$. Added calcium was not required for production; indeed, about four times the titre of CDA was produced in the absence of added Ca(NO$_3$)$_2$ than in the presence of 8 mM-Ca(NO$_3$)$_2$.

**Requirements of metal salts for CDA activity**

The results in Fig. 2 show the effect of Ca(NO$_3$)$_2$ concentration in NA on the activity of CDA against *B. mycoides*. Maximum activity was reached at a concentration of about 16 mM, with an undetectable effect at or below 0.4 mM. Other salts of calcium – CaSO$_4$ and CaCl$_2$ – had the same effect as Ca(NO$_3$)$_2$. 

---

**Fig. 1.** Zones of inhibition of *B. mycoides* by CDA. Left, in the presence of 8 mM-calcium nitrate; right, no added calcium. Upper discs, unboiled samples; lower discs, boiled samples.

**Fig. 2.** Antibiotic activities of CDA against *B. mycoides* as a function of calcium nitrate concentration.

**Fig. 3.** Dependence of bilayer conductance (G) on concentration (C) of CDA. Bilayers were formed in 100 mM-NaCl, 15 mM-CaCl$_2$ at 26.5 °C and conductance was measured 5 min after formation. Each point represents the mean of five membranes. The gradient of the best straight line fitted by the method of least squares is 3.7 ± 0.2.
Table 1. Resistance or sensitivity of various micro-organisms to CDA from Streptomyces coelicolor A3(2)

<table>
<thead>
<tr>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td>Bacillus mycoides</td>
<td>Rhizobium leguminosarum</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Streptomyces coelicolor 204 F*</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>Streptomyces coelicolor violaceoruber Kutzner 673†</td>
</tr>
<tr>
<td>Corynebacterium glutamicum</td>
<td>Streptomyces coelicolor ISS*</td>
</tr>
<tr>
<td>Streptomyces coelicolor Müller</td>
<td>Streptomyces coelicolor 199**</td>
</tr>
<tr>
<td>Streptomyces rimosus NRRL 2234</td>
<td>Streptomyces lividans 66*</td>
</tr>
<tr>
<td>Streptomyces parvulus ATCC 12434‡</td>
<td></td>
</tr>
<tr>
<td>Streptomyces acriomycini IPV 1610</td>
<td></td>
</tr>
<tr>
<td>Streptomyces albus G</td>
<td></td>
</tr>
<tr>
<td>Streptomyces glaucescens ETH 22794</td>
<td></td>
</tr>
<tr>
<td>Streptomyces griseus ATCC 10137</td>
<td></td>
</tr>
<tr>
<td>Streptomyces fradiae NRRL B3357</td>
<td></td>
</tr>
<tr>
<td>Streptomyces kanamyceticus NRRL B2535</td>
<td></td>
</tr>
<tr>
<td>Streptomyces vinaceus NCIB 8852</td>
<td></td>
</tr>
<tr>
<td>Streptomyces varsoviensis ISP 5346 (CUB 308)</td>
<td></td>
</tr>
</tbody>
</table>

* Streptomyces violaceoruber according to Kutzner & Waksman (1959).
† Producers of CDA.
‡ Slightly sensitive.

The effect of six other cations was tested by substituting Ca(NO₃)₂ by chlorides of sodium, potassium, magnesium, manganese, barium and strontium at 4 mM (and in some cases also higher) concentration. No antibacterial activity of CDA was seen in the presence of any of these six metals salts.

**Antimicrobial spectrum of CDA**

CDA was inactive against the three strains of Gram-negative bacteria tested and also against the yeast strain but was active against all the Gram-positive unicellular bacteria tested (Table 1). Amongst streptomycetes, five strains closely related to S. coelicolor A3(2) [S. lividans, S. violaceoruber and three strains named S. coelicolor but, like S. coelicolor A3(2), resembling S. violaceoruber according to Kutzner & Waksman (1959)] were resistant (two produced CDA, see above) but the other streptomycetes tested were sensitive (S. parvulus ATCC 12434, which may be fairly closely related to S. coelicolor A3(2), was only slightly sensitive).

**Chemical properties of CDA**

Simple tests showed that CDA is a highly polar compound. Samples (20 ml) of CDA solution (pH 9) were evaporated to dryness in a rotary evaporator and the dried material was washed with 2 ml volumes of water, methanol, ethyl acetate or chloroform. These solutions were filtered and again evaporated to dryness. Water-soluble material was re-dissolved in 2 ml volumes of water and the resulting solutions were assayed for CDA activity. The sample taken up originally in water showed essentially quantitative recovery of CDA compared with the starting solution, while no detectable activity passed through ethyl acetate or chloroform. Methanol extracted a very small amount of CDA.

In an attempt to find conditions for extraction of CDA into a water-immiscible solvent, samples of CDA solution were adjusted to pH 9, pH 7, pH 4 and pH 2 and shaken with equal volumes of butanol or chloroform. After separation, the aqueous phases were adjusted to pH 7 and the organic phases were shaken with equal volumes of water buffered to pH 7. On assay of all the aqueous samples for CDA activity it was found that no extraction occurred into chloroform at any pH value tested. Complete extraction into butanol occurred at pH 2, with re-extraction into water at pH 7; slight extraction into butanol occurred at pH 4 and none at pH 7 or pH 9.
**Fig. 4**. CDA-induced conductance as a function of calcium chloride concentration. △, Bilayers formed in 100 mM-NaCl; ▲, bilayers formed in 100 mM-NaCl, 3% (v/v) CDA. Each point is the average of five membranes measured 5 min after formation.

**Fig. 5**. Arrhenius plot. Log bilayer conductance (G) plotted as a function of 1/T. Bilayers were formed in 100 mM-NaCl, 10 mM-CaCl₂, 3% (v/v) CDA. Each point represents the mean of five membranes. Conductance was measured 5 min after formation. The least squares gradient is $-6900 \pm 600$ and intercept $17.7 \pm 2.0$.

### Effect of CDA on membrane conductivity

Certain previously described antibiotics, such as X537A and A23187 (Hyono et al., 1975; Reed & Lardy, 1972), act as ionophores capable of promoting the transport of calcium ions across membranes. We therefore tested CDA for its effect on the conductivity of artificial membranes. The results of these experiments showed that CDA does indeed promote conductance, but only in the presence of calcium; monovalent cations, rather than calcium itself, are transported.

The results in Fig. 3 show the dependence of membrane specific conductivity on concentration of CDA in 100 mM-NaCl, 15 mM-CaCl₂ at 26.5 °C. The means from sets of five membranes have been plotted using a log/log plot. The least squares fit of a straight line to this set of data gives a gradient of $3.7 \pm 0.2$. Hence the specific conductance is dependent on the third to fourth power of concentration of CDA. The most obvious explanation for the observed dependence of conductance on concentration is that the exponent represents the number of monomers interacting to form a conducting unit, as has been suggested for the polyene antibiotics (Finkelstein & Cass, 1968), gramicidin A (Veatch et al., 1975) and many other carrier-type antibiotics. Such an explanation, attractive as it might seem, needs to be regarded cautiously in the light of the fact that co-operative (Cass et al., 1970) and other effects (Lea & Croghan, 1969; McLaughlin, 1972) between conducting units or subunits may influence their interpretation. Until detailed studies have been made of random fluctuations of current under voltage clamp, these suggestions remain speculative.

As a control in the above experiments, a solution was prepared under identical conditions from a culture of a CDA non-producing mutant, cda-1 (strain 2355; Hopwood & Wright, 1983). Negligible conductance was induced in the membranes.

The conductivity induced in bilayers by a fixed antibiotic concentration increased with increasing calcium chloride concentration (Fig. 4). The curve saturated at a calcium concentration of approximately 16 mM. The total increase was slightly more than one order of magnitude, a change that cannot be accounted for by the increase in conductivity caused by increasing the total ionic concentration of the solution.

Conductivity of bilayers in 3% (v/v) CDA, 100 mM-NaCl, 10 mM-CaCl₂ at 24 °C was compared for bilayers containing zero and 50% (w/v) cholesterol. The mean conductance for egg lecithin–cholesterol bilayers under these conditions was $(4.57 \pm 2.43) \times 10^{-2}$ S m⁻² (mean ±
s.e. of the mean) for eight readings and for egg lecithin only bilayers the value was $(4.41 \pm 2.02) \times 10^{-2}$ S m$^{-2}$ (mean ± s.e. of the mean) for eight readings. Unlike polyene antibiotics (Finkelstein & Cass, 1968) it seems unlikely that CDA depends on cholesterol for its pore forming activity.

**Dependence of conductivity on temperature**

It was noted that, to obtain reliable values for conductivity, it was necessary to control the temperature in the membrane apparatus. In a separate experiment, membrane conductance, $G$, was measured as a function of temperature over the range 5–40 °C. A very slight increase in control conductance occurred but this was overshadowed by a steep increase in the conductance due to CDA. The probable relationship is that of an Arrhenius equation:

$$\log G = -E(G)/RT$$

where $E(G)$ is the activation energy of the conductance, and the results are displayed in the form of an Arrhenius plot (Fig. 5). The gradient of this plot gives a value for the activation energy of the conductivity mechanism of 57 kJ mol$^{-1}$.

The conductance of the bilayer can be expressed in the form

$$G = nA$$

where $n$ is the number of channels and $A$ is the conductivity of a single channel. If, in addition, multimeric channels ($M$) are formed from non-channel forming monomers ($A$) by a reaction of the form

$$mA \rightleftharpoons M$$

where $m$ is the order of the reaction, then

$$M = K_A A^m$$

where $K_A$ is the equilibrium constant of the polymerization reaction. If the number of open channels is proportional to the concentration of multimers, i.e.

$$n = kK_A A^m$$

where $k$ is another equilibrium constant, then

$$G = A(kK_A)A^m$$

taking logs,

$$\log (G) = \log (A) + \log (kK_A) + m \log (A)$$

differentiating with respect to $1/T$

$$E(G) = E(A) + \Delta H(kK_A)$$

where $E(A)$ is the activation energy for the conductivity through a single channel and $H(kK_A)$ the enthalpy of channel formation. These procedures may be compared with those of Bamberg & Lauger (1974). The activation energy of the conductivity through a single channel was assumed to be the same as that for the conductivity of Na$^+$ in aqueous solution (16.7 kJ mol$^{-1}$). This means that $\Delta H \approx 40$ kJ mol$^{-1}$, i.e. the enthalpy of channel formation is strongly endothermic, consistent perhaps with breaking of hydrogen bonds in aqueous solution and formation of much weaker bonds of a (much less stable) channel structure. In the presence of small amounts of CDA, the current passing through membranes held under a voltage of 50 mV exhibited discrete fluctuating behaviour characteristic of randomly opening and closing molecular channels (Fig. 6). In 100 mM-NaCl, 25 mM-CaCl$_2$, mean channel conductivity was $1.0 \times 10^{-10}$ S. Taking the equivalent conductances of Na$^+$ and Cl$^-$ at 25 °C to be 50.9 S cm$^{-1}$ and 75.5 S cm$^{-1}$, respectively, and a channel length of 5.0 nm (the thickness of the bilayer membrane), the predicted channel diameter is 0.6 nm.
**S. coelicolor A3(2) CDA channels**

Fig. 6. Single channel record for an egg lecithin–cholesterol (2:1) membrane containing a small amount of CDA. The membrane was voltage clamped at 50 mV and the bathing solution was 100 mM-NaCl, 25 mM-CaCl₂; temperature, 20 °C. The mean channel conductance was 0.1 nS.

Fig. 7. Zero-current membrane potentials \( V_2 - V_1 \) for NaCl as a function of \( \ln (C_o/C_i) \), the ratio of salt concentration outside the tube to that inside. Initially the membranes were formed in 25 mM-NaCl, 5 mM-CaCl₂. External NaCl concentration was increased by adding aliquots of 2.5 mM-NaCl. At each concentration four membranes were used, each value being plotted separately. The line is drawn according to equation (8) and corresponds to a \( P_X/P_C \) ratio of 3.0.

### Table 2. Selectivity of antibiotic channels

The third column shows the selectivity of antibiotic channels expressed as a ratio of cation to chloride permeability calculated from measurements of membrane p.d. at zero current arising from different concentrations of salt on opposite sides of the membrane. Abbreviations: TMA, tetramethyl ammonium; Ch, choline.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Relative mass of cation (Dal)</th>
<th>( P_X/P_C )</th>
<th>Cation crystal radius (nm)*</th>
<th>Conductivity at infinite dilution (25 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>6-9</td>
<td>3-2</td>
<td>0-60</td>
<td>38-6*</td>
</tr>
<tr>
<td>NaCl</td>
<td>23</td>
<td>3-0</td>
<td>0-95</td>
<td>50-1*</td>
</tr>
<tr>
<td>KCl</td>
<td>39</td>
<td>3-5</td>
<td>1-33</td>
<td>73-5*</td>
</tr>
<tr>
<td>CsCl</td>
<td>133</td>
<td>3-1</td>
<td>1-69</td>
<td>72-2*</td>
</tr>
<tr>
<td>TMACl</td>
<td>74-1</td>
<td>1-0</td>
<td>—</td>
<td>34-7</td>
</tr>
<tr>
<td>ChCl</td>
<td>104-1</td>
<td>1-0</td>
<td>—</td>
<td>30-7</td>
</tr>
</tbody>
</table>

* Data of Robinson & Stokes (1959).

### Ion selectivity measurements

For each of a number of salts the concentration on the inside of the membrane tube was fixed at 25 mM while the concentration outside was increased in a series of steps. After each change the membrane p.d. was recorded. The results are shown for NaCl in Fig. 7, and for the series of salts in Table 2. The transmembrane p.d. can be described by the Goldman equation (Goldman, 1943; Hodgkin & Katz, 1949; Sandblom & Eisenman, 1967):

\[
V_2 - V_1 = \frac{-RT}{F} \ln \frac{\alpha_X [X_2] + [Cl_1]}{\alpha_C [X_1] + [Cl_2]}
\]

where subscripts refer to opposite sides of the membrane and \( \alpha_\text{X} \) is the ratio of permeability coefficient \( P_X \) and \( P_C \) for \( \text{X}^+ \) and \( \text{Cl}^- \) respectively; \([X]\) and \([Cl]\) are activity values. For all the univalent cations studied except for tetramethyl ammonium and choline the preferential selectivity for cations over anions was of the order of three, suggesting a negatively charged channel (Table 2). Selectivities were measured by fitting a line of best fit calculated using the Goldman equation.
A number of antibiotics exhibit ion-selective membrane channel activity. Some are cation-selective, e.g., gramicidin A (Hladky & Haydon, 1972), monazomycin (Bamberg & Janko, 1976) and alamethicin (Eisenberg et al., 1963), while others are anion-selective and cholesterol-dependent, e.g., nystatin (Cass et al., 1970) and amphotericin B (Ermishkin et al., 1977).

Ionophore antibiotics which exhibit ion carrier activity include valinomycin and the actins which are monovalent cation-specific (Hladky et al., 1974) and X537A and A23187 which are calcium-specific (Hyono et al., 1975; Reed & Lardy, 1972).

So far as we are aware, CDA has properties different from those of previously described antibiotics which react with biological membranes because it forms channels which conduct monovalent cations and which depend on the presence of calcium for their activity.

It is reasonable to suppose that the antibacterial activity of CDA depends on trans-membrane leakage of ions in the presence of calcium, since in vivo inhibition of B. mycoides (Fig. 2) and in vitro ion transport (Fig. 4) showed a strongly similar dependence on calcium concentration. The finding that the conductance of channels in cholesterol-free membranes did not differ from that in cholesterol-containing membranes is compatible with the inhibitory effect of CDA on bacteria (which lack membrane sterols); it contrasts with the activity of the polyene antibiotic nystatin, which requires cholesterol for channel conductivity (Finkelstein & Holz, 1973).

REFERENCES


LOMOVSKAYA, N. A., MKRTUMIAN, N. M., GOSTIMSHAYA, N. L. & DANILENKO, V. N. (1972). Characterization of temperate actinophage φC31 isolated...
S. coelicolor A3(2) CDA channels

from Streptomyces coelicolor A3(2). Journal of Virology 9, 258–262.