Synergistic Transport of Pr^{3+} across Lipid Bilayers in the Presence of Two Chemically Distinct Ionophores

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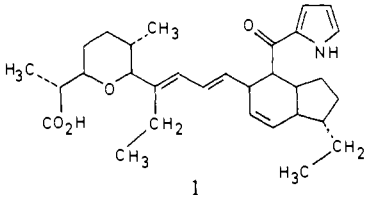
Abstract: A pronounced positive cooperativity, amounting to a rate enhancement by a factor of 3-12, is found for Pr^{3+} transport across egg yolk phospholipidic vesicles in the presence of two chemically distinct ionophores. These effects are not due to a greater thermodynamic stability of the hybrid (Pr^{3+}-AB) complex as compared to the Pr^{3+}-A_2 or Pr^{3+}-B_2 complexes. Facilitated proton (or sodium ion) efflux in the presence of the mixed (Pr^{3+}-AB) complex could explain the observed synergism.

We have already reported[1] our finding of a remarkable positive cooperativity (which we shall denote here by synergism, for short) in the transport of Pr^{3+} ions across phosphatidylcholine vesicles[2-4] when etheromycin and lasalocid (X-537A), or monensin and lasalocid, are both present in the lipid bilayer. In the present paper, we generalize these observations to a number of other ionophore antibiotics. These are molecules not only of prime importance for transport phenomena but with important pharmaceutical applications, in the realm of animal diseases for instance.[5] These molecules transport Pr^{3+} ions according to first-order (1:1 complexes) or to second-order (2:1 complexes) kinetics. We find that *the velocity of Pr^{3+} transport is always enhanced markedly, relative to that with a single ionophore, if a 2:1 hybrid complex can be formed.* Such an observation is reminiscent of the synergistic liquid-liquid extraction of cations by synthetic[6-8] or by natural[9] ionic complexants. Accordingly, we report also here our determinations of the synergism between two distinct ionophores in translocating Pr^{3+} from an aqueous to a chloroformic solution, together with evidence upon formation (or lack of formation) of hybrid complexes[10,11] between Pr^{3+} and the pairs of ionophores that display synergistic transport. We use model membranes, which are lipid bilayers in the shape of globular vesicles and which approximate the behavior of real cell membranes much more closely than the liquid chloroform (or methylene chloride)-water "membranes" often found in studies of carrier-mediated transport.

Materials and Methods

(A) Preparation of Vesicles. Four milliliters of a chloroformic solution of 400 mg of egg yolk phosphatidylcholine (Sigma) are evaporated under nitrogen flow. After vacuum evaporation removal of solvent residues, an aqueous solution (12 mL of H_2O plus 3 mL of D_2O) of 40 mg of 2-(*N*-morpholino)ethanesulfonic acid (MES) is admixed with the lipidic phase. To prevent small pH variations during the transport process, MES is an adequate buffer at the pH (~6.1) of these studies. This mixture is then sonicated for 50 min according to the Barenholz et al. procedure.[12] After pH adjustment to 6.1, using aqueous NaOH, further sonication is performed for 5 min to remove any proton gradient. Finally, ultracentrifugation at 45 K for 4 h is performed[12] (ultracentrifuge Beckmann L2-65B). The phosphate concentration of these solutions was typically 25-30 mM, corresponding to ca. 75% yield. Suspensions of vesicles were stored in the refrigerator under nitrogen and were used within a few hours of preparations.

(B) Solutions of the Ionophores. Samples were obtained as follows: X-537A (**8**) (Aldrich); etheromycin[13] (**7**) (Pfizer); A23187 (**6**) (Calbiochem); X-14766a,[14] X-14547A[15] (**1**), X-206,[16] and X-14540A[17] (Hoffmann-La Roche); M139603[18] (**2**) (ICI); tetronomycin[19] (**5**) (Sandoz); nigericin, (**4**), monensin and A204[20] (Eli Lilly); emericid and narasin[21] (**3**) Rhône-Poulenc). All these



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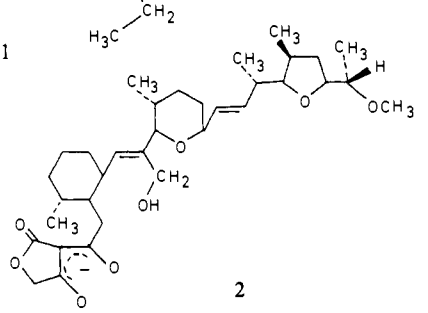

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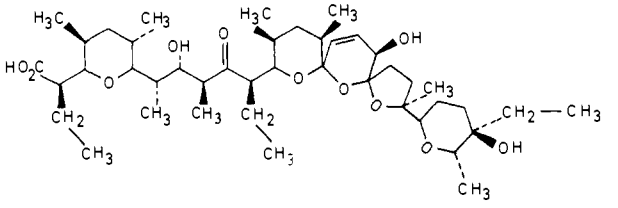

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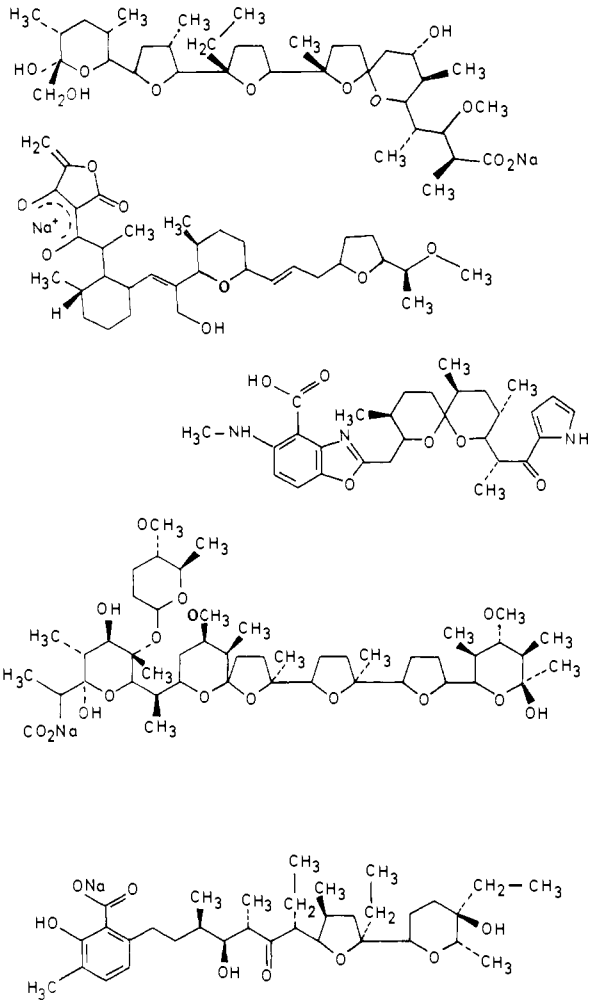

antibiotics were dissolved (20 mM) in methanol—except for A23187 (4.7 mM, as determined spectrophotometrically: $\epsilon = 9500$ at 378 nm and 20800 at 278 nm in methanol), M139603, and tetronomycin (3 mM)—prior to their incorporation in the phospholipidic vesicles. This was accomplished by incubation during 15 min, at the temperature of the experiment, of a known quantity of these methanolic solutions together with the suspension of vesicles. Under these conditions, the ionophores are quantitatively incorporated in the vesicles. To check it, we have incubated, as indicated above, identical solutions of the ionophores A23187 and X-537A as used in the transport experiments. After ultrafiltration of the vesicles, the UV spectrum of the filtrate failed to show any of the above absorptions characteristic of these antibiotics.

(C) Transport Rate Measurements. We use here, as previously,[1,3,4] a technique pioneered by the group of Bystrov:[2] The praseodymium cation Pr^{3+} serves both as the transported cation and as a paramagnetic marker. Due to a pseudocontact interaction, the ^{31}P resonances for the polar head groups of the phospholipidic molecules at the interface with the Pr^{3+}-containing aqueous solution shift downfield. In this manner, the two ^{31}P resonances, corresponding to the inner and the outer compartments of the vesicles, are shifted apart if only one of these compartments has been doped with Pr^{3+}. This method has also been used by other groups.[22,25]

We first check that no leakage occurs through the vesicles in the absence of an ionophore: the two ^{31}P absorptions remain stable for periods of at least 24 h. When the vesicular membranes contain ionophore molecules, transport of the Pr^{3+} ions is monitored conveniently from the change in relative position of the two ^{31}P resonances with time.

In the experiments reported here, Pr^{3+} ions were present initially in the outer compartment only, and the position of the inner peak was measured as a function of time, in the midtime of each run. For this purpose 8 μL of an aqueous solution of $PrCl_3$, prepared from Pr_6O_{11} (Alpha Products), were added to the solution prepared as in B. The ^{31}P spectra were recorded with a Bruker WP-80 multinuclear spectrometer. A 45° pulse width of 4.5 μs was used. 254 scans (2.5 min) were performed, on 4K data points, with a line-broadening factor of 10 Hz, and Fourier transformed (16 K). Each run lasted ~1 h. A minimum of 5 data points were recorded for each run at a given concentration. In all cases (Tables II and III), good linearity was observed, with correlation coefficients routinely above 0.99 (5–7 experimental points). While some differences in the *absolute* rate values could occur from one vesicle preparation to the next,[3] the *relative* values obtained on a single preparation are entirely significant. Furthermore, to enable quantitative comparisons between different vesicle preparations, a transport experiment with X-537A has been performed each time. The temperature of the experiments (Tables I–III) was 307 K.

(D) Extraction from Water into Chloroform. A chloroform solution of the ionophore (10 mM) was stirred with an aqueous solution of $PrCl_3$ (3–6 mM). The organic solution was then dried over anhydrous Na_2SO_4 (UCB), filtered, and transferred to an NMR tube. ^1H spectra, obtained with the same WP-80 spectrometer before and after contact with the solution of the paramagnetic ions, were then matched in order to test for Pr^{3+} extraction by the ionophore into the organic phase.

Results and Discussion

By determining the velocity of transport of Pr^{3+} across membrane models constituted of vesicles of phospholipids, we discriminate between those substances capable of transport *under these conditions* and those unable of serving as carriers. In this latter class, one finds X-206, X-14540A, X-14766A, A204, nigericin, and emericid. Most probably, under other conditions of concentration, pH, temperature, etc., these substances may act as Pr^{3+} carriers. In the former case, i.e., with bona fide carriers, we have ascertained that ionic transport is linear with time at a fixed concentration. We have also determined, from comparison of the velocities of transport at a few carrier concentrations, whether the transporting complex has 1:1 or 2:1 stoichiometry (Table I). The data point to 1:1 stoichiometries with M139603, X-14547A, and tetronomycin, while a 2:1 stoichiometry characterizes narasin-mediated transport, which is second order in the ionophore concentration. Thus, narasin resembles in this respect lasalocid[22] and etheromycin.[3] The stoichiometry (2:1) observed here with X-537A, using Pr^{3+}, is the same as reported by Degani[24] with Mn^{2+} ions.

Transport stoichiometry can be concentration-range dependent,[23] and we have been careful to work in a relatively narrow span of concentrations (Table I). Another factor, of possible influence on the measurements, is the spread in pK_a values for the various ionophores whose effects we examine: with carboxylic ionophores such as studied here, the kinetically-active species is the anionic conjugate base. At a fixed pH (6.1 for this work), an antibiotic ionophore characterized by a lower pK_a value is more extensively dissociated than a carrier with a less acidic group. Also and incidentally, use of the ionophores as the sodium salts contributes less than 1% to the total sodium concentration and thus does not affect the results. The measured transport rates (Table I) reflect some degree of competition between Na^+ (~30–50 mM) and Pr^{3+} (1.15 mM) transport. However, even with "monovalent" ionophores such as monensin, praseodymium transport is highly favored.

Looking at the chemical structures of these antibiotic ionophores one is struck by the contrast between A204 (**9**) and etheromycin:[3] despite the great similarity in the two structures, the former does not act as a carrier, the latter does.

We consider now Pr^{3+} transport mediated by a mixture of two ionophores (Table II). With the X-537–narasin combination the

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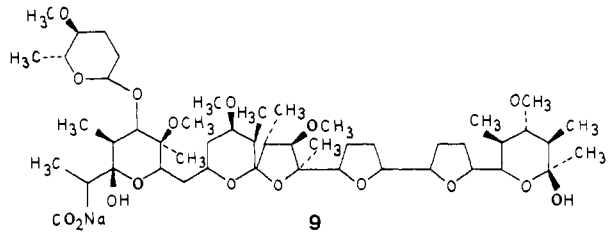

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effect is very obvious: one observes a significant enhancement in the transport rate when two, rather than a single, ionophores are introduced jointly in the membrane. Qualitatively, such a synergism is found to be quite general, with most of the entries in Table II.

Quantitation of the effect requires additional assumption to be made. The simplest are probably the following:

(A) If transport occurs with 2:1 stoichiometry of carrier molecules to the Pr^3 cation, the intrinsic velocity with a hybrid AB complex is the arithmetic mean of the two velocities with the pure A_2 and B_2 transporting species.

(B) Admixture of equimolar quantities of A and B leads to a statistical distribution $A_2 + 2AB + B_2$.

By making these two assumptions, one can compare the observed velocities of Table II with calculated velocities assuming additivity, i.e.:

$$\text{velocity(calcd)} = k_{A_2}[A]^2 + 2k_{AB}[A][B] + k_{B_2}[B]^2 \quad (1)$$

when [A] = [B] (Table II). Note that some entries in Table II do not correspond to equimolar mixtures of A and B: in such cases, the statistical distribution will be such that [AB] < 2[A], if A is the more concentrated of the two ionophores; eq 1 will thus lead to an *over*estimate of the calculated velocity, so that the *actual* rate enhancements are greater than appears from the comparison of the observed and calculated values in Table II.

Two of the ionophore combinations tried do not lead to a positive cooperativity in transport: X-537A–X-206 and X-14547A–monensin. All the other nine combinations (Table II) lead to most impressive velocity enhancements, ranging between 3 and 12, under the above assumptions.

The lack of cooperativity displayed by the X-537A–X-206 system is not surprising: under the conditions of our experiments, X-206 does *not* act as a carrier. As for the X-14547A–monensin system, each of these two ionophores taken alone displays a 1:1 stoichiometry for Pr^{3+} transport. Hence, the result in Table II implies simply that a 1:1 stoichiometry also prevails with their mixture.

For all the other entries in Table II, at least *one* of the two partners (indicated as A), if taken alone, shows a 2:1 stoichiometry for Pr^{3+} transport. In all these cases, a boost occurs in the transport velocity, due to a synergistic effect from the conjunction of the two ionophores (Table II).

A comment is in order here. Since in the competition experiments the concentrations of M-139603, tetronomycin, and A-23187 are markedly lower, by as much as an order of magnitude, than that of X-537A (Table II), one may question the attribution of the rate enhancement to formation of a hybrid complex. To check this point, we have performed transport studies at different pH values also, with both M-139603 (pK_a = 1.8 in 1:9 CH_3OH–H_2O[17]) and X-537A ($pK_a \sim 5.5$ in 9:1 CH_3OH–H_2O[26]) (Table III). Lowering the pH from 6.47 to 5.23 should decrease the amount of the conjugate base of X-537A by one order of magnitude, at least. Conversely, the transport-active anionic species derived from M-139603 should be barely affected in concentration by this pH change. Indeed, transport by X-537A *alone* becomes insignificant at the lower pH. However, at this lower pH, with similar concentrations of the anionic forms of X-537A and M-139603, the synergistic rate enhancement is blatant.

Table I. Velocity of Carrier-Mediated Transport of Pr^{3+} through Model Membranes Made of Phospholipidic Bilayers, in Egg Yolk Lecithin Vesicles

ionophore	concentration, mM	velocity,[b] Hz/min^{-1} ($\pm\sigma$)
A23187	[a]	0.65 (0.06)
M139603	0.013	1.80 (0.23)
	0.020	2.40 (0.23)
X-14547A	0.084	0.60 (0.04)
	0.168	1.22 (0.14)
narasin	0.177	0.45 (0.03)
	0.354	1.80 (0.10)
tetronomycin	0.011	0.90 (0.03)
	0.022	1.53 (0.11)

[a] 5 μL of a saturated methanolic solution. [b] $[Pr^{3+}]_{out}$ = 1.15 mM.

Table II. Observed and Calculated Velocities of Transport of Pr^{3+} in the Presence of Two Chemically Distinct Ionophores[b]

ionophore A (mM)	ionophore B (mM)	velocity,[a] Hz min^{-1} obsd ($\pm\sigma$)	calcd
	pH 6.1		
X-537A (0.133)		0.44 (0.03)	
X-537A (0.133)	narasin (0.118)	4.18 (0.13)	0.32
X-537A (0.133)	tetronomycin (0.011)	4.23 (0.22)	0.67
X-537A (0.133)	A23387 (0.015)[c]	1.65 (0.09)	0.54
X-537A (0.168)	X-206 (0.168)	0.75 (0.06)	0.70
X-537A (0.084)	X-14547A (0.084)	1.88 (0.34)	0.38
etheromycin (0.267)		0.45 (0.03)	
etheromycin (0.133)	narasin (0.118)	0.66 (0.03)	0.15
etheromycin (0.084)	X-14547A (0.084)	1.96 (0.14)	0.32
	pH 6.35		
X-537A (0.146)		1.25 (0.03)	
X-537A (0.073)	M139603 (0.013)	3.00 (0.20)	1.05
etheromycin (0.185)		0.80 (0.04)	
X-537A (0.073)	etheromycin (0.093)	2.40 (0.11)	0.72
	pH 6.1		
X-14547A (0.267)		0.85 (0.07)	
monensin (0.267)		0.87 (0.13)	
X-14547A (0.133)	monensin (0.133)	0.83 (0.12)	0.86

[a] $[Pr^{3+}]_{out}$ = 1.15 mM; phospholipid content 27.5 ± 0.5 mM. [b] See text for details of calculation. [c] 1:1 stoichiometry.[25]

Table III. Influence of the pH of the Solution Containing the Vesicle Suspension upon the Velocity of Transport and the Observed Synergisms[a]

[M139603], mM	[X537A]	pH	velocity, Hz min^{-1} obsd (σ)	calcd
0.0075		6.47	2.1 (0.12)	
	0.13	6.47	1.26 (0.16)	
0.0075	0.067	6.47	3.91 (0.25)	1.20
0.0075		5.23	1.33 (0.10)	
	0.067	5.23	<0.05	
0.0075	0.067	5.23	2.23 (0.25)	0.60
0.0075		4.05	<0.05	
	0.067	4.05	<0.05	
0.0075	0.067	4.05	0.849 (0.02)	

[a] A sonication has been performed in order to ensure uniformity of the pH inside and outside the vesicles.

We have attempted to determined more accurately the magnitude of this positive cooperativity. If all the transporting species are 2:1 with respect to Pr^{3+}, the observed velocity V is the weighted average:

$$V = p_{A_2}V_{A_2} + p_{AB}V_{AB} + p_{B_2}V_{B_2} \quad (2)$$

with

$$p_{A_2} + p_{AB} + p_{B_2} = 1 \quad (3)$$

Hence, by determining V as a function of the relative concentrations of A and B one should be able in principle to determine the four unknowns k_{AB}, K_{A_2}, K_{B_2}, and K_{AB}, where the k_i's are the

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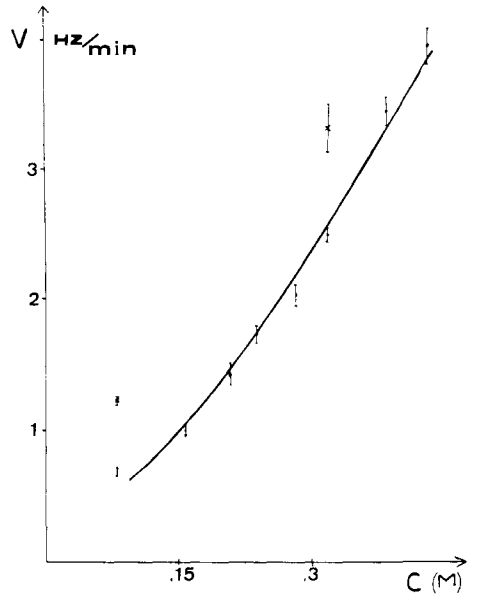

Figure 1. Transport rates of Pr^{3+} across phosphatidylcholine vesicles against the concentration of ionophore in the bilayer (●) [etheromycin] = 0.4 M, (X) [X-537A] = 0.4 M (these two points do *not* belong with the underlying curve).

rate constants for transport by the 2:1 entities and the K_j's are the apparent equilibrium constants for the formation of these 2:1 ternary complexes (k_{A_2} and k_{B_2} are determined separately from experiment). However, the multiparametric Simplex optimization[27] did not lead to accurate and reliable results: the nonrandom scatter of the data in Figure 1 is a warning that the attempt was only partly successful. There are several reasons for this limited success: (i) If one wishes to determine reliably *four* unknowns, 10 data points at least are required; however, it is difficult with each vesicle preparation to go beyond a maximum of seven experiments. (ii) The uncertainty on the measured velocities (see Tables I and II), 5–10% according to the run, is rather large. (iii) There may be, at the lower concentrations, contributions from species with other than 2:1 stoichiometries.[23]

By working with the lasalocid (X-537A)–etheromycin system, using 10 data points, we have nevertheless analyzed the data according to eq 2 and 3 and obtained the following calculated parameters (these suffer from large uncertainties and should be interpreted with much caution; they ought to be taken merely as order-of-magnitude estimates): the intrinsic rate constant k for Pr^{3+} transport mediated by lasalocid, etheromycin, and the hybrid complex respectively are calculated as ~2.85, ~1.25, and ca. 500 Hz mn^{-1} mol^{-2}. They clearly show a greatly enhanced transport velocity for the hybrid, as compared to the pure complexes. The apparent stability constants for the formation of these complexes are ~250, ~500, and ~15 M^{-2}. Taken together, both sets of numbers are consistent with the usual inverse correlation between stability and lability for such complexes; the less stable complexes have the highest cation transport rates. This observation is not restricted to the lasalocid–etheromycin system. In order to make it more general, by studying ^1H or ^{13}C NMR spectra for equimolar mixtures in $CDCl_3$ solution of two ionophores A and B, we have ascertained if a thermodynamic mixed AB complex forms in the presence of Pr^{3+} ions (Table IV and V). Whenever spectra were simply the superposition of the individual A and B spectra, they pointed to the absence of any (AB–Pr^{3+}) complex. Conversely, spectra deviating from additivity of the component spectra were evidence consistent with AB–Pr^{3+} complex formation. As expected, there is no relationship between the kinetically active species and the equilibrium situation (Table IV): transport cooperativity does not reflect formation of a stable hybrid complex in chloroform solution.

The synergism reported here (Table II) is highly reminiscent of the synergism observed in cation liquid–liquid extraction when a mixture of two extractants is used.[6-9] This last effect can be quite large: for instance, synergistic factors varying between 3 and 400 were reported[8] in the extraction (H_2O(pH 4.0) → C_6H_6) of alkali metal ions by combined use of crown ethers (0.24 M) and bis(2-ethylhexyl) phosphate (0.125 M) and were attributed to a greater solubility of the hybrid complex in the organic phase.

We entertain another hypothesis as the explanation for the observed synergism in cation transport. Let us first remark that the observed cooperativity (Table II) is either zero or positive; in none of the cases investigated does it have a negative value. Let now the reader be reminded that the translocation of Pr^{3+} across phospholipidic membranes, from the outer to the inner compartments, must be accompanied either by anionic entry or by efflux of protons (or other cations) (in → out). This is required by the necessary maintainance of electroneutrality. We have shown earlier[3,4] that the latter phenomenon (proton efflux) occurs in our membrane models ("antiport" mechanism).[28]

The slow step in the transport process (2:1 stoichiometry, Table II) involves a positively charged species. We have found no evidence in this work for intervention, in the explored range of

Table IV. Comparison between the Observed Synergistic Effects from Table IV and the Observation from ^1H NMR (or Lack Thereof) in Chloroform Solution of Formation of a Mixed AB–Pr^{3+} Complex[a]

ionophores	synergism	stable complex in $CDCl_3$
X-537A–X-206	–	+
X-537A–X-14547A	+	+
X-537A–M139603	+	–
X-537A–narasin	+	?
X-537A–etheromycin	+	–

[a] $[Pr^{3+}]$ = 3–6 mM; [X-537A] = [A] = 10 mM; [B] = 10 mM.

Table V. Low-Field Portions of the ^{13}C Spectra in Deuteriochloroform (Chemical Shifts in Hz from Me_4Si) for the Ionophores after Extraction of $PrCl_3$ (0.03 M) Showing That the Spectrum of the Mixture is Identical with the Sum of the Two Individual Spectra

X-537A[a]	etheromycin[b]	X-5371 + etheromycin[c]
4315 (3551)		4317
	3635	3628
3545 (3234)		3549
3296 (2882)		3293
2953 (2641)		2950
2692 (2474)		2696
2517 (2404)		2514
2456 (2373)		2455
	2194	2194
	1999	2000
	1977	1979
	1913	1913
1727 (1754)	1729	1726[d]
	1713	1713
	1702	1702
	1689	1688
	1671	1671
1662 (1663)	1661	1663[d]
	1619	1620
	1607	1603

[a] Before Pr^{3+} extraction, to show the magnitude of the resulting shifts. [b] The spectrum was unaffected by Pr^{3+} extraction. [c] Spectrum of an equimolar mixture (0.15 M) after Pr^{3+} extraction. [d] Resonance arising from both ionophores.

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ionophore concentrations, of any 3:1 species. Therefore, a compensating process is needed for maintenance of electroneutrality: either anionic cotransport or cationic counter transport is required to complete the transport cycle.

We have checked this by adding picric acid, which indeed facilitates proton equilibration across the membrane. Addition of picric acid (0.4 mM) to a vesicle solution containing X-537A (2×10^{-4} M) increases the Pr^{3+} transport velocity by a factor of 1.8—when picric acid alone is unable to effect Pr^{3+} transport under our experimental conditions. This observation is consistent with operation of a counter transport of protons from the inside to the outside of the vesicles.[28]

The A and B molecules are carboxylic acids, as is the case for all the ionophores studied here (see the formulas), except for M139603 and tetronomycin, which are acyl ylidene tetronic acids and therefore closely related to carboxylic acids. Hence, with all the ionophores studied here, while the A_2 and B_2 systems have each a single pK_a, the AB hybrid has *two* pK_a values. This is a unique distinguishing feature of any AB system, as compared to the A_2 and B_2 cases. Therefore, when a proton migrating outward from the inner compartment of a vesicle encounters such an AB hybrid, the proton transfer A-COOH + B-COO^-, $\rightarrow$ A-COO^- + B-COOH will assist in the *penetration* of this proton inside the membrane. A similar statement applies to the sodium counterions, and many authors have shown that the rate-determining step, in ionic transport phenomena, occurs at the water–membrane interface.

This hypothesis leads to the prediction that A and B need not be both ionophore antibiotics for synergistic ionic transport across membranes to be set up. Indeed mixtures of lasalocid and crown ether carboxylic acids[29] give rise to similar positive cooperativities[30,31] as reported here. Furthermore, at the pH 6.1 of our experiments, tetronomycin (pK_a = 2.52) and M139603 (pK_a = 1.8 in 1:9 methanol–water) are fully ionized, which is consistent with the high transport rates found with these carriers (Table I). Lasalocid (pK_a = 5.13 in 66% dimethylformamide) differs significantly in acidity from the other ionophores with which it gives rise to synergistic transport (Table II): it is less acidic than the two above-quoted molecules and more acidic than X-206, X-14547A, monensin, and A21387, according to the published pK_a values (obviously, these pK_a's will differ in the lipidic environment, this has been shown for fatty acids,[32] but the sequence of relative acidities should remain the same). Hence, a likely origin for the observed synergistic transport appears to be the pK_a differences that are set as soon as two chemically distinct ionophores are comixed in a membrane environment. The pH dependence of the synergism (Table III) is also consistent with the mechanism implied here for Pr^{3+} transport.

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Registry No. A23187, 52665-69-7; M139603, 75139-05-8; X-14547A, 66513-28-8; X-537A, 25999-31-9; narasin, 55134-13-9; tetronomycin, 82206-10-8; etheromycin, 59149-05-2; monensin, 17090-79-8; praseodymium, 7440-10-0; sodium, 7440-23-5.

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Oxygen Transfer from Oxaziridines: A Chemical Model for Flavin-Dependent Monooxygenases[1]

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Abstract: The ability of several aryloxaziridines to transfer an oxygen atom to phenolates was examined. 2-(*p*-Nitrophenyl)-3-*tert*-butyloxaziridine (**1**) was found to oxidize potassium 2,6-dialkylphenolates to the corresponding *p*-benzoquinones. Product studies and an observed ESR signal suggest an electron-transfer mechanism for these oxidations. ^{18}O-Labeled oxaziridine **30** was prepared. Oxidations of phenolates with **30** rigorously establish the oxaziridine ring oxygen as the atom that is transferred to substrate. Kinetic studies with oxaziridine **1** and the isomeric nitrone **15** rule out the nitrone as an obligate intermediate in the oxygen-transfer reaction. In the oxidation of substrate, a single electron transfer from phenolate to oxaziridine is thought to generate a phenoxy/nitroxyl radical pair, which upon coupling and fragmentation achieves the oxygen transfer. These oxygen-transfer reactions serve as models for the proposed flavin-based oxaziridine **34** in enzyme-mediated monooxygenations.

The flavin-dependent monooxygenases catalyze the incorporation of one atom of molecular oxygen into a substrate with concomitant reduction of the second atom to water.[2] The binding and activation of molecular oxygen is central to flavin monooxygenase reactivity, yet the nature of the active oxygenating species remains unknown. Recent evidence indicates that 4α-hydroperoxyflavin (**33**, Scheme III) is an initial intermediate in these oxidations; this intermediate and derived species have been offered to explain the flavin, oxygen-transferring systems.[3] We

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