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Note

Macrocyclic polyfunctional Lewis bases

XII. Influence of complex formation on chromatographic migration

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Macrocyclic polyethers, their aza analogues, cryptands, some podands and numerous groups of biologically important compounds (such as the antibiotics valinomycin and nigericin) form complexes with metal ions, especially of alkali and alkaline earth metals, and ammonium cations[1]. The determination of the stability of these complexes permits a better understanding of the properties of ligand or the qualification of their biological action.

In crown ether chemistry[2-5] some qualitative tests have been introduced to establish whether the given ligand forms complexes with the cation under consideration. A simple thin-layer chromatographic (TLC) test described previously[6] is based on changes on the chromatographic R_F values of ligands caused by impregnation of the support with salts. The influence of complex formation on R_F values has been reported for many cations[7].

In this paper we present the results of experiments on and a simplified mathematical treatment of the chromatographic behaviour of ligands 1–13 (Fig. 1) in the presence of salts. Experiments were performed using TLC and high-performance liquid chromatography (HPLC) using impregnated silica gel as the stationary phase.

EXPERIMENTAL

Silufol plates (3 × 8 cm) were impregnated on one half with 2% solutions of alkali metal, strontium or barium chlorides by developing them perpendicularly along the longer edge. The plates were then dried and heated at 105°C for 2 h. The ligands were applied on the impregnated and unimpregnated parts of the plate and developed along the longer edge. The spots on the chromatograms were detected with iodine. The solvent systems used were selected to obtain R_F^0 values of 0.6–0.8. The results are summarized in Table I.

HPLC experiments were performed using a KB-5101 (Kabid-Warszawa) apparatus equipped with a UV detector (254 nm). The columns (100 × 3.3 mm I.D.) were dry packed with silica gel (particle diameter, d_P = 33 µm) obtained by multiple sedimentation of an H-60 (Machery, Nagel & Co.) gel. The solid phase was impregnated in the following way: a 1-g portion of silica gel was mixed with a solution of
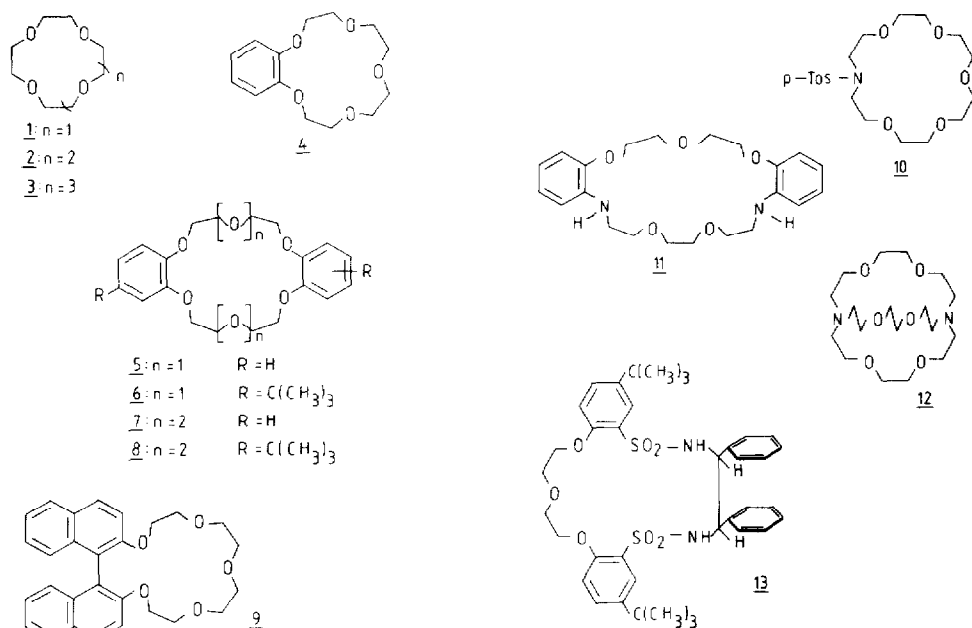
Fig. 1. Structures of compounds 1–13.

0.1 g of the respective salt in water (1 cm^3) and the mixture was dried for 2 h at 105°C. The results of the HPLC experiments are summarized in Table II.

RESULTS AND DISCUSSION

The changes in R_F values[8] may be expressed by the equation

$$\Delta R_M = \log\,(1/R_F^1 - 1) - \log\,(1/R_F^0 - 1) \tag{1}$$

where R_F^0 denotes the R_F valve on the unimpregnated support and R_F^1 that on the salt-impregnated support.

In the HPLC technique we should consider analogously the equation

$$\Delta V_M = \log\,(V_R^1/V^D - 1) - \log\,(V_R^0/V^D - 1) \tag{2}$$

where V_R^0 is the retention volume on the unimpregnated support. V_R^1 the retention volume on the salt-impregnated support and V^D the dead volume.

Migration in the presence of a salt differs because of complex formation. Most ligands exhibit retardation on the impregnated compared with the unimpregnated support. The greatest retardation was observed for ligands that form the strongest complexes. Ligand 12 is an interesting exception where acceleration is observed.

In order to explain this behaviour, let us assume that the chromatographic process is described by a partition mechanism and the species ligand L, metal ion M, complex ML and anion A exist in the stationary phase (water) and ligand L, ion pair

TABLE I

RESULTS OF TLC MEASUREMENTS

Mobile phase*	Ligand	R_F^0	R_F^1 and ΔR_M	Salt							
				LiCl	NaCl	KCl	NH_4Cl	RbCl	CsCl	$SrCl_2$	$BaCl_2$
I	1	0.75	R_F^1	0.68	0.73	0.80	0.65	0.73	0.83	–	–
			ΔR_M	0.15	0.05	– 0.12	0.21	0.05	– 0.21		
I	2	0.80	R_F^1	0.65	0.63	0.65	0.60	0.70	0.75	–	
			ΔR_M	0.33	0.37	0.33	0.42	0.23	0.12		
I	3	0.80	R_F^1	0.88	0.65	0.55	0.68	0.60	0.60	–	–
			ΔR_M	– 0.27	0.33	0.51	0.27	0.42	0.42		
II	4	0.60	R_F^1	0.10	0.13	0.15	0.23	0.08	0.33	0.13	0.18
			ΔR_M	1.13	1.01	0.93	0.7	1.24	0.49	1.01	0.84
II	5	0.63	R_F^1	0.60	0.10	0.08	0.13	0.45	0.43	–	–
			ΔR_M	0.05	1.18	1.29	1.06	0.32	0.35		
II	6	0.62	R_F^1	0.80	0.20	0.15	0.22	0.20	0.37	–	–
			ΔR_M	– 0.39	0.81	0.96	0.76	0.81	0.44		
II	7	0.62	R_F^1	0.50	0.27	0.20	0.25	0.15	0.17	0.52	0.20
			ΔR_M	0.21	0.64	0.81	0.69	0.96	0.9	0.18	0.81
II	8	0.70	R_F^1	0.62	0.27	0.22	0.30	0.17	0.15	–	–
			ΔR_M	0.16	0.8	0.92	0.74	1.06	1.12		
II	9	0.65	R_F^1	0.70	0.53	0.68	0.75	0.68	0.70	–	
			ΔR_M	– 0.1	0.22	– 0.06	– 0.21	– 0.06	– 0.1		
I	10	0.80	R_F^1	0.88	0.70	0.68	0.75	0.78	0.83	–	–
			ΔR_M	– 0.27	0.23	0.27	0.12	0.05	– 0.09		
III	11	0.74	R_F^1	0.74	0.70	0.47	–	0.25	0.29	0.56	0.74
			ΔR_M	0	0.08	0.5		0.93	0.84	0.35	0
IV	12	0.16	R_F^1	0.16	0.56	0.75	–	0.64	0.55	–	–
			ΔR_M	0	– 0.82	– 1.2		– 0.97	– 0.81		
III	13	0.81	R_F^1	–	–	0.78	–	–	–	–	–
			ΔR_M			0.08					

* Composition of mobile phases: I = chloroform–methanol (4:2); II = acetone–chloroform (3:2); III = acetone–chloroform (1:4); IV = acetone–methanol (1:1).

TABLE II

RESULTS OF HPLC MEASUREMENTS

Mobile phase for all ligands = dioxane–heptane (4:1).

Ligand	V_R^0	V_R^1 and ΔV_M	Salt		
			NaCl	KCl	CsCl
1	0.74	V_R^1	1.87	1.41	1.10
		ΔV_M	0.59	0.43	0.28
6	1.14	V_R^1	3.43	4.32	1.53
		ΔV_M	0.59	0.7	0.18
7	0.52	V_R^1	0.93	1.30	2.36
		ΔV_M	0.53	0.74	1.07
8	0.45	V_R^1	0.70	1.00	1.99
		ΔV_M	0.54	0.81	1.21
9	0.39	V_R^1	0.39	0.41	0.40
		ΔV_M	0	0.17	0.09

MA and ion pair MLA in the organic mobile phase. Let us define equilibrium constants:

$$k_L = (L)/[L]; k_{MLA} = (ML)(A)/[MLA] \tag{3}$$

where (X) is the concentration of substance X in the stationary phase and $[X]$ is the concentration of X in the mobile phase. The average partition coefficient of the ligand L in the presence of salt MA is expressed by

$$k^{av} = [(L) + (ML)]/([L] + [MLA]) \tag{4}$$

Applying the known equations

$$V_R^1 = V^D (1 + k^{av})$$
$$V_R^0 = V^D (1 + k_L) \tag{5}$$

we obtain

$$\Delta V_M = \log k^{av} - \log k_L \tag{6}$$

The relative difference between k^{av} and k_L:

$$(k^{av} - k_L)/k_L = \frac{(k_{MLA} - k_L(A)) (ML)}{k_{MLA} (L) + k_L (A) (ML)} \tag{7}$$

$$k^{av} < k_L \Leftrightarrow k_{MLA} < k_L (A) \tag{8}$$

$$k^{av} > k_L \Leftrightarrow k_{MLA} > k_L (A) \tag{9}$$

According to eqns. 5, chromatographic acceleration, as a consequence of impregnation, takes place for those salts MA and ligands L for which relation 8 holds. Consequently, retardation occurs when relation 9 is valid. Relation 8 means that the complex prefers the mobile phase to the stationary one more than the free ligand does, and relation 9 means the opposite.

The model described is of course a simplified one. To prove its adequacy we chose compound 13 of macrocyclic structure, *i.e.*, similar to other compounds considered in this work. It has been reported[9] that it does not form complexes with potassium ion at any pH value. Results of TLC measurements on this substance (Table I) confirmed that for non-complexing agents the R_F values are the same for both sides of the plate. We have therefore assumed that complexation is the decisive, essential factor influencing the R_F changes, *i.e.*, modification of the physical properties of the phases due to impregnation may be neglected.

For ligands 1–11 we usually observe a retardation of migration, implying that relation 9 is valid. However, impregnation with lithium salts in some instances causes a reversed behaviour of the ligand. For compound 9, which forms weak complexes, the ΔR_M values are usually negative, implying a grain effect, which can be neglected for strong complexes.

Cryptand [2.2.2] is a special case. It illustrates the other possibility expressed by relation 8, *i.e.*, that the complex is less polar than the free ligand. It is possible to explain this fact by structural conversion from the out–out structure of the ligand to the in–in conformation of its complexes[10].

CONCLUSIONS

The chromatographic method described permits the following:

(a) the estimation of the complex-forming ability;

(b) the identification of the ionophoric properties of compounds present in a reaction mixture (coronands, cryptands, some podands) or in a mixture of natural products (antibiotics);

(c) the separation of compounds of identical R_F values if at least one of them forms complexes with salts; this may be important for the purification of ionophores by column chromatography or preparative HPLC;

(d) information to be obtained about the interactions of the complex and the free ligand with the solvent.

The determinations may be carried out quickly and with the use of trace amounts of pure compounds in addition to mixtures of compounds.

ACKNOWLEDGEMENT

REFERENCES

1 Yu. A. Ovchinnikov, I. T. Ivanov and A. M. Shkrob, *Membranoaktivnije Kompleksoni*, Nauka, Moscow, 1974.
2 M. M. Htay and O. Meth-Cohn, *Tetrahedron Lett.*, (1976) 469.
3 A. R. Butler, *J. Chem. Soc., Perkin Trans. 1*, (1975) 1557.
4 V. Takaki, T. E. Hogen Esch and J. Smid, *J. Am. Chem. Soc.*, 93 (1971) 6760.
5 N. S. Poonia, *J. Am. Chem. Soc.*, 96 (1974) 1012.
6 J. F. Biernat and T. Wilczewski, *Pol. J. Chem.*, 53 (1979) 513.
7 B. Vonach and G. Schomburg, *J. Chromatogr.*, 149 (1978) 417.
8 K. Macek and Z. J. Vejdelek, *Nature (London)*, 176 (1955) 1173.
9 J. S. Bradshaw, H. Koyama, N. K. Dalley, R. M. Izatt, J. F. Biernat and M. Bocheńska, *J. Heterocycl. Chem.*, 24 (1987) 1077.
10 J. Cheney, J. P. Kintzinger and J. M. Lehn, *Nouv. J. Chim.*, 2 (1978) 411.