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NOTE

[4-^{3}H]-LABELLING OF THE CALCIUM IONOPHORE A 23187 (CALCIMYCIN)

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SUMMARY

[4-^{3}H-labelled]-A 23187 (or calcimycin) has been obtained as a magnesium salt from the corresponding 4-bromo derivative using tritiated hydrogen gas in the presence of 10% Pd/C catalyst. The specific radioactivity proved to be 50.6 Ci/mmole (1872.2 GBq/m mole).

Key Words: A 23187: calcimycin: tritiation: debromination: calcium carrier.

INTRODUCTION

A 23187 or calcimycin 1 (fig.1) is a carboxylic polyether ionophore isolated from a strain of *Streptomyces chartreusis* (NRRL 3882)[1]. Since its discovery, it has drawn a constant interest as its

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structure is remarkably suited to the transport of alkaline-earth cations through membranes, with a specificity highly in favour of calcium[2]. The commonly accepted antiport mechanism by which a divalent cation is exchanged versus two protons by this mobile carrier is represented in figure 2.

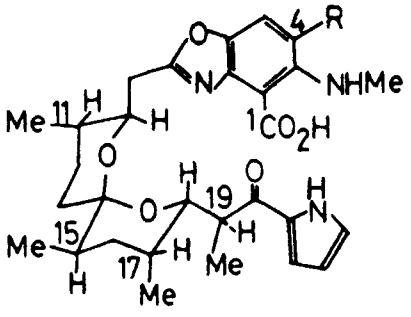

Fig. 1

A 23187 or calcimycin 1 (R = H); 4-bromo-derivative 2 (R = Br)

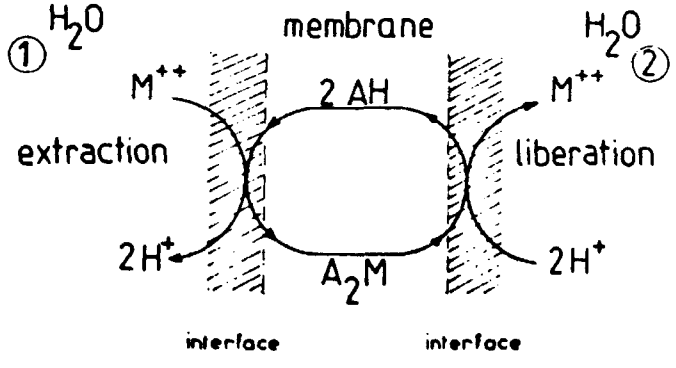

Fig. 2

Schematic representation of the transport mechanism M^{++} <-> $2H^{+}$ postulated for calcimycin. AH stands for the protonated form of the ionophore

Owing to the ubiquitous role of calcium in biological events, this ionophore has been universally used as a tool to modify the free intracellular concentration of this cation second messenger. Thousands of works have been published using this approach. However, its utilisation raises several questions ; among these the localization of the ionophore should be questioned when multi-

compartment biological systems are studied. We thought a radioactive labelled A 23187 could help to examin this question, for this purpose we set out an efficient tritium labelling.

Tritium-labelled calcimycin was prepared by debromination of the 4-bromo derivative 2 (fig.1). During our investigations on the synthesis of calcimycin analogs[3] we observed for the preparation of the aromatic precursors that when an halogen atom was present together with a nitro substituent on the aromatic ring, the halogen was lost during the hydrogenation step of the $-NO_2$ (unpublished), so it appeared interesting to study the derivative 2. The regioselective halogenation of calcimycin has been nicely carried out by DEBONO et al.[4] using the 2:1 magnesium complex of this ionophore (fig.3). We used their method to prepare 2. Similarly, the Br/^{3}H replacement was performed on the magnesium complex of 2 which was more stable than the free acid.

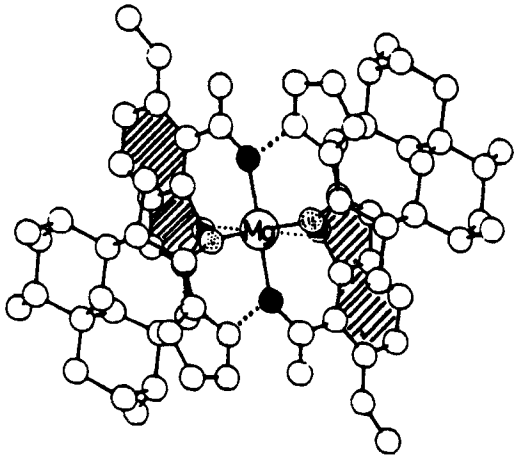

Fig. 3

Representation of the (calcimycin)$_2$Mg complex, following ref.7.

EXPERIMENTAL

I- Preliminary experiments with unlabelled materials

The following abbreviations are used: EtOAc, ethyl acetate ; RT, room temperature ; TLC, thin layer chromatography, Tnmr, Tritium nuclear magnetic resonance ; MeOH, methanol.

Bromo-calcimycin 2 was prepared from the bacterial metabolite 1 (stock sample of our laboratory[5]), following the procedure of DEBONO above-mentioned[4] and characterized by its NMR and mass spectra.

Compound 2 (50.0 mg magnesium salt; 41 µmoles) was dissolved in ethyl acetate (15 ml) and reduced in a Parr apparatus (H_2 pressure 20 psi) on 10% Pd/C (10 mg). The catalyst was removed by filtration and the solvent evaporated. Purification of the solid obtained was done by thin layer chromatography (silicagel plates F 254 Schleicher and Schuell, eluent: cyclohexane-ethyl acetate 5:5). The crystalline compound obtained proved to be the magnesium salt of calcimycin by comparison with the TLC spot of an authentic sample and from its ^{1}H-NMR spectrum (Bruker MSL 300) which showed the aromatic resonance signals described by ANTEUNIS[6], especially 4-H at 6.68 ppm (doublet, J=9.1 Hz) which was missing in the spectrum of 2.

II - Tritium labelling

II - 1 Materials and methods.

Pure tritium gas was supplied by the Commissariat à l'Energie Atomique (France). The catalyst Pd/C (10%) was supplied by Fluka (Switzerland). The automatic gas transfer unit used for catalytic tritiation has been previously described[8]. The catalyst was separated from the reaction solution by filtration through a Millipore filter (Millex F.G., 0.2 µ, USA). The chemical purity was checked by thin layer chromatography on silicagel plates (F 254 Schleicher and Schuell in the following eluent system (v/v): ethyl acetate-cyclohexan (6:4). Absorption spectra were obtained with a DU-70 (Beckman, USA). Radioscans of TLC were performed with a Berthold Scanner II (W. Germany). Autoradiography of TLC was achieved on X-O mat XAR5 (Kodak, USA). Radioactive countings (^{3}H) were determined in a liquid scintillation counter Beta V (Kontron, France).

NMR spectra of tritiated calcimycin were recorded on a Bruker AC 300 spectrometer operating at 320.134 MHz (W. Germany). All other chemicals and solvents were of analytical grade from Prolabo (France), BDH (U.K.), Merck (W. Germany) or Fluka (Switzerland).

II - 2 Tritiation.

4-Bromo-calcimycin magnesium salt (5 µmoles, 6.11mg) was dissolved in 1ml of EtOAc and then frozen by liquid nitrogen. 11.1mg of the catalyst (Pd/C 10%) was dispersed on the frozen solution surface and the reaction vial was connected to the automatic tritium gas transfer unit.After the vial and all tubing had been evacuated (10^{-3} - 10^{-4} Torr), 30 to 40 Curies (1147 - 1480 GBq) of pure tritium gas (>99.9%) were introduced and compressed until 1.35 Bar (pressure read at low temperature - 196°C). After thawing, the reaction mixture was vigorously stirred at RT for 4.13 hours. The adsorption of tritium gas (measured at low temperature) was not detectable. The catalyst was removed by filtration through a Millipore filter (FG) and washed with 2x5 ml of pure MeOH. The labile tritium atoms were removed by successive flash rotative evaporations with 40ml of MeOH (2x20ml). Total radioactivity recovered was 101 mCuries (3737 MBq).

II - 3 Results and discussion.

The first attempt to purify the crude labelled material was performed using TLC on silicagel (EtOAc-cyclohexane 6:4, v/v, Rf: 0.78). The main peak (detected by U.V. and ^{3}H-scannings) co-migrating with the authentic calcimycin was collected by ethanol elution (6x2 ml). Tnmr spectrum revealed a complete halogen-tritium replacement (position 4 of aromatic ring ; doublet at 6.68 ppm, J = 9.7 Hz). The specific radioactivity was determined by UV spectrophotometric titration from UV spectra performed with the unlabelled compound. Quantitative and comparative estimations indicated that the specific radioactivity was found to be 50.6 Ci/m

mole (1872.2 GBq:mmole, dimeric form). After two months of storage in liquid nitrogen, [4-^{3}H]-calcimycin retained both its chemical and biological properties.

ACKNOWLEDGEMENTS

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