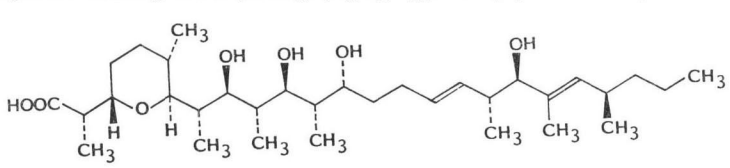
THE STRUCTURE AND ABSOLUTE STEREOCHEMISTRY OF ZINCOPHORIN (ANTIBIOTIC M144255): A MONOBASIC CARBOXYLIC ACID IONOPHORE HAVING A REMARKABLE SPECIFICITY FOR DIVALENT CATIONS[1]

Sir:

In the course of our search for natural ionophores with ruminant growth promoting properties, we have previously reported the structure of antibiotic M139603[2], the first tetronic acid-containing polyether. We now wish to describe antibiotic M144255, a monocarboxylic acid type ionophore from a strain of *Streptomyces griseus*. It has a high affinity for divalent cations especially zinc, for which reason it was given the trivial name, zincophorin. The molecular structure and absolute stereochemistry (Fig. 1) were determined by X-ray analysis on the mixed zinc-magnesium salt (see below)[1]. Although zincophorin has several unusual structural features which distinguish it from divalent cation ionophores, such as the diacidic ionomycin[3] or the A23187/X-14885A[4] group, it has the same gross structure as griseochelin[5]. Both antibiotics are produced by strains of *S. griseus* and the ^{1}H NMR ($CDCl_3$) of zincophorin (Fig. 2) is very similar to that of griseochelin. They are therefore stereoisomers or possibly even identical. The publication of griseochelin during the preparation of our full paper on zincophorin prompts the present communication.

A strain of *S. griseus* NCIB 11504 was shaken at 25°C for 120 hours in 2-liter Erlenmeyer shake flasks, each containing 1 liter of a medium consisting of glycerol 3.0%, Bacteriological peptone ("Oxoid" L37 — Oxo Ltd.) 2.0%, $CaCO_3$ 0.1%, KH_2PO_4 0.024%, $MgSO_4 \cdot 7H_2O$ 0.02%, minor elements concentrate 0.1%. This inoculum was used in a fermenter containing 30 liters of a medium consisting of glucose syrup 3.0%, glycerol 1.0%, soya protein ("BSP 70" —British Soya Products Ltd.) 1.5%, $CaCO_3$ 0.25%, NaCl 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, KH_2PO_4 0.02%, minor elements concentrate 0.1%. After stirring at 28°C for 45 hours, the whole broth was extracted with an equal volume of ethyl acetate at natural pH (*ca.* 7.0), dried (Na_2SO_4), and the solvent removed *in vacuo*. Using vanillin/EtOH-H_2SO_4 spray reagent, a biologically active com-

Fig. 1. Zincophorin (antibiotic M144255): absolute configuration.
Perspective drawing of Zn (or Mg) $(C_{33}H_{59}O_7)_2$. Only $\frac{1}{2}$ of the complex is shown.



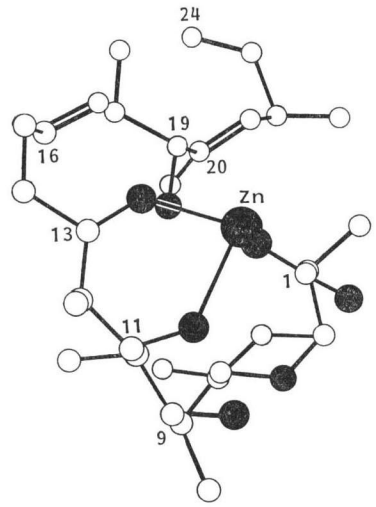
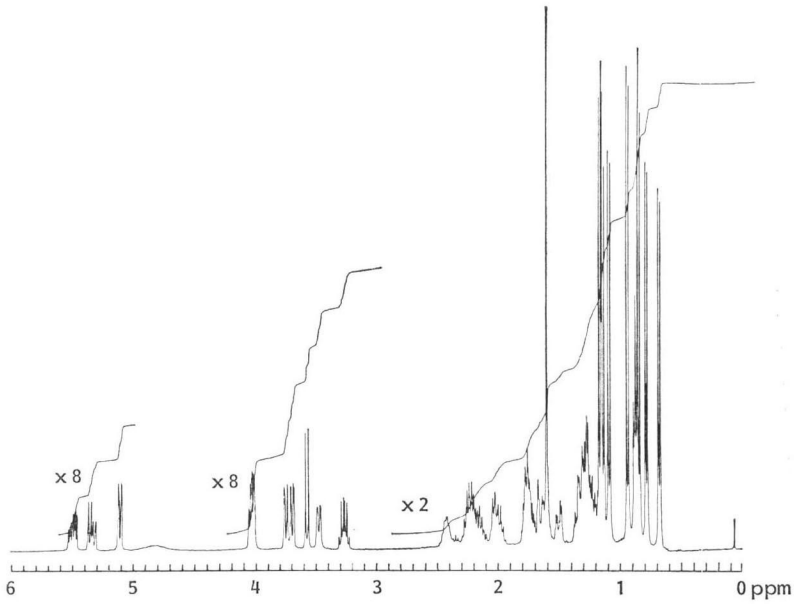


Fig. 2. ^{1}H NMR spectrum of zincophorin Zn-Mg salt at 400 MHz in $CDCl_3$.



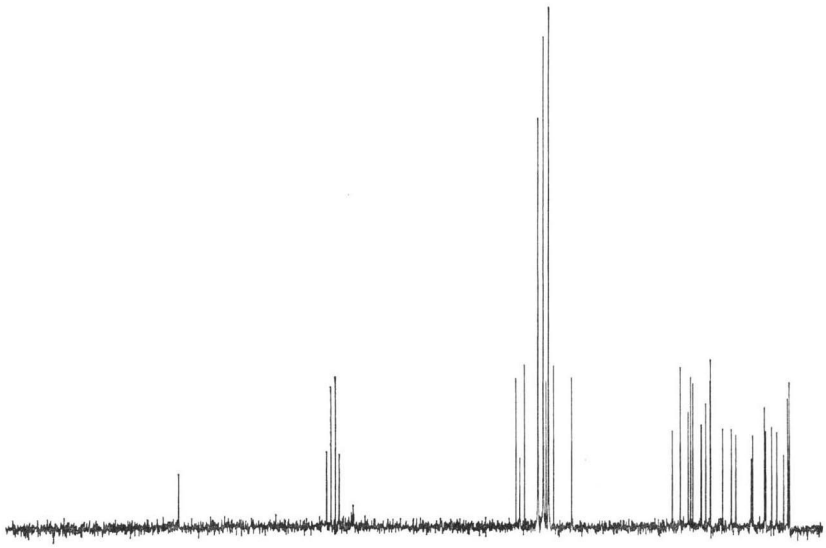
ponent was detected on TLC, giving a bright purple coloration, quite different from colors given by other polyethers. It could be separated from isopalmitic acid, tri-isopalmitin and other contaminants by silica gel chromatography, eluting with ethyl acetate - petroleum ether - acetic acid mixtures (10: 89: 1) ~ (20: 79: 1). The appropriate fractions were combined and concentrated. Further purification by preparative TLC (SiO_2, 2 mm) eluting with chloroform - acetone - acetic acid (95: 4: 1), gave the component of Rf *ca.* 0.15 as a gum, which was triturated with acetone. The resulting solid was recrystallized from ethyl acetate - acetone to give zincophorin as a (1: 1) zinc-magnesium salt, suitable for X-ray crystallographic studies (see below): mp 221 ~ 222°C, $[\alpha]_D^{24}$ +17.9° (*c* 2, $CHCl_3$), IR ν_{max} (Nujol) 3370, 3250, 2700 ~ 2600 and 1580 cm^{-1}, UV λ_{max} end absorption only. EI and CI mass spectra indicated a molecular ion for zincophorin of 550 *m/z* (like griseochelin[5]) (Calcd for $C_{33}H_{58}O_6$: 550.4233. Found: 550.4225), but the FD mass spectrum allowed this to be corrected to $C_{33}H_{60}O_7$. *Anal* Calcd for $(C_{33}H_{59}O_7)_4Zn$-Mg: C 67.2, H 10.0, Zn 2.8, Mg 1.0. Found: C 67.2, H 10.3, Zn 2.6, Mg 0.9.

Zincophorin free acid was prepared by treatment of a petroleum ether solution of the zinc-magnesium salt with an aqueous solution of EDTA. It was a crystalline solid, mp 66 ~ 70°C, IR ν_{max} (Nujol) 3400, 3240 and 1735 cm^{-1}, ^{13}C NMR spectrum (Fig. 3). Zincophorin and its salts are soluble in most organic solvents, but not in water. Solutions of zincophorin free acid could be dried over Na_2SO_4 and recovered as the free acid.

Reaction of zincophorin as the free acid with ethereal diazomethane gave a monomethyl ester as a waxy solid, mp *ca.* 100°C, $[\alpha]_D^{24}$ +20.9° (*c* 2, $CHCl_3$), $M^{+\cdot}$ $-H_2O$ at *m/z* 564. The major product of acetylation of the methyl ester (acetic anhydride, pyridine) was a hydroxytriacetate, indicating four hydroxyl groups, one of which is hindered in some way. Catalytic hydrogenation of zincophorin free acid (two isolated olefinic bonds) gave a dihydro derivative, mp 104 ~ 105°C, $[\alpha]_D^{23}$ +10.5° (*c* 2, $CHCl_3$) containing one tri-substituted double bond, again indicative of some steric hindrance. These chemical transformations account for six of the seven oxygen atoms and three of the four double bond equivalents, suggesting a single ether ring or oxirane (in the absence of carbonyl stretches other than carboxylate in the IR spectra).

These findings were confirmed by an X-ray crystallographic study on the zinc-magnesium salt (Fig. 1): Crystal data: $(C_{33}H_{59}O_7)_2Zn(Mg)$, M = 1180.5, monoclinic, a = 12.629 (4), b = 12.259 (4), c = 22.958 (6) Å, β = 90.4 (1)°, U = 3554.2 Å^3. Dc = 1.095 g cm^{-3}, Z = 2, F (000) = 1294. Space

Fig. 3. ^{13}C NMR spectrum of the free acid in $CDCl_3$.



group $P2_1$, Mo-K_α radiation (graphite monochromator) $\lambda=0.71069$ Å, $\mu=2.5$ cm^{-1}.

Reflections were measured to $\theta=25°$. A total of 6611 intensities were measured of which 4400 had a net count $\geq 2\sigma(I)$ and were used in the full matrix least squares refinement to give a final R of 11.95%. This relatively large value is due to the small number of strong reflections. Lorentz and polarization corrections were made but no absorption corrections were applied. It is noteworthy that the terminal methyl group of the backbone (C_{25}) had an abnormally large temperature factor and is not shown in Fig. 1.

The absolute configuration of zincophorin was deduced (as being that shown) by HAMILTON's statistical method[6]. The discriminating ratio was 1.003 and for a one-dimensional hypothesis and 4000 degrees of freedom a ratio of 1.001 is significant at the 0.005 level. The calculations were carried out using identical co-ordinates by changing the sign of the imaginary part of the anomalous dispersion for zinc. Friedel reflections were not measured.

Chelation to the cation is octahedrally through the carboxylate and the hydroxyls on C_{11} and C_{13} of two molecules of zincophorin. The pyran oxygen and the hydroxyls on C_9 and C_{19} do not co-ordinate to the metal ion. Complexation studies have established a stability order of zinc≈cadmium>magnesium>strontium≈barium≈calcium.

Zincophorin showed good *in vitro* activity against Gram-positive bacteria and against *Clostridium welchii* at ≤1 ppm, at which levels it also inhibited methane production and favourably altered volatile fatty acid ratios in *in vitro* rumen fermentations. It showed some anticoccidial activity against *Eimeria tenella* in chicks.

The LD_{50} in rats (iv) is between 0.5 and 5 mg/kg. It is not acutely toxic when given orally to mice at 350 mg/kg.

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