

Communications to the Editor

New Synthetic Calcium Selective Ionophores. Design, Synthesis, and Transport Properties

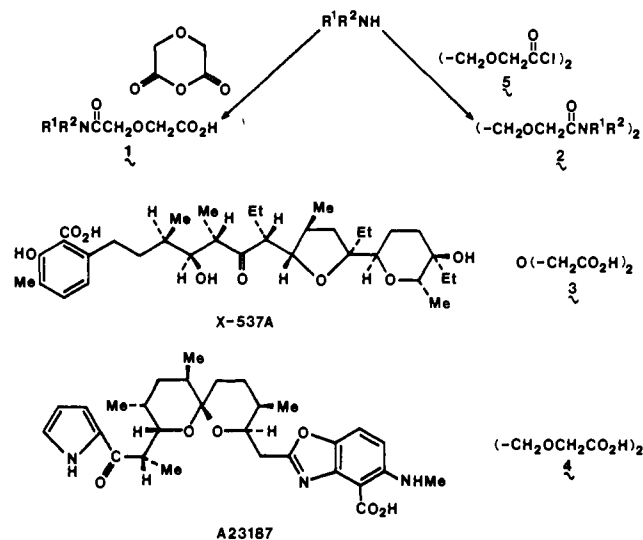
Sir:

The importance of intracellular calcium as a fundamental physiological regulator is now well recognized, and the detailed mechanisms by which intracellular calcium is controlled are the object of intensive biochemical research.¹ Within the past 5 years, the antibiotic ionophores X-537A and A23187 have emerged as valuable tools in helping to elucidate the mechanisms of Ca²⁺-dependent physiological events and have themselves shown therapeutic potential as cardiotoxic agents.²

With the purpose of developing totally synthetic, calcium selective ionophores as both biochemical tools and potential therapeutic agents, we have prepared, by conventional organic chemistry procedures (Scheme I), a group of diglycolamic acids **1** and diamides **2** which are the subject of this report. The design of these new molecules was based on consideration of known calcium chelators and the desirable characteristics³⁻⁵ (e.g., ion selectivity, favorable calcium-ionophore stability constants, and Ca²⁺ on/off kinetics, etc.) of an ideal calcium ionophore.

Both diglycolic acid **3** and diacid **4** are known to be good, selective (as compared to the biologically important Mg²⁺) calcium chelators.⁶ In addition, a group of related diamides **2** prepared by Simon and co-workers^{7,8} were found to function as Ca²⁺ selective sensors in ion selective electrodes. We decided to attach selected lipophilic groups to these calcium chelating skeletons, employing reactions⁹ shown in Scheme I. The resulting amides collected in Table I were tested for cation transport in the Pressman cell.¹⁰⁻¹² The transport properties of the classical, structurally complex antibiotic ionophores X-537A and

Scheme I



A23187 were measured for comparative purposes. Of the compounds shown in Table I, **1b,e** and **2b-d** exhibited significant Ca²⁺ transport as illustrated in Figure 1. Diglycolamic acid **1e** was clearly the best new calcium ionophore functioning equally well with or without picrate, suggestive of a 1:2 complex (Ca²⁺, **1e**) as the transported species. Diglycolamic acid **1b** was less efficient than **1e** and functioned to transport Ca²⁺ only with picrate added, suggestive that here a 1:1 complex was the transported species. Of the diamides **2** examined, **2b** produced the most demonstrable effect on Ca²⁺ transport, and it, like **2c** and **2d**, required the lipophilic picrate anion. None of the new molecules studied were capable of transporting Na⁺. Some Mg²⁺ transport was observed for **1b,e** and **2b**;

Table I. Diglycolamic Acids **1** and Diamides **2**

No.	R¹R²N	Mp, ^a °C	Yield, ^b %	Formula ^c	Crystn solvent
1a	1-Adamantylamino	181-183	31	C ₁₄ H ₂₁ NO ₄	2-Propanol-H ₂ O
1b	C ₆ H ₁₁ CH ₂ CH(CH ₃)N(CH ₃)	90-91	68	C ₁₄ H ₂₅ NO ₄	EtOAc-hexane
1c	C ₆ H ₅ C ₂ H ₄ NH	105-106.5	59	C ₁₂ H ₁₅ NO ₄	EtOAc
1d	C ₆ H ₅ C ₂ H ₄ N(CH ₃)	71-73	56	C ₁₃ H ₁₇ NO ₄	H ₂ O
1e	4-Diphenylmethyl-1-piperidinyl	139.5-141	55	C ₂₂ H ₂₅ NO ₄	EtOAc-hexane
2a	1-Adamantylamino	109-110.5	55	C ₂₆ H ₄₀ N ₂ O ₄	EtOH-H ₂ O
2b	<i>o</i> -OCH ₃ -C ₆ H ₄ CH ₂ CH(CH ₃)N(CH ₃)	<i>d</i>	87 ^d	C ₂₈ H ₄₀ N ₂ O ₆	<i>d</i>
2c	C ₆ H ₁₁ CH ₂ CH(CH ₃)N(CH ₃)	<i>d</i>	84 ^d	C ₂₆ H ₄₈ N ₂ O ₄	<i>d</i>
2d	5 <i>H</i> -Dibenz[<i>b,f</i>]azepin-5-yl	125-126.5	25	C ₃₄ H ₃₂ N ₂ O ₄ ^e	EtOH-H ₂ O
2e	2-(3-Indolyl)ethylamino	125.5-126.5	33	C ₂₆ H ₃₀ N ₂ O ₄	Me ₂ CO-hexane
2f	C ₆ H ₅ C ₂ H ₄ NH	82-83	46	C ₂₂ H ₂₈ N ₂ O ₄	EtOAc-hexane

^a Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are corrected. ^b Yields refer to purified compounds. ^c All compounds were analyzed for C, H, and N. Analytical results for these elements were within ±0.4% of calculated values. IR, ¹H NMR, and UV spectral data were consistent with assigned structures. ^d A diastereomeric mixture was obtained and employed without separation. ^e Water of crystallization (0.25 mol).

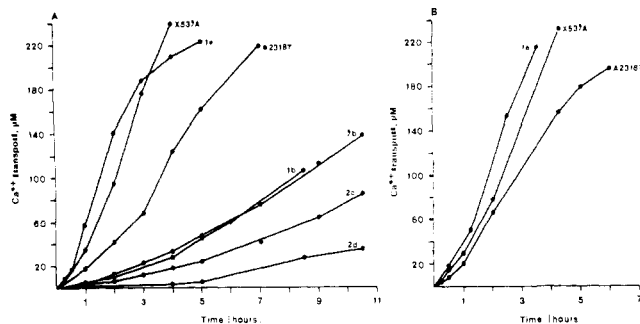


Figure 1. Ionophore-induced Ca^{2+} transport across a chloroform layer (A) with picric acid and (B) without picric acid. U-tube vessels similar to those described previously¹² were used at room temperature. A chloroform solution (30 mL), 200 μM in ionophore, continuously stirred, separated two 5-mL water phases, 10 mM in Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.0. One water compartment was initially 200 mM in CaCl_2 and, in Figure 1A only, also 500 mM in picric acid. A 500- μL sample was withdrawn from the initially Ca^{2+} free compartment at each of the times indicated in the figure, diluted with 500 μL of distilled water, and assayed for Ca^{2+} concentration by atomic absorption. The results of these assays are reported in the figure as Ca^{2+} transport. No detectable Ca^{2+} transport was seen in the absence of ionophore.

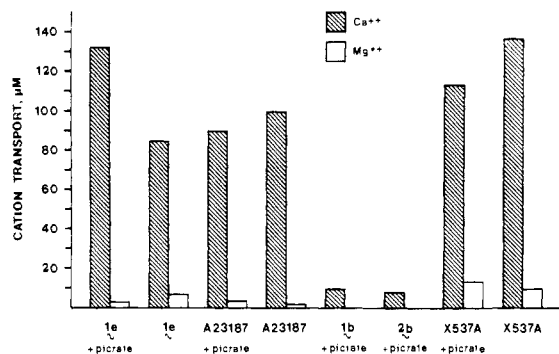


Figure 2. Ca^{2+} selectivity of ionophores. Data were obtained as described in Figure 1, modified by removing an aliquot from the initially metal cation free compartment at 3 h only. Mg^{2+} transport induced by **1b** and **2b** was insignificant at 3 h but was observable at longer times.

however, the selectivity of these new ionophores for Ca^{2+} was extremely high (Figure 2).

Diglycolamic acids **1b** and **1e** are the first totally synthetic calcium selective carboxylic acid ionophores.¹³ Diamides **2b** and **2c** are the first neutral calcium ionophores to demonstrate activity in the Pressman cell.¹⁴ Studies are in progress to fully characterize the pharmacology of these new ionophores and to demonstrate their utility as biochemical tools. The structural simplicity of these new calcium ionophores allows for ready molecular modification.

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References and Notes

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- (9) Acid chloride **5** was prepared as described by B. Dietrich, J. M. Lehn, J. P. Sauvage, and J. Blanzat, *Tetrahedron*, **29**, 1629 (1973).
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- (13) 5-(Tetradecyloxy)-2-furancarboxylic acid has been reported [G. Ribereau-Gayon, P. Vitali, and J. Wagner, Abstracts, FEBS (Federation of European Biochemical Societies) Symposium on "Biochemistry of Membrane Transport", Zurich, Switzerland, July 1976, No. P418] to transport Ca^{2+} from an aqueous phase to a bulk organic phase; however, we found no evidence for Ca^{2+} transport when this compound was employed in our Pressman cell experiments.
- (14) A related group of neutral diamides has recently been shown to exhibit calcium ionophore properties when studied in bilayer membranes. See (a) G. Amblard and C. Gavach, *Biochim. Biophys. Acta*, **448**, 284 (1976); (b) P. Vuilleumier, P. Gazzotti, E. Carafoli, and W. Simon, *ibid.*, **467**, 12 (1977); (c) T. Wun and R. Bittman, *Biochemistry*, **16**, 2080 (1977).

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