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SYNTHESIS OF A BIOMIMETIC MODEL OF CALCIMYCIN (A 23187) WITH A DEMETHYLATED SKELETON

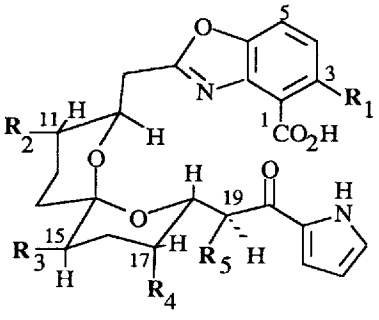
Jean-Gabriel Gourcy, Michelle Prudhomme, Gérard Dauphin and Georges Jeminet*

Université Blaise Pascal
Laboratoire de Chimie Organique Biologique, associé au CNRS
24, Avenue des Landais
63177 Aubière Cedex, France

Summary : A demethylated model (**15**) of Calcimycin (**1**) has been synthesized from the known 2,8-dibromo-1,7 dioxaspiro (5.5) undecane (**6**) ; biphasic extraction experiments indicated a net decrease of affinity towards Mg++ and Ca++ for (**15**) versus (**1**).

A 23187 or Calcimycin (**1**) is a carboxylic polyether antibiotic which has drawn constant attention since its discovery sixteen years ago[1], as it is an efficient carrier of calcium through membranes, universally used for biological investigations[2] and also as a stimulating synthetic target for chemists[3]. Interestingly, closely related analogues (**2-5**) were recently isolated from new strains of *Streptomyces*. The differences in the structures lie only in the nature of the R_1-R_5 groups : Scheme 1.

Scheme 1

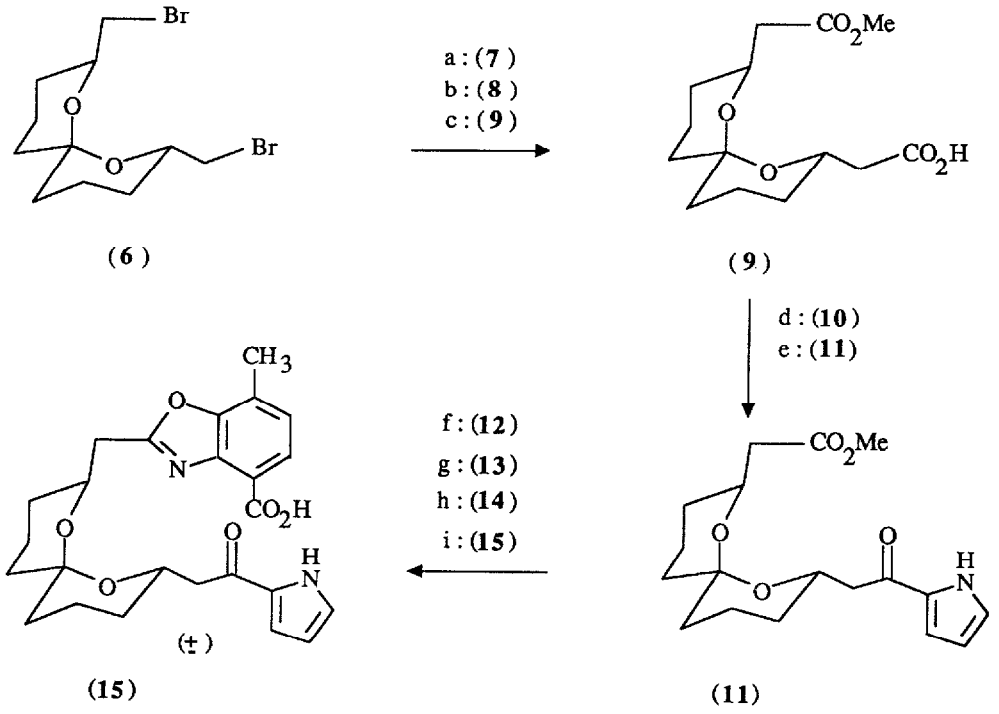


(**1**) R_1 = NHMe, R_2 = R_3 = R_4 = R_5 = Me (Calcimycin, A 23187)[1]
(**2**) R_1 = H, -id- (Cezomycin)[4]
(**3**) R_1 = OH, -id- (AC 7230)[5]
(**4**) R_1 = OH, R_2 = R_4 = R_5 = Me, R_3 = H (X 14885A)[6]
(**5**) R_1 = OH, R_2 = R_3 = H, R_4 = R_5 = Me (CP 61405)[7]

In a previous work we have designed the partial synthesis of calcimycin analogues bearing modified benzoxazole ring substituents[8] ; their ionophoric and biological properties showed the prominent role of the 2-carboxy-benzoxazole sequence in the formation and stability of complexes with calcium and magnesium[9]. Furthermore, from a study of several of these analogues by ^{1}H NMR, we observed that the conformation of the methylene-benzoxazole moiety differed, for the acid form, going from $CDCl_3$ to CD_3OD and was greatly affected by the cation complexation process. Conversely, the H_{18}-H_{19} protons remained antiperiplanar under all conditions, suggesting a relatively rigid C_{18}-C_{19}-C_{20} framework due especially to the interaction of the C_{17} and C_{19} methyl substituents[10].

The difference in the conformational behaviour of the two arms bearing the coordination sites, as shown by cristallographic studies[11], could be of importance for the transporting ability of calcimycin. To examine this point, we undertook the synthesis of a model incorporating an identical overall stereochemistry in its framework, but with lacking methyl groups in positions 11, 15, 17 and 19[10]. While this work was in progress, Nakahara et al.[12] achieved the enantiospecific synthesis of tetranormethyl Calcimycin (Scheme 1 ; $R_1 = NHCH_3$, $R_2 = R_3 = R_4 = R_5 = H$). This prompted us to report our different synthetic route. Preliminary results from extraction experiments with calcium and magnesium are also given. Our approach is outlined in Scheme 2.

Scheme 2



for (6) see ref. 13. a: NaCN (3eq.), HMPA, 25°C, 16h, 88 % yield; b: KOH 30 %, H_2O_2 (110vol.), 40°C, 2h, then reflux, 3h, 70 % yield ; c: CH_2N_2, Ether,25°C, 75 % yield; d: 2,2'-dipyridyl-disulfide, PPh_3, CH_2Cl_2, 25°C, 3h, 75 % yield ; e: pyrrolylmagnesium bromide, Ether-THF (1:1),CuI.THF, 0°C under argon 0.5h then (10) in dry THF 1.5h, 0°C, 80 % yield ; f: KOH, EtOH, 3h, 25°C, 95 % yield ; g: methyl 4-methyl-3-hydroxy anthranilate hydrochloride, DMF, Et_3N, BOP, 50°C under argon[8], 4h, 65 % yield ; h: then EPP, $CHCl_3$, under argon, 60°C, 1h[8], 21 % yield ; i: KOH, EtOH, 30°C, 1h, 98 % yield.

As a convenient precursor, we opted for the 2,8-dibromomethyl-1,7 dioxaspiro (5.5) undecane (6), easily prepared following the pioneering work of Cresp et al.[13]. The dinitrile (7) preparation, also quoted by the above-mentioned authors, was improved by using hexamethylphosphoramide as solvent. Its hydrolysis by aqueous KOH in the presence of H_2O_2 (30 %) gave the cristalline diacid (8). Attempts to directly convert (8) to the keto-pyrrole derivative failed. However, the monoesterification was achieved by careful addition of diazomethane, giving (9). The 2-pyridyl-thioester (10) was prepared and reacted with pyrrolylmagnesium bromide, in the presence of cuprous iodide, to give the methyl ester (11) which was finally hydrolysed giving (12). At this stage, for this first investigation, we decided to couple (12) with the easily available 4-methyl-3-hydroxy

anthranilic methyl ester[8] to obtain (**13**). The benzoxazole structure (**14**) was obtained by application of the method we have described[8]. In this Scheme, the benzoxazole ring closure of (**13**) with ethyl-polyphosphate (EPP) was the only reaction with poor yield. This was due to the formation of three isomers at the spiroacetal level in respective percentages 7, 30, 63 and also of byproducts which were discarded. The cyclisation products were analysed by NMR spectroscopy, using ^1H-^1H and ^1H-^{13}C 2D-correlations, and compared with substituted spiroacetals obtained in another work[14]. The major product proved to have the required overall stereochemistry. The final hydrolysis of this ester by KOH in ethanol yielded the model calcimycin (**15**)[15].

Complexing properties for (**15**) were compared with Calcimycin (**1**) and its previously prepared[8] analogue (**16**, Scheme 1, R_1 = H, CH_3 in 5-position, $R_2 = R_3 = R_4 = R_5 = CH_3$), by ionic exchanges in a two-phases extraction system : water (aq.) / toluene-butanol 70:30 v/v (org.), according to the technique used by Pfeiffer et al.[16] and by us[8]. The equilibrium for the formation of a 2:1 neutral complex can be written :

$$2AH_{org.} + M^{++}_{aq.} \underset{}{\overset{K_{ex.}}{\rightleftharpoons}} A_2M_{org.} + 2H^+_{aq.}$$

(AH stands for the protonated form of the ionophore)

$K_{ex.} = [A_2M]_{org.}[H^+]^2_{aq.} / [AH]^2_{org.}[M^{++}]_{aq.}$, measured as previously mentioned[8], were the following :

compounds	Ca^{++}	Mg^{++}
(**15**)	$7.9\ 10^{-11}$	$7.9\ 10^{-10}$
(**1**)	$3.7\ 10^{-7}$	$1.3\ 10^{-7}$
(**16**)	$1.3\ 10^{-7}$	$1.3\ 10^{-7}$

These data indicated a net decrease of affinity towards the two cations for (**15**), especially on comparison with (**16**) which differs only by the methyl groups R_2-R_5 and also possesses the absolute Calcimycin configuration. *In vitro* antimicrobial tests carried out jointly confirmed the loss of activity of the model compound (unpublished). All the results stress the important role of methyl groups -17 and -19, since a recent study of the microbial metabolite (**5**) had shown that this compound, with the methyl groups -17 and -19 remaining, exhibited a marked biological activity[7]. This strongly supports the necessity of a preferential conformation of the keto-pyrolle arm to form stable 2:1 complexes with divalent cations.

Additional investigations are necessary to examine in particular : a) a -19 methylated model ; b) if the change of the skeleton lipophilicity has to be considered, since this parameter is reduced in (**15**) and c) as the complex involves two molecules of ionophore then the fact that (**15**) is racemic could be important. Work is in progress to examine these questions.

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15 The i.r., mass spectra, ^{1}H and ^{13}C n.m.r. spectra were consistent with the assigned structures for the intermediary compounds.

Selected n.m.r. data for (**15**).

^{1}H n.m.r. . (300.13 MHz, $CDCl_3$) ; δ p.p.m. ; 10.0 to 9.8 (broad, 2H, NH pyrrole and COOH) ; 8.0 and 7.25 (syst. AB, 2H, H_3 and H_4) ; 7.05 (m, 1H, H_{24}) ; 6.90 (m, 1H, H_{22}) ; 6.25 (m, 1H, H_{23}) ; 4.44 (m, 1H, H_{10}) ; 4.18 (m, 1H, H_{18}) ; 3.12 (m, 2H, H_{9A}, H_{9B}) ; 3.07 (m, 1H, H_{19A}) ; 2.62 (m, 1H, H_{19B}) ; 2.61 (s, 3H, Me) ; 1.2-1.8 (m, 12H, 6 CH_2 spiroacetal).

^{13}C n.m.r. . (75.47 MHz, $CDCl_3$); δ p.p.m.; 15.53 (Me_5); 18.50 (C_{16}); 18.76 (C_{12}); 30.93 (C_{17}); 31.03 (C_{11}); 34.93 (C_{15}); 35.07 (C_{13}); 35.85 (C_9); 44.43 (C_{19}); 66.9 (C_{18}); 67.10 (C_{10}); 96.66 (C_{14}); 110.47 (C_{23}); 116.67 (C_{22}); 117.72 (C_2); 124.78 (C_{24}); 126.23 and 127.04 (C_3,C_4); 132.82 (C_{21}); 140.03 (C_7); 149.55 (C_6); 165.18 (C_8); 167.03 (C_1); 188.36 (C_{20}).

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