

BINDING OF SOLUTES TO POLY(VINYLBENZOCROWN ETHER)S AND  
POLY(VINYLBENZOGLYME)S IN AQUEOUS MEDIA

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**Abstract** - Certain polystyrene-based polymers with pendent crown ether and glyme ligands can act in aqueous media as polysoaps. This is demonstrated by the strong binding of optical and fluorescent organic solutes to poly(vinylbenzo-18-crown-6) and to a poly(vinylbenzogleyme). The binding of ionic solutes to the poly(crown ether) can be regulated by crown-complexable cations which converts the neutral polymer into a polycation of variable charge density depending on the type and concentration of added salt. The binding can also be used for cation-controlled catalysis of reactants whose reaction rates are sensitive to the polarity of the medium. The two polymers also form insoluble complexes with polyacids and, for the charged poly(crown ether), with polyanions.

INTRODUCTION

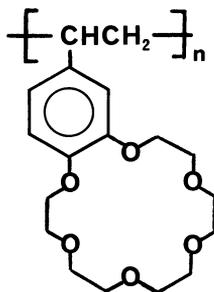
The macroheterocyclic crown ethers and cryptands as well as the acyclic podands (e.g., the glymes) are well recognized for their powerful and selective complexation of a variety of cations. The synthesis and properties of these interesting chelating agents have been extensively reviewed (1-9 and others) and will not be elaborated on in this paper. Anchoring these ligands to linear polymers or immobilized polymer supports not only facilitates their recovery but frequently modifies their properties. For example, crown ethers often form stable 2:1 sandwich-type ligand-cation complexes, especially when the cation diameter exceeds that of the crown cavity. The formation of these complexes is facilitated when the two ligands are already in close proximity, for example, when anchored to a polymer chain as pendent groups. Also, comonomer substituents can hinder or enhance the binding of cations to polymer-bound ligands.

Several studies have been reported where macroheterocyclic or acyclic ligands are either incorporated in the backbone of the polymer chain or are attached as pendent moieties (10-26). In most cases difunctional monomers or vinyl derivatives of glymes, crown ethers or cryptands have been used in the synthesis of these polymers. For immobilized supports, macroheterocyclic or acyclic ligands with appropriate functional groups have been reacted with supports such as crosslinked chloromethylated polystyrenes or inorganic materials. Resins have also been synthesized by condensation polymerization (e.g., formaldehyde with benzocrown ethers) or by polymerizing vinylcrown ethers in the presence of crosslinking agents.

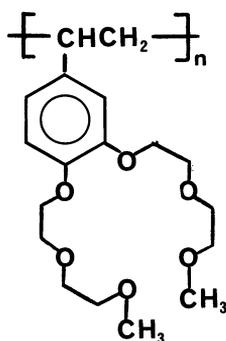
Our research in this area has focused chiefly on the synthesis and properties of both linear and crosslinked polymers derived from crown ether derivatives of styrene or methacrylic acid (23-29). Measurements on the solute binding and catalytic properties of these materials were carried out in aqueous media as well as in nonaqueous solvents such as benzene, dioxane, acetone and tetrahydrofuran, and included potentiometric, spectrophotometric, fluorometric, conductometric and viscometric techniques. This paper will be limited to a discussion of recent work on the polysoap-type properties of some water-soluble polymeric crown ether and glyme ligands, especially their interaction with optical and fluorescent probes and with polyacids and polyanions.

POLY(VINYLBENZOCROWN ETHER)S AND POLY(VINYLBENZOGLYME)S AS POLYSOAPS

Polymers derived from vinylbenzocrown ethers are usually poorly soluble in water, but poly(vinylbenzo-18-crown-6) is a notable exception. This polymer, abbreviated as P18C6 (see structure below) has at 25°C a water solubility of 8.1 g/100 cm<sup>3</sup> for  $M_n = 106,000$  and exhibits inverse temperature solubility, its cloud point being 37°C (30, 31).



P18C6



PVBG

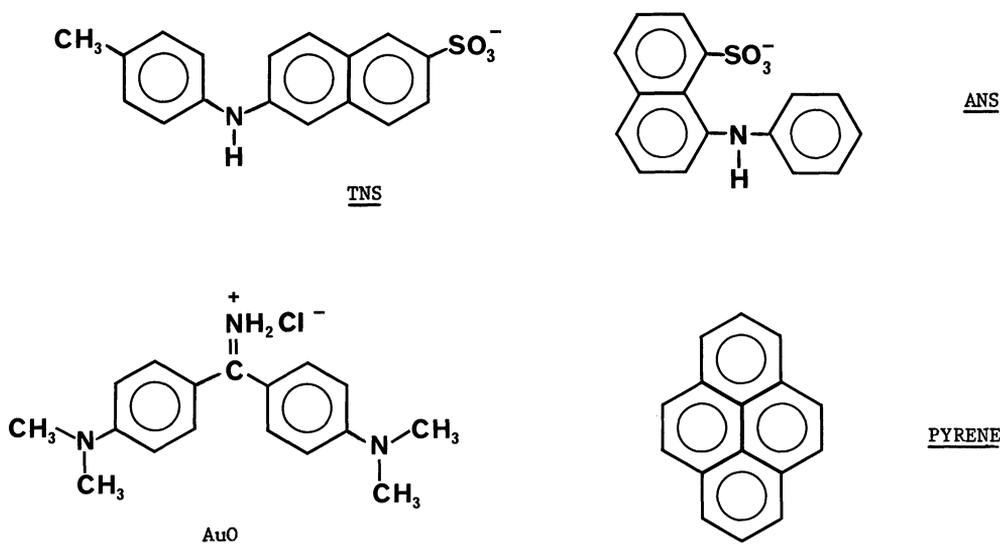
The intrinsic viscosity of this polymer in water is only 0.107 (30) and approaches that of globular proteins. The chains are tightly coiled, each forming a microdomain consisting of a rather hydrophobic interior formed by the polystyrene backbone and an exterior which probably contains many crown ligands hydrogen-bonded to the surrounding water molecules. A similar behavior is shown by polymers with a polystyrene backbone and containing short  $O(CH_2CH_2O)_nCH_3$  chains attached to the benzene rings (32). The structure of one of these poly(vinylbenzocrown ether)s most frequently used in our work (PVBG) is shown above. The compactness of the polysoap-type polycrown ether or polybenzocrown ether chains can be modified by incorporating into the polymer structure more hydrophobic or hydrophilic monomers, or by changing the length of the oligo-oxyethylene (glyme) chains.

The polymers P18C6 and PVBG, and those of similar structure, behave as typical neutral polysoaps as demonstrated by their ability to strongly interact with neutral and ionic organic solutes (31, 32). In this respect they resemble nonionic surfactants containing ethylene oxide units (33). The P18C6 polysoap has the interesting feature that it can easily be converted into a polycation on addition of crown ether-complexable cations such as  $K^+$ ,  $Cs^+$ ,  $Tl^+$ ,  $Ba^{2+}$ ,  $Hg^{2+}$  etc. (31, 34). The number of cation-crown complexes on such a polymer chain and, therefore, the charge density depends on the nature and concentration of added salt. This means that in binding ionic organic solutes such as dyes the polycrown-bound cations add an electrostatic component to the largely hydrophobic-type of interaction exerted by the neutral P18C6 chain. This can augment or attenuate the binding of these solutes depending on whether they are anionic or cationic. The possibility to regulate the binding by adding salts does not exist for the poly(vinylbenzocrown ether)s, since cation-binding to glyme ligands in water is very weak even at high cation concentration (32). Binding can be strong in solvents such as chloroform or tetrahydrofuran, and the cation binding to polymers such as PVEG is frequently much stronger than to the corresponding monomeric analogues due to cooperative effects involving neighboring glyme chains. However, in comparison with P18C6, the binding constants of cations to PVBG are lower by about two to three orders of magnitude.

#### BINDING OF OPTICAL AND FLUORESCENT PROBES TO P18C6 AND PVBG

Several optical and fluorescent probes have been used in the study of solute absorption to P18C6 and PVBG, both in the absence and presence of crown ether-complexable cations. Our earlier work chiefly dealt with optical probes such as picrate anions and methyl orange. The first dye gives a bathochromic shift from 354 nm in water to 384 nm when bound to P18C6 or PVBG. The latter absorption maximum is nearly identical to that found for the free picrate anion in solvents such as acetone or THF. The polymer-bound methyl orange molecule absorbs at 435 nm, as compared to 465 nm in water. The 435 nm peak is close to that found for this dye bound to bovine serum albumin. Very large spectral shifts are observed for the dye 2-(4'-hydroxybenzeneazo) benzoate. Its azo structure in water ( $\lambda_m$  347 nm) changes to a hydrazone structure ( $\lambda_m$  480-510 nm) when bound to P18C6 (34) similar to what has been reported for the behavior of this dye in the presence of detergents and proteins (35,36). We have recently also investigated the binding of the neutral pyrene molecule to P18C6 by optical spectroscopy. Its lowest energy transition peak at 334 nm in water changes to 341 nm when the hydrocarbon is solubilized into the P18C6 domain. Both P18C6 and PVBG show a strong capacity to increase the solubility of pyrene in water (37).

More recent work with P18C6 and PVBC has included anionic, cationic and neutral fluorophores such as 1,8-anilinonaphthalene sulfonate (ANS), 2,6-toluidinonaphthalene sulfonate (TNS), auramine O (AuO) and pyrene (39-41). These compounds have been extensively used in probing the structures of proteins, synthetic polymers and micelles.



Many fluorophores have a very low fluorescence quantum yield in water, but exhibit strongly enhanced intensities in less polar media or when bound to polymers (5). The change in environment can also cause a shift in the emission maximum of the fluorophore. Assuming Langmuir absorption behavior, it can be shown that for binding of fluorophores to sites on polymer chains the inverse of the fluorescence intensity,  $F$  (in arbitrary units) is linearly related to the inverse of the dye concentration at constant polymer concentration. A similar relationship exists between  $F$  and the polymer concentration at constant dye concentration. Two representative plots for TNS are shown in Figures 1 and 2, and similar

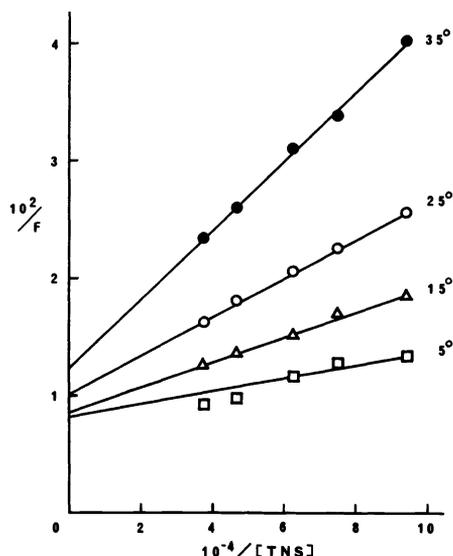


Fig. 1. Plots of  $1/F$  (in arbitrary units) vs  $1/TNS_0$  for TNS binding to P18C6 at 5° (□), 15° (△), 25° (○) and 35° C (●).  $[P18C6]_0 = 9.32 \times 10^{-6}$  M;  $[TNS]_0 = 1.06 \times 10^{-5} - 4.26 \times 10^{-5}$  M. (Ref. 41).

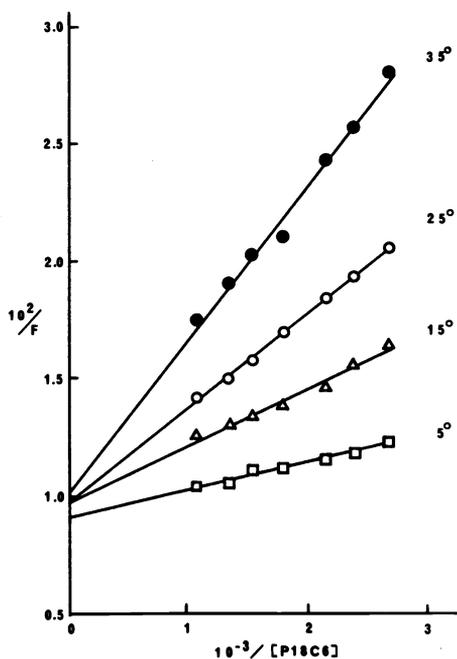


Fig. 2. Plots of  $1/F$  vs  $1/[P18C6]_0$  for  $2.72 \times 10^{-7}$  M TNS at 5 ( $\square$ ), 15 ( $\Delta$ ), 25 ( $\circ$ ) and 35°C ( $\bullet$ ).  $[P18C6]_0 = 3.73 \times 10^{-4} - 9.32 \times 10^{-4}$  M. (Ref. 41).

plots were obtained for ANS and AuO (39-41). The respective slopes and intercepts yield values for the intrinsic ( $K$ ) and first ( $K_1$ ) binding constant and for the average minimum number of monomer units ( $N$ ) per bound dye molecule. Values of  $K$ ,  $K_1$  and  $N$  for a number of optical and fluorescent probes are collected in Table 1. Also listed are values for the enthalpies and entropies of binding. Temperature dependent studies were usually carried

TABLE 1. Intrinsic ( $K$ ) and first ( $K_1$ ) binding constants (25°C) and enthalpies and entropies of binding of some optical and fluorescent probes to P18C6 and PVBG.

Polymer	Solute	$K$ $\times 10^{-4}$ M $^{-1}$	$K_1$ $\times 10^{-2}$ M $^{-1}$	$N$	$\Delta H$ Kcal/mol	$\Delta S$ eu	Ref.
P18C6	Picrate	13.4	31.9	42	-11.9	-17	(31)
	MO	9.6	11.3	85	- 3.9	9.7	(31)
	TNS	6.18	24.1	26	- 6.7	- 0.64	(41)
	ANS	5.38	6.73	80	- 2.7	12.4	(41)
	AuO	2.2	3.17	67			(39)
	Pyrene	29	284	10			(37)
PVBG	Picrate	23	60.5	38	-26.3	-64	(32)
	MO	6.9	23.8	29	- 7.8	- 3.8	(32)
	TNS	11.9	27.3	44	- 4.6	7.7	(41)
	AuO	1.2	0.6	200			(39)
	Pyrene	32.6	326	10			(37)

out over a temperature range of 5 - 35°C. In all systems studied so far, the binding of organic solutes to P18C6 and to PVBG could be described by assuming simple Langmuir adsorption behavior, that is, the "binding sites" act as independent entities, and binding itself is only determined by statistical factors. Since at saturation only a few ionic solute molecules are present in the polymer domain, electrostatic repulsion is negligibly small. This is not the case when alkali ions are bound to the crown moieties of P18C6. In this case up to almost half the crown ligands can contain a cation, and repulsion between bound cations causes a clear deviation from the Langmuir adsorption behavior at higher cation concentrations (34).

The intrinsic binding constants lie in the range of  $10^4 \text{ M}^{-1}$  -  $33 \times 10^4 \text{ M}^{-1}$ , and compare favorably with those reported for the binding of these solutes to proteins and to polymers such as poly(vinylpyrrolidone). The solutes yield quite different  $N$  values, ranging from 10 for pyrene to 200 for AuO binding to PVBG. A high  $N$  value means that the maximum number of solute molecules per polymer chain will be low, although the intrinsic binding constant may be high. For example, to a PVBG macromolecule of  $DP = 200$  only one AuO molecule can be bound but as many as twenty pyrene molecules. A more systematic study is needed to determine the factors that cause a high or low  $N$  value for these polymers. The presence and location of polar or ionic substituents in the solute molecule are factors that will likely influence the solubility of the solute into the polymer domain and whether it will be located in the interior of this domain or more on its periphery close to the aqueous phase. The rather strong exothermicities found for some of the solute binding reactions imply that in addition to hydrophobic interactions, other, more specific effects resulting from Van der Waals or polar interactions between solute and monomer units may exist. The positive entropy change found for some of the solutes suggest a predominance of hydrophobic contributions to the binding process.

For most solutes, PVBG appears to yield slightly higher binding constants than P18C6, while  $N$  values can also be quite different. AuO appears to deviate from this pattern, since both  $K$  and  $K_1$  are lower for PVBG than for P18C6, the  $K_1$  value by as much as a factor five. This dye contains a  $\text{>C} = \text{N}^+\text{H}_2$  ion, and it is not unreasonable to suggest that it may interact specifically with a crown ether ligand as is also found for primary ammonium and guanidinium cations. In fact, it was found that 4'-methylbenzo-18-crown-6, the monomeric analogue of P18C6, considerably increases the solubility of AuO in toluene. Hence, binding of AuO to P18C6 may include a contribution resulting from a specific interaction between the  $\text{>C} = \text{N}^+\text{H}_2$  ion and a crown ligand of P18C6 in addition to hydrophobic interactions. A more detailed discussion of the binding of the various solutes to P18C6 and PVBG is given in the references listed in Table 1.

#### EFFECT OF ADDED SALT AND ORGANIC SOLVENT

When added to an aqueous solution of P18C6, cations which can complex to the benzo-18-crown-6 ligand can convert the neutral P18C6 macromolecule into a polycation. The number of cation-crown complexes per chain will depend on the type and concentration of cation. This conversion into a positively charged polymer enhances the binding of anionic solutes such as picrate, ANS or TNS, but cationic solutes like AuO should experience a repulsive effect. Figures 3 and 4 show the effects of added salts on the fractions,  $F/F_m$ , of TNS and AuO bound to P18C6,  $F_m$  being the fluorescence intensity under conditions when all solute is bound to polymer. As expected, the fraction of bound solute on adding salts increases for TNS and decreases for AuO, the effect being most pronounced for cesium ions. The effectiveness in regulating the binding of ionic solutes to P18C6 decreases for

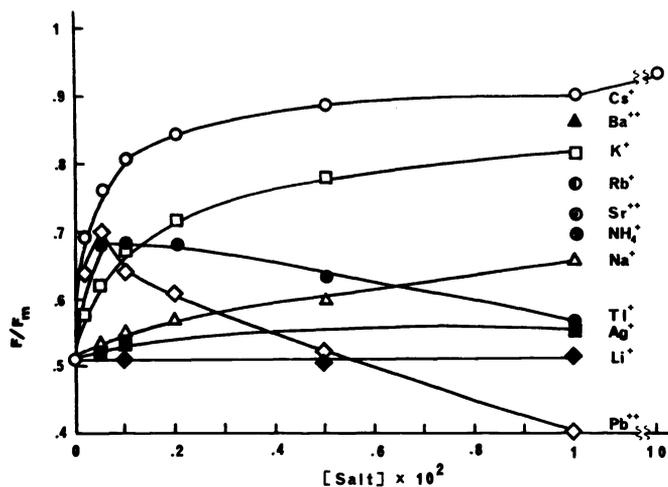


Fig. 3. Cation effect on the fraction of P18C6-bound TNS in water at 25°C.  $[\text{TNS}] = 2.72 \times 10^{-7} \text{ M}$ ;  $[\text{P18C6}] = 3.73 \times 10^{-4} \text{ M}$  (Ref. 39,41).

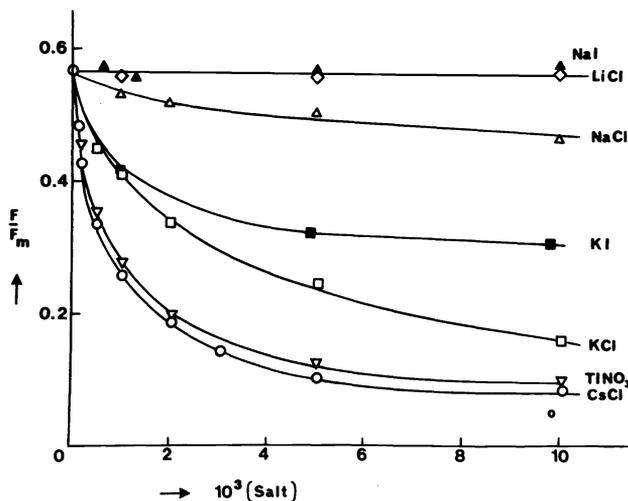


Fig. 4. Cation effect on the fraction of P18C6-bound auramine O in water at 25°C.  $[AuO] = 1.08 \times 10^{-5} M$ ;  $[P18C6] = 6.45 \times 10^{-3} M$ . (Ref. 40,37).

alkali ions in the order  $Cs^+ > K^+ > Na^+ \gg Li^+$ ,  $Li^+$  having no effect on the binding up to 0.5 M LiCl. The sequence reflects the decrease in the binding constants of the respective cations to P18C6 in water. Their values are  $300 M^{-1}$  for  $Cs^+$ ,  $110 M^{-1}$  for  $K^+$  and  $2.4 M^{-1}$  for  $Na^+$  (34). The relatively high binding constant for  $Cs^+$  is due not only to its lower dehydration energy, but its complex with P18C6 is known to involve two crown ligands (27, 31). The peculiar behavior of  $Tl^+$  and  $Pb^{2+}$  (Fig. 3) results from fluorescence quenching of the P18C6-bound TNS by  $Tl^+$  or  $Pb^{2+}$  cations bound to the macromolecule. In such a system the ratio  $F/F_m$  does not equal the fraction of bound solute. Although more TNS becomes bound, the expected increase in  $F$  is eventually offset by quenching as the average distance between crown-bound  $Tl^+$  or  $Pb^{2+}$  and the TNS in the polymer domain becomes shorter at higher cation concentration.

It was demonstrated that the logarithm of the intrinsic binding constant of picrate to P18C6 in the presence of potassium chloride is proportional to the number of potassium ions bound per P18C6 chain (34). This result can be predicted theoretically assuming that on binding potassium ions to P18C6 the average diameter of the tightly coiled polysoap molecule does not change. Viscosity data for P18C6 in the absence and presence of potassium chloride suggest that this assumption is justified. At very high salt concentration the optical spectrum of picrate bound to P18C6 shifts back to an absorption maximum closer to that found for picrate in water (31), although dialysis measurements do not indicate a release of picrate anions from the polymer domain. Apparently, when the polymer particle is highly charged with cations, some water may penetrate, causing partial hydration of the picrate anion. A similar phenomenon was observed in the catalytic application of these systems (see below).

The effect of added salt on the binding of dye also depends on the nature of the counter-anion. For example, the effects of iodides on AuO binding is considerably less than those for the corresponding chlorides (Fig. 4). More effective counterion shielding by the more lipophilic anions reduces the electrostatic repulsion or attraction between polycation and the incoming solute. Anions such as tetraphenylboron and dodecyl sulfate simply replace the bound dye. Such competitive measurements can be used to determine the binding constants of anions where spectrophotometric or fluorometric techniques fail.

Addition of organic solvents to an aqueous solution of P18C6 or PVBG should lead to a more expanded polymer chain, permitting water to penetrate into the polymer domain. With sufficient quantities of a good solvent, the polysoap-type behavior of these polymers eventually will disappear. An example of such a behavior is depicted in Fig. 5, where the change of the fluorescence intensity and the emission maximum of TNS in the presence of P18C6 is plotted as a function of the alcohol or dioxane content of the mixture. The observed rapid decrease in the fluorescence intensity on adding alcohol or dioxane does not necessarily imply that TNS is released from the polymer domain. Water penetration into the expanding polymer coil could quench the TNS fluorescence while at least initially most of the solute remains bound to the polymer. Note also that the drop in  $F$  is not simultaneously accompanied by a change in the emission maximum. For example,  $F$  (in arbitrary units) decreases from 55 to about 15 on addition of 10% dioxane or 20% alcohol, while at the same time the

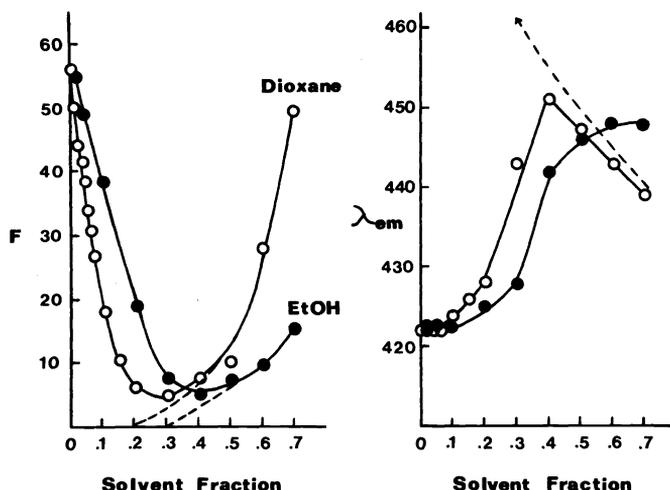
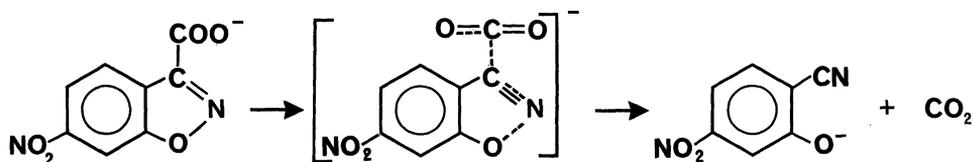


Fig. 5 Effect of increasing volume fractions of ethanol (○) and dioxane (●) on the fluorescence intensity,  $F$ , (in arbitrary units) and the emission maximum,  $\lambda_{em}$ , of TNS in water at 25°C in the presence of P18C6.  $[TNS]_0 = 2.5 \times 10^{-7}$  M;  $[P18C6]_0 = 4 \times 10^{-4}$  M. Dotted lines refer to changes in  $F$  and  $\lambda_{em}$  of TNS on addition of alcohol or dioxane but in the absence of P18C6. (Ref. 41)

emission maximum remains at 423 nm, the value found for P18C6-bound TNS in pure water. It appears that compact hydrophobic regions within the polymer coil continue to exist up to an alcohol or dioxane content of at least 0.2. In the absence of P18C6 the emission maximum of TNS is known to change nearly linearly with dioxane content, its value being 500 nm in water and 434 nm in 90/10 dioxane/water. Data show that the fluorescence intensity and the emission maximum of TNS in the presence of P18C6 approach the corresponding values found in the absence of P18C6 when the alcohol fraction is 0.4 and the dioxane fraction 0.3. With ethanol, the same was found for picrate binding to P18C6, that is, the 384 nm peak for the bound picrate had shifted back to the 354 nm peak characteristic for free picrate in water when 40% of alcohol was added (31). Dialysis measurements would be more conclusive in determining the relationship between solute binding and solvent composition.

#### CATALYTIC PROPERTIES OF P18C6 IN WATER

Micelles and polysoaps have frequently been utilized to catalyze reactions in aqueous media. Transfer of reactant molecules to a micelle or polysoap provide a less polar and often more reactive environment for the solute. Therefore, macromolecules like P18C6 or PVBG could be expected to function in a similar manner. A convenient reaction to study such a catalytic system is the decarboxylation of 6-nitrobenzoxazole-3-carboxylate. This solvent-sensitive reaction proceeds quantitatively and in a unimolecular fashion, producing 2-cyano-5-nitrophenolate which can be monitored spectrophotometrically (see below). Rate



constants at 30°C are reported to be  $k$  ( $s^{-1}$ ) =  $7.4 \times 10^{-6}$  in water,  $4.8 \times 10^{-3}$  in benzene, 4.0 in THF and about 700 in HMPA (43). A change to an aprotic or less polar solvent destabilizes the carboxylate reactant more than the delocalized transition state.

Addition of P18C6 or PVBG to an aqueous solution of this carboxylate strongly enhances its decarboxylation rate. The reaction follows typical micellar kinetics from which the binding constant of the carboxylate to P18C6 and the rate constant for decarboxylation ( $k_m$ )

of the P18C6-bound reactant can be derived. The latter was found to exceed the rate constant in water by a factor 2300. This compares with a catalytic factor of 95 for the cetyltrimethylammonium bromide catalyzed decomposition of this carboxylate (45) and a factor 1300 reported for the same reaction catalyzed by a polysoap-type derivative of poly(ethylene imine) (46). Phosphonium salts immobilized on silicagel are also effective micellar and phase transfer catalysts for this reaction (47).

The binding constant of this carboxylate to P18C6 at 25°C is only  $340 \text{ M}^{-1}$ , much lower than those for the more hydrophobic solutes listed in Table 1. However, the binding can be enhanced by P18C6-complexable cations in a manner similar to that found for other anionic solutes (see Fig. 3). The rate behavior of this cation-controlled decarboxylation as a function of salt concentration is shown in Fig. 6 for  $\text{K}^+$  and  $\text{Cs}^+$ , utilizing conditions

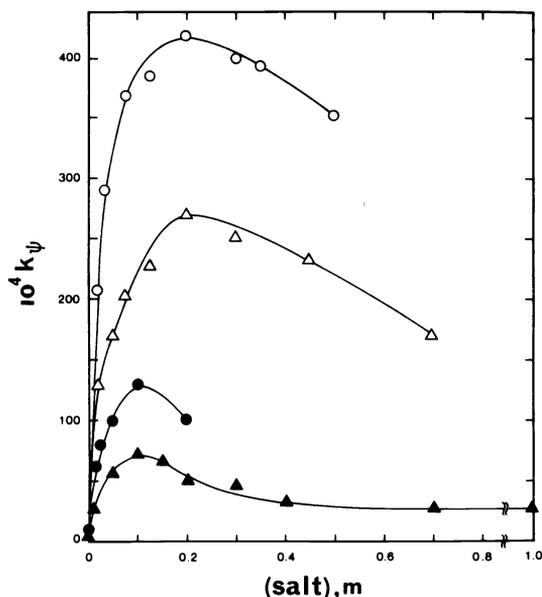


Fig. 6. Observed decarboxylation rate constant,  $k_p$ , for 6-nitrobenzisoazole-3-carboxylate in water at 25°C in the presence of P18C6 as a function of KCl or CsCl concentration, and of the ratio,  $r$ , of P18C6 to carboxylate. (O) CsCl,  $r = 50$ ; ( $\Delta$ ) CsCl,  $r = 20$ ; ( $\bullet$ ) KCl,  $r = 50$ ; ( $\blacktriangle$ ) KCl,  $r = 20$  (Ref. 44).

where in the absence of salt only a small fraction of carboxylate is bound. The sharp increase in the observed rate constant for  $\text{Cs}^+$  is again evidence for the relatively high binding constant of this cation to P18C6. The increase is due to two factors: an increase in the fraction of bound carboxylate, and a higher decarboxylation rate constant when the carboxylate is bound to a  $\text{Cs}^+$ -charged P18C6 macromolecule. The catalytic factor for this system is 14,000. The lower rate constants found at values of  $r = \text{P18C6}/\text{COO}^- = 20$  (see fig. 6) result from saturation. The  $N$  value for the carboxylate is about 40, that is, at least forty monomer units per carboxylate anion are needed to avoid saturation. This condition is fulfilled for experiments where  $r$  equals 50.

The increased reactivity of polymer-bound carboxylate results from a decrease in the activation enthalpy of the reaction, the values being 32.0 kcal/mol for the reaction in the absence of polymer, 21.1 kcal/mol for the reactant bound to neutral P18C6 and 16.1 kcal/mol with the  $\text{Cs}^+$ -charged polymer (44). The respective activation entropies are 19, 2.3 and -10.7 e.u. The significance of these values have been discussed previously (44). Figure 6 also shows a rate decrease at very high salt concentrations. Addition of the same concentration of lithium chloride added to a P18C6/CsCl solution where the rate is maximum has no effect. This implies that high counterion concentration is not a factor in the rate decrease. The decrease may be caused by an effect similar to that observed for picrate. Very high cation loading of P18C6 may cause water molecules to penetrate the P18C6 domain and decrease the reactivity of the bound carboxylate.

## POLYACID AND POLYSALT COMPLEXES WITH P18C6 AND PVBG

Turbidity or precipitation is observed when aqueous mixtures of P18C6 and poly(acrylic acid) (PAA) are acidified (48, 49). These phenomena already occur at P18C6 and PAA concentrations below  $10^{-4}$  M (expressed in monomer units). Figure 7 depicts a plot of the fraction of

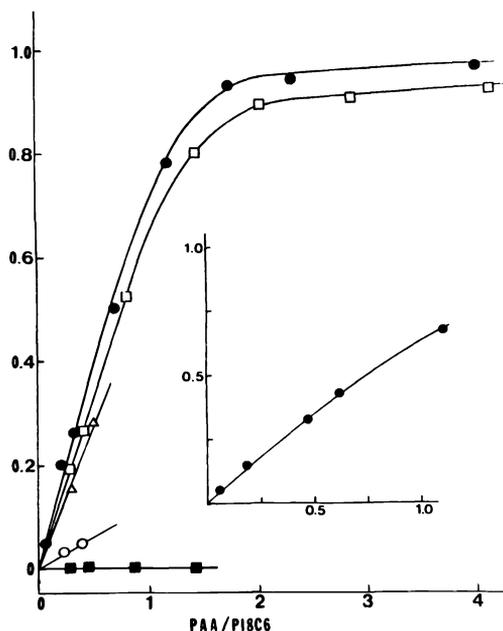


Fig. 7. Plot of the fraction of precipitated P18C6 as a function of the ratio of poly(acrylic acid) to P18C6 in water at 25°C in the presence and absence of HCl. ( $\square$ ) 0.1 M HCl; ( $\bullet$ ) 0.01 M HCl; ( $\Delta$ ) 0.001 M HCl; ( $\circ$ )  $10^{-5}$  M HCl; ( $\blacksquare$ ) no HCl added.  $[P18C6]_0 = 10^{-4}$  M. Insert: same plot as at 0.01 N HCl but for low ratios of PAA/P18C6 (Ref. 48, 49).

precipitated P18C6 as a function of the ratio PAA (MW 250,000)/P18C6 (MW 6,000) at  $[P18C6] = 1 \times 10^{-4}$  M for different concentrations of HCl. Reversing the mixing order or time of shaking has no effect on the results. Precipitation of the complexes reaches a maximum effectiveness at a pH of about two, although the results are not very different at pH 1. At pH equal three or higher more PAA is needed to precipitate the P18C6. Turbidity but no precipitation is observed in the absence of HCl but only when  $PAA/P18C6 > 1.5$ . Clear solutions exist in polymer mixtures containing lithium hydroxide, but insoluble polysalt complexes are formed when in neutral or basic PAA/P18C6 mixtures crown ether-complexable cations such as  $K^+$ ,  $Cs^+$  or  $Ba^{2+}$  are added.

The type of complexes formed between PAA and P18C6 in acidic media resemble those found in aqueous solutions of polyacids with oxygen-containing macromolecules like poly(ethylene oxide) (50), poly(vinylpyrrolidone) (51) and nonionic surfactants (33). Cooperative hydrogen bonding between the crown oxygen atoms and the carboxyl groups of the polyacid, aided by hydrophobic interactions, produce complexes of low hydrophilicity and poor water solubility. Effective precipitation occurs when long sequences of hydrogen bonds are formed at low pH. At higher pH the sequences are interrupted by carboxylate anions, and water penetration into the charged particle is facilitated. Sufficient ionization results in complete solubilization of the complexes. PVBG shows a behavior similar to that of P18C6, while poly(methacrylic acid) (PMMA) can be substituted for PAA. In spite of its more hydrophobic character, precipitation in PMAA/P18C6 mixtures is not as efficient and requires more PMAA. The less flexible structure of PMMA may make it more difficult to form sequences of hydrogen-bonded complexes.

Precipitates recovered from solutions containing mixtures of PAA and P18C6 in the ratio of 0.5, 1.0 or 2.0 yielded a composition of 1.8 COOH groups per crown ligand. At a PAA/P18C6 ratio of 4.0 all PAA was precipitated with the P18C6. Apparently, P18C6 can precipitate considerable quantities of PAA, and a relatively small fraction of COOH groups need to interact with the poly(crown ether) to cause flocculation. Changes in molecular weights

of the two polymers do not appear to be an important factor. Mixtures of P18C6 (75,000)/PAA (250,000) and P18C6 (75,000)/PAA (5,000) give precipitation curves not much different from those shown in Fig. 7 for P18C6 (6000)/PAA (250,000).

Films composed of PAA-P18C6 complexes can be formed at the interphase of a toluene solution of P18C6 in contact with a 0.01 M HCl solution of PAA. These complexes are more stable than those recovered from precipitation experiments. The film can be removed as a fiber which, when dried, is insoluble in dioxane, a solvent for both P18C6 and PAA. The interfacial complex formation also permits the use of water-insoluble polymers such as poly(vinylbenzo-15-crown-5) or styrene copolymers of vinylbenzocrown ethers. The rate of film formation at the same crown concentration is much slower when the copolymer contains a large amount of styrene comonomer.

The formation of complexes between PAA and PVBG suggests that crown ligands are not essential for the hydrogen bonding to occur. However, the ligands play a unique role when salts are added. A crown-bound cation prevents hydrogen-bond formation between the oxygen atoms of that ligand and a carboxyl group. Also, the complex becomes positively charged. Both factors make precipitation more difficult. For example,  $10^{-4}$  M mixtures of P18C6 and PAA at pH 2 do not give a precipitate but only become turbid in the presence of 0.01 M KCl. The mixture remains clear in 0.1 M KCl or 0.01 M CsCl. Hence, precipitated complexes can be solubilized in acidified water as long as a sufficient concentration of P18C6-complexable cations is present.

Insoluble polysalt complexes are formed in the presence of crown-complexable cations and at a pH where a sufficient fraction of COOH groups are ionized. Complex formation between polymers of opposite charge is a common phenomenon observed with both synthetic and natural polymers. The structure and properties of such complexes depend on the charge density of the polyions, chain conformation and flexibility, the presence of electrolytes, charge equivalence and other variables. Insoluble polysalt complexes are formed at concentrations as low as  $10^{-4}$  M between cation-charged P18C6 and salts of carboxymethylcellulose (CMC) (52), poly(styrene sulfonate) (53), poly(acrylates) (53) and polynucleotides (54). An example of the precipitation of P18C6 with sodium CMC in the presence of potassium chloride is shown in Fig. 8. The precipitation is sensitive to the ratio CMC/P18C6

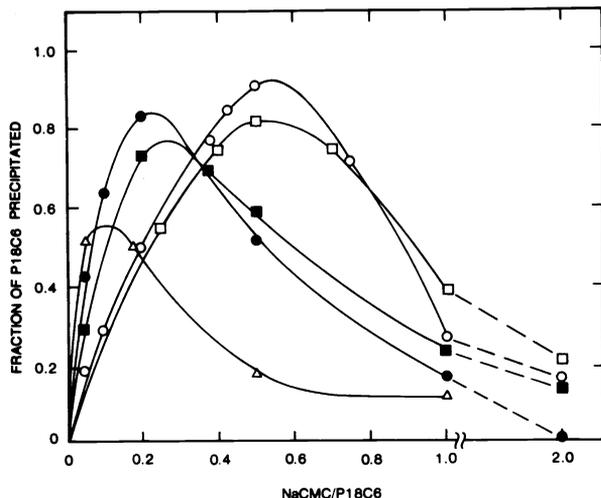


Fig. 8. Precipitation of polysalt complex of P18C6 with sodium carboxymethylcellulose (CMC) as a function of the ratio CMC/P18C6 at different KCl concentrations: ( $\Delta$ ) 0.002 M, ( $\bullet$ ) 0.005 M; ( $\blacksquare$ ) 0.02 M; ( $\circ$ ) 0.1 M; ( $\square$ ) 0.2 M. (Ref. 52).

and is at its maximum in 0.1 M KCl when this ratio is approximately 0.5-0.6. Under these conditions, about one out of two crown ligands contains a  $K^+$  cation, hence, charge equivalence is reached at this point (52). At low KCl concentrations, e.g. 0.005 M, fewer crown ligands contain a cation, and less sodium CMC is needed to reach charge equivalence and to obtain maximum precipitation of P18C6. High salt concentrations weaken the polycation-polyanion interaction due to counterion shielding, and in 0.5 M KCl there is no

precipitation in  $10^{-4}$  M mixtures of P18C6 and sodium CMC. This is also the reason why no precipitation occurs in the presence of NaCl. The weak binding of  $\text{Na}^+$  to P18C6 means that at least 0.1 M NaCl is needed to obtain the same number of crown-bound cations as with 0.005 M KCl. The high ionic strength, coupled with the low concentration of cation-crown complexes, and therefore, charged contact points, prevents the precipitation in 0.1 M NaCl.

Polymer-polymer complexes are especially of interest in template polymerization processes, for example, the polymerization of vinylpyrrolidone in dimethylformamide in the presence of poly(methacrylic acid) (55). An interesting case where P18C6 interferes with such polymerization concerns the use of polynucleotides as templates in nucleic acid polymerase. Figure 9 depicts the inhibitory effect of P18C6 on the RNA-directed DNA polymerase activity from murine leukemia virus system. While the monomeric benzo-18-crown-6 does not effect the elongation of oligodeoxynucleotides templated with its complimentary polynucleotide (e.g., polyriboadenylate), addition of  $10^{-3}$  M P18C6 nearly completely deactivates the reaction (Fig. 9). The inhibition occurs both in the absence and presence of crown ether-complexable cations. Apparently, neutral P18C6 interacts sufficiently strong with the polynucleotide to affect inhibition, although no polymer complex precipitates. In the presence of KCl or CsCl, insoluble polysalt complexes are formed under conditions similar to that found for other polyanions. There is no spectrophotometric evidence for the formation of P18C6-polynucleotide complexes in the absence of crown-bound cations, but when poly(riboadenylate) is attached to sepharose beads it strongly binds P18C6 in both

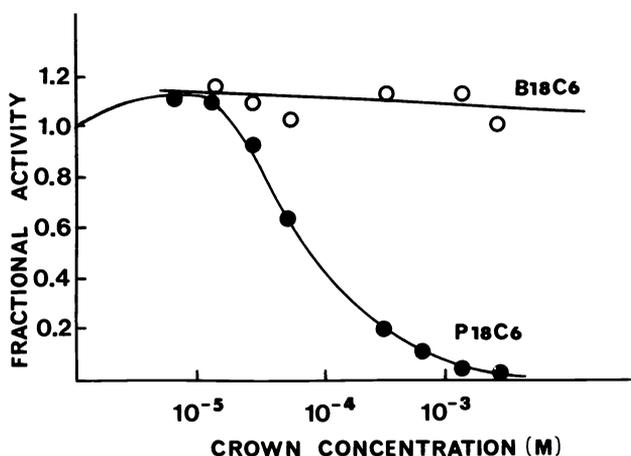


Fig. 9. Effect of P18C6 and B18C6 on the RNA-directed DNA polymerase activity from murine leukemia virus system. (O) B18C6 and poly(rA) template; (●) P18C6 and poly(rA) template (Ref. 54).

the absence and presence of KCl. No absorption of P18C6 to sepharose itself is observed. The inhibition of DNA polymerase is reversed on addition of excess polynucleotide. The poly(crown ether) did not interact with the studied polymerases or with bovine serum albumin, suggesting that P18C6 may be a reasonably selective reagent for nucleic acids.

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#### REFERENCES

1. R. M. Izatt and J. J. Christensen, Eds, Progress in Macrocyclic Chemistry, Wiley Interscience, New York, Vol. I (1979), Vol. II (1981).
2. R. M. Izatt and J. J. Christensen, Eds., Synthetic Multidentate Macrocyclic Compounds, Academic Press, New York, (1978).
3. I. M. Kolthoff, Anal. Chem. Rev. **51**, 1R-22R (1979).
4. N. S. Poonia and A. V. Bajaj, Chem. Rev. **79**, 389-445 (1979).
5. J. M. Lehn, Pure Appl. Chem. **50**, 871-892 (1978).
6. J. S. Bradshaw and P. E. Stott, Tetrahedron **36**, 461-510 (1980).

7. J. F. Stoddart, Quart. Rev., 85-142 (1979).
8. C. J. Pedersen and H. K. Frensdorff, Angew. Chem., Int. Ed. Engl. 81, 16-25 (1972).
9. W. Raszhofer, W. M. Müller and F. Vögtle, Chem. Ber. 112, 2095-2119 (1979).
10. F. Montanari and P. Tundo, J. Org. Chem. 46, 2125-2130 (1981).
11. S. Akabori, S. Miyamoto and H. Tanabe, J. Polym. Sci. Polym. Chem. Edit. 17, 3933-3937 (1979).
12. K. Kimura, T. Maeda and T. Shono, Makromol. Chem. 182, 1579-1586 (1981).
13. Jun-ichi Anzai, A. Ueno and T. Osa, Makromol. Chem., Rapid Comm. 1, 741-743 (1980).
14. P. Kutchukov, A. Ricard and C. Quivoron, Eur. Polym. J. 16, 753-758 (1980).
15. G. Manecke and A. Krämer, Makromol. Chem. 182, 3017-3030 (1981).
16. K. Yagi and M. C. Sanchez, Makromol. Chem., Rapid Comm. 2, 311-315 (1981).
17. S. Yanagida, K. Takahashi and M. Okahara, J. Org. Chem. 44, 1099-1103 (1979).
18. S. L. Regen, Angew. Chem. Int. Ed. Engl. 18, 421-429 (1979).
19. E. Blasius, K. P. Janzen, M. Keller, H. Lander, T. Nguyen-Tien and G. Scholten, Talanta, 27, 107-126 (1980).
20. E. Shchori and J. Jagur-Grodzinski, J. Appl. Polym. Sci., 20, 773-788 (1976).
21. Ph. Gramain and Y. Frère, Macromolecules, 12, 1038-1042 (1979).
22. A. Akelah and D. C. Sherrington, Chem. Rev., 81, 557-587 (1981).
23. J. Smid, Ind. Eng. Chem. Prod. Res. & Dev., 19, 364-371 (1980).
24. J. Smid, Makromol. Chem. Suppl. 5, 203-215 (1981).
25. J. Smid, Pure Appl. Chem. 48, 343-353 (1976).
26. J. Smid, "Solute Binding to Polymers with Macroheterocyclic Ligands", Chapter 3 of reference 1, Vol. II (1981).
27. K. H. Wong, K. Yagi and J. Smid, J. Membr. Biol. 18, 379-397 (1974).
28. J. Smid, A. J. Varma and S. C. Shah, J. Am. Chem. Soc. 101, 5764-5769 (1979).
29. R. Sinta and J. Smid, J. Am. Chem. Soc. 103, 6962-6963 (1981).
30. S. Kopolow, T. E. Hogen-Esch and J. Smid, Macromolecules 6, 133-142 (1973).
31. L. Wong and J. Smid, J. Am. Chem. Soc. 99, 5637-5642 (1977).
32. R. Sinta and J. Smid, Macromolecules, 13, 339-345 (1980).
33. S. Saito, Coll. Polym. Sci. 257, 266-272 (1979).
34. L. Wong and J. Smid, Polymer 21, 195-200 (1980).
35. J. H. Baxter, Arch. Biochem. Biophys. 108, 375-383 (1964).
36. I. Moriguchi, S. Fushimi, C. Oshima and N. Kaneniwa, Chem. Pharm. Bull. 18, 2447-2452 (1970).
37. K. Kimura and J. Smid, unpublished results from this laboratory.
38. G. M. Edelman and W. O. McClure, Acc. Chem. Res. 1, 65-70 (1968).
39. K. Kimura and J. Smid, Makromol. Chem., Rapid Comm. 2, 235-240 (1981).
40. K. Kimura, K. H. Wong and J. Smid, Polymer Bull. 6, 395-399 (1982).
41. K. Kimura and J. Smid, Macromolecules, in press.
42. P. Molyneux and H. P. Frank, J. Am. Chem. Soc. 83, 3169-3180 (1961).
43. D. S. Kemp and K. G. Paul, J. Am. Chem. Soc. 97, 7305-7312 (1975).
44. S. C. Shah and J. Smid, J. Am. Chem. Soc. 100, 1426-1432 (1978).
45. C. A. Bunton, A. A. Kamego, M. J. Minch and J. L. Wright, J. Org. Chem. 40, 1321-1327 (1975).
46. J. Suh, I. S. Scarpa and I. M. Klotz, J. Am. Chem. Soc. 98, 7060-7064 (1976).
47. P. Tundo and P. Venturello, Tetrahydron Lett. 21, 2581-2584 (1980).
48. G. D. Jaycox and J. Smid, Makromol. Chem. Rapid Comm. 2, 299-303 (1981).
49. G. D. Jaycox, R. Sinta and J. Smid, J. Polym. Sci. Polym. Chem. Edit., in press.
50. Y. Osada and M. Sato, J. Polym. Sci., Polym. Lett. Edit. 14, 129-134 (1976).
51. L. A. Bimendina, V. V. Roganov and E. A. Bekturov, Vysokomol. Soedin A16, 2810-2814 (1974).
52. A. J. Varma and J. Smid, J. Polym. Sci., Polym. Chem. Edit. 15, 1189-1197 (1977).
53. A. J. Varma, T. Majewicz and J. Smid, J. Polym. Sci., Polym. Chem. Edit. 17, 1573-1581 (1979).
54. J. Pitha and J. Smid, Biochim. Biophys. Acta 425, 287-295 (1976).
55. D. W. Koetsier, Y. Y. Tan and G. Challa, J. Polym. Sci., Polym. Chem. Edit. 18, 1933-1943 (1980).