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CHIRAL AMINOACID CONTAINING ACYCLIC LIGANDS - II. COMPLEXATION OF ALKALINE EARTH CATIONS

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Abstract — The binding abilities of the title ligands for alkali and alkaline earth cations are estimated by ^1H and ^{13}C NMR and by picrate extraction from water to methylene chloride. These ligands are shown to be excellent complexing agents for alkaline earth cations. Complexation occurs preferably at the ether and amide carbonyl groups. The stoichiometry of complexation is evaluated by ^1H NMR.

We have previously reported[1] the syntheses of new chiral aminoacid containing acyclic ligands, whose general structure is reported in Fig. 1.

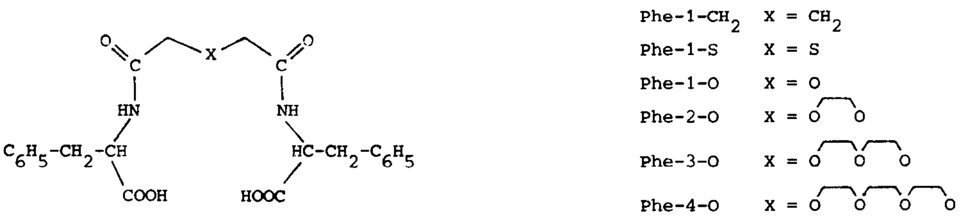


Fig. 1. General structure of the ligands. The abbreviations Phe-1-O for the ligand possessing one ether oxygen, Phe-2-O two ether oxygens, etc. will be used hereinafter.

They are characterized by the presence of two terminal carboxyl groups, which frequently act as binding sites for complexation by enzymes and antibiotics, and seem to be specially required in complexing the alkali and alkaline earth metal cations and effecting their transfer across natural or synthetic membranes.[2]

These ligands were shown[1] to assume preferential conformations in different solvents, according to the possibility of establishing H bonding within themselves or with the solvent. Moreover, in apolar solvents they could enforce their open structures into pseudocyclic conformations *via* strong intramolecular H bonding, thus enhancing the complex stability.

In the present paper we discuss the ability of these ligands to bind alkali and alkaline earth cations by ^1H and ^{13}C NMR spectroscopy and by picrate extraction from water into methylene chloride.

^1H NMR studies on complexation. ^1H and ^{13}C NMR spectroscopy proved to be extremely useful for recognizing complexation at certain binding sites in solution.[3-5] We used anhydrous methanol, a solvent for which many data are available for crown ethers[6] and other ligands.[7] A qualitative estimate of the extent of complexation was obtained by adding a large excess of a solid salt of divalent cations (Ca^{++}, Sr^{++}, and Ba^{++}) to a methanol solution of each ligand. No new signal appears in the spectra, whereas those due to the methylene adjacent to the ether and to the amide carbonyl groups undergo downfield shifts, in agreement with the literature[3,4] (see Fig. 2). We take this behaviour as evidence

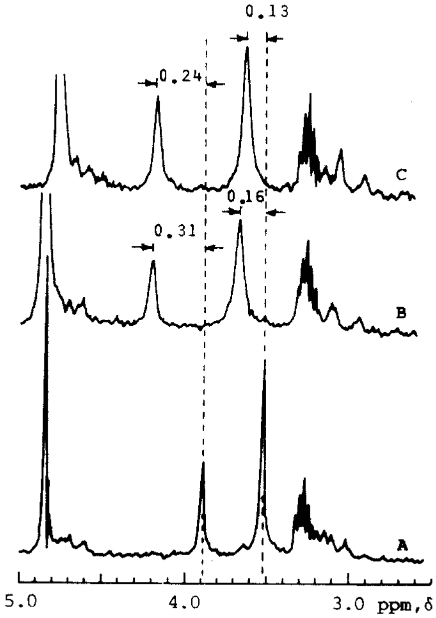


Fig. 2. ^{1}H NMR spectra of 0.1 M solution of Phe-3-O in CD_3OD: (A) free ligand; (B) with $Ca(SCN)_2$ [M]/[L] = 1; (C) with $Ba(SCN)_2$ [M] / [L] = 1.

for the occurrence of complexation, ether and amide carbonyl oxygens acting as cooperative binding sites for the cations.

In contrast, no (or very small) complexation is observed with the alkali cations (Li^+, Na^+, and K^+) (see Table 1).

Four anions, SCN^-, Cl^-, Br^-, and ClO_4^- were used in these studies and were found to have little effect on the spectral characteristics of the complexes, indicating that ion pairing is unimportant in this solvent.

The presence of the oxygens in the bridge seems to be specifically required for the binding of alkaline earth cations. In fact, very small or no shifts are observed for the analogous ligands Phe-1-CH_2 and Phe-1-S.

There is also a certain size-dependence in the complexation, since ligand Phe-1-O exhibits the largest induced shift (0.37 ppm) for Ca^{++} and ligand Phe-4-O shows a strong interaction with Ba^{++} and Sr^{++} (0.30 ppm) (see Table 1).

Stoichiometry of complexation. ^{1}H NMR spectroscopy was utilized to obtain the stoichiometry

Table 1. ^{1}H NMR salt-induced chemical shifts for complexes[a] in CD_3OD

	Phe-1-CH_2	Phe-1-O	Phe-1-O	Phe-2-O		Phe-3-O		Phe-4-O	
	CH_2CH_2CO	SCH_2CO	OCH_2CO	OCH_2CO	OCH_2CH_2O[b]	OCH_2CO	OCH_2CH_2O[b]	OCH_2CO	OCH_2CH_2O[b]
Free Ligand	*2.10*	*3.30*	*3.86*	*3.86*	*3.48*	*3.91*	*3.54*	*3.86*	*3.55*
$LiClO_4$	2.10	3.30	3.86	3.86	3.48	3.91	3.54	3.86	3.55
NaSCN	2.10	3.30	3.87	3.88	3.48	3.93	3.55	3.87	3.56
KSCN	2.10	3.30	3.87	3.86	3.48	3.95	3.55	3.90	3.56
$Ca(SCN)_2$	2.10	3.36	4.23	4.15	3.69	4.22	3.70	4.09	3.66
$CaCl_2$	2.10	3.34	4.19	4.15	3.66	4.22	3.67	4.09	3.63
$SrBr_2$	2.10	3.34	4.10	4.12	3.61	4.13	3.63	4.16	3.66
$Ba(SCN)_2$	2.10	3.36	4.10	4.06	3.58	4.15	3.67	4.16	3.66

[a] Values (in ppm) obtained at a M/L = 1 molar ratio. [b] Center of multiplet.

of complexation.[4] Subsequent increments of a salt are added to the ligand until there is no further change in the spectrum. The induced shifts are linear up to a 1:1 cation:ligand ratio for both $COCH_2O$ and OCH_2CH_2O protons for Phe-2-O, Phe-3-O and Phe-4-O with Ca^{++} and Ba^{+} Thus it is possible to conclude that the stoichiometry of complexation is 1:1 in methanol when the concentration of the ligand is kept at a value of 0.1 M (see Fig. 3).

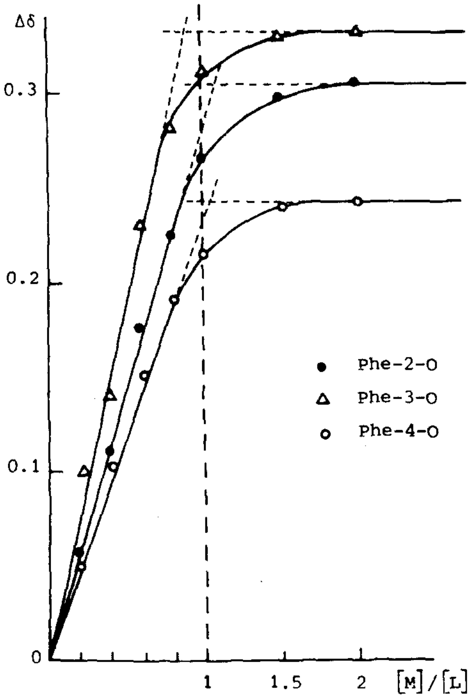


Fig.3. ^1H NMR chemical shift variation of the OCH_2CO signals of Phe-2-O, Phe-3-O and Phe-4-O *vs* $[Ca(SCN)_2]/[L]$ ratio in CD_3OD

In contrast, ligand Phe-1-O forms a 1:2 (metal: ligand) complex with calcium and barium thiocianates (see Fig. 4).

^{13}C NMR studies on complexation. ^{13}C chemical shifts and line assignments for the ligands and their Ca^{++} complexes in methanol are given in Table 2 and in Fig. 5. As it can be seen, the

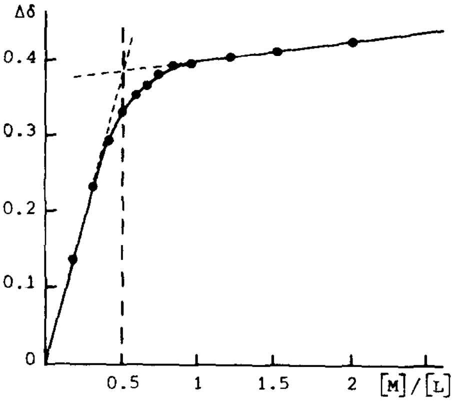


Fig. 4. ^1H NMR chemical shift variation of the OCH_2CO signal of Phe-1-O *vs* $[Ca(SCN)_2]/[L]$ ratio in CD_3OD

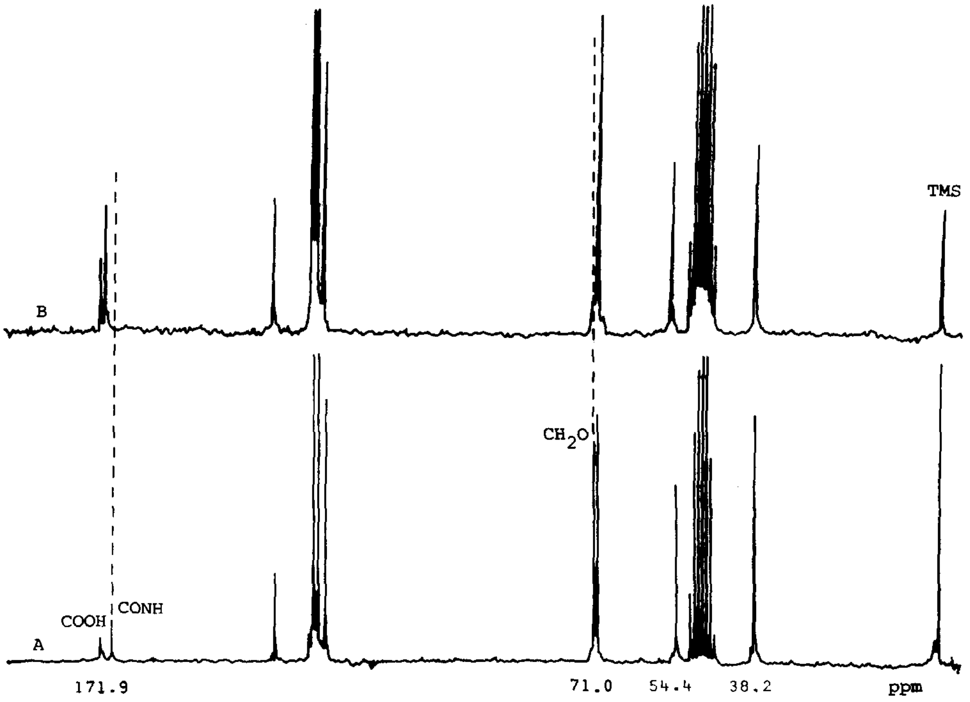
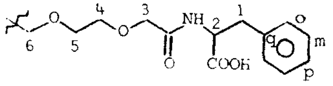


Fig.5. ^{13}C NMR spectra of Phe-2-O in CD_3OD: (A) free ligand; (B) with $Ca(SCN)_2[M]/[L] = 1$

Table 2. ^{13}C NMR shifts of ligands in their free form and in their Ca^{++} complexes[a] in CD_3OD

Carbons[b]	Phe-1-CH_2		Phe-1-O		Phe-2-O		Phe-3-O		Phe-4-O	
COOH	175.6	[175.6]	174.0	[173.7]	174.2	[173.7]	174.0	[173.6]	173.8	[173.8]
CONH	175.1	[175.1]	171.6	[172.9]	171.9	[172.9]	171.9	[172.9]	171.6	[172.8]
C_q	138.1	[138.1]	137.8	[137.7]	138.0	[137.8]	137.9	[137.7]	137.8	[137.8]
C_{meta}	130.0	[130.0]	130.0	[130.0]	130.2	[130.0]	130.1	[130.0]	130.0	[130.0]
C_{ortho}	129.2	[129.2]	129.0	[129.3]	129.3	[129.3]	129.1	[129.3]	129.3	[129.9]
C_{para}	127.6	[127.6]	127.2	[127.6]	127.7	[127.6]	127.5	[127.6]	127.6	[127.7]
C_3	34.9	[34.9]	71.8	[70.6]	71.6	[70.5]	71.6	[70.6]	71.6	[70.1]
C_4					71.0	[70.3]	71.0	[70.4]	71.0	[70.1]
C_5							70.7	[70.2]	70.7	[69.6]
C_6									70.5	[69.6]
C_2	55.2	55.2	54.2	55.3	54.4	[55.2]	54.2	[55.3]	54.2	[55.2]
C_1	38.1	38.1	38.2	38.1	38.2	[38.0]	38.1	[38.1]	38.1	[38.1]

[a] At the ratio [M]/[L]=1 for M = $Ca(SCN)_2$ reported in brackets. [b] Numbers of carbons indicate their assignments as follows:



resonances of the methylene groups adjacent to the amide carbonyl and to the ether oxygens present a distinctive upfield shift, as expected for binding at the oxygen.[5] Moreover, a significant downfield shift (1 ppm) is observed for the amide carbonyl, which is, therefore, greatly involved as a specific binding site in the complexation.

In contrast, the carboxyl groups seem to be little affected by complexation, even when using a large excess of salt. Neither they are depotronated, since their chemical shift is completely different from that of the same ligand at pH = 6 (177.7 ppm), where carboxylates are expected to be formed.

These results seem to be in favour of a cation-neutral ligand complex, where the two terminal carboxyl groups are not involved in complexation, being completely solvated by methanol.

Picrate extraction experiments. In order to have a rapid screening of the relative complexing abilities of the different ligands, we used the technique of extracting metal cation picrates from water into an organic phase (methylne chloride).[8] Relatively high concentration of ligands ($7.5 \cdot 10^{-2}$ M) and salts ($1.5 \cdot 10^{-2}$ M) were used so that complexes might be considered fully associated in the organic phase.[9] All ligands, but Phe-1-O, are sufficiently lipophilic to allow extraction experiments to be made at such concentrations. Cations were evaluated by atomic absorption and the anion (picrate) by UV-VIS spectroscopy and gave values practically consistent so that it appears that neutral ligand coordinated ion pairs are present in the apolar solvent.

The stoichiometry of the complexes in methylene chloride was tentatively assigned by evaluating the maximum of the main optical absorption band of picrates in this phase and by assuming the formation of a 1:1 ligand coordinated tight ion pair complex when λ_{max} ~350 nm and a 2:1 ligand separated ion pair complex when λ_{max} ~377 nm, in agreement with what reported in the literature.[10]

Ligand Phe-4-O gives tight ion pair complexes 1:1 with all cations (λ_{max} = 342 nm for Li^+ to λ_{max} = 354 nm for Ba^{++} and K^+). Phe-3-O seems to give 1:1 complexes with divalent cations (λ_{max} = 346 nm for Ca^{++}; λ_{max} = 352 nm for Ba^{++}) and 2:1 complexes with alkali cations (λ_{max} = 373 nm). With Phe-2-O a weaker bathochromic shift is observed for Ba^{++} (λ_{max} = 363 nm) so that both 1:1 and 2:1 complexes might be simultaneously present under these conditions. Formation of a 1:1 complex for Ca^{++} and Phe-2-O (λ_{max} = 355 nm) is not surprising since there is evidence (^1H NMR) that water molecules are also extracted in the organic phase, which could complete the coordination spheres of the cations. The preferencial formation of 2:1 complexes with alkali metals (λ_{max} ~372 nm) is in agreement with the reported participation of only one amidic carbonyl of analogous

Table 3. Extraction constants (K_e)[a] of picrates of alkali and alkaline earth cations by ligands Phe-n-O in methylene chloride[b]

	Li^+		Na^+		K^+		Ca^{++}	Ba^{++}	
Ligand	(1:1) M^{-2}	(2:1) M^{-3}	(1:1) M^{-2}	(2:1) M^{-3}	(1:1) M^{-2}	(2:1) M^{-3}	(1:1) M^{-3}	(1:1) M^{-3}	(2:1) M^{-4}
Phe-2-O	-	$6.7 \cdot 10^2$	-	$3.21 \cdot 10^3$	-	$1.00 \cdot 10^3$	$1.08 \cdot 10^5$	$1.14 \cdot 10^5$	$5.03 \cdot 10^6$
Phe-2-O-EE[c]	-	-	-	-	-	-	$1.61 \cdot 10^3$	$1.96 \cdot 10^3$	$2.66 \cdot 10^4$
Phe-3-O	-	$7.27 \cdot 10^2$	-	$6.59 \cdot 10^3$	-	$2.14 \cdot 10^3$	$5.67 \cdot 10^4$	$5.40 \cdot 10^5$	-
Phe-4-O	$2.9 \cdot 10$	-	$6.26 \cdot 10$	-	$1.95 \cdot 10^2$	-	$1.22 \cdot 10^5$	$1.84 \cdot 10^6$	-
Phe-4-O-EB[d]	-	-	-	-	-	-	$8.13 \cdot 10^3$	$8.94 \cdot 10^5$	-

[a] K_e values are given by an average of three measurements; complex stoichiometry is given in brackets.

[b] Initial concentrations: $[Ligand]_{org} = 7.5 \cdot 10^{-2}$ M; $[Metal\ Picrate]_{aq} = 1.5 \cdot 10^{-2}$ M; it is assumed that ligands remain diprotonated in the organic phase and that the metal picrate ligand complex is not soluble in water. In order to allow a dimensionally equivalent comparison M^{++}/M^+ we report here the percentages of extraction (%): Phe-2-O Li^+ 3, Na^+ 15, K^+ 6, Ca^{++} 38, Ba^{++} 49; Phe-2-O-EE Ca^{++} 2.5, Ba^{++} 3; Phe-3-O Li^+ 2, Na^+ 16, K^+ 12, Ca^{++} 30, Ba^{++} 58; Phe-4-O Li^+ 3, Na^+ 6, K^+ 15, Ca^{++} 40, Ba^{++} 70; Phe-4-O-EB Ca^{++} 10, Ba^{++} 65.

[c] Ethyl ester.

[d] Benzyl ester.

ligands[11] to complexation.

The extraction equilibrium constants K_e determined by using eq. 1 in the Experimental section are reported in Table 3. The K_e values are a composite of several equilibria which taken together define the amount of ions extracted from an aqueous phase to an organic phase.[12]

All ligands show a high M^{++}/M^{+} selectivity, thus confirming the role played by the ligand thickness[13] and the importance of the basic system featuring ether oxygens and two amide groups.[14,15] The selectivity $Ba^{++} > Ca^{++}$ is not surprising when considering the free energies of hydration of the cations[16] and the relatively large picrate counter ion.[17]

However, a very high extraction constants ($K_e = 1.31 \cdot 10^{8}\ M^{-4}$) is obtained for a Phe-2-O: Ca^{++} 2:1 complex when using a larger excess of ligand (10:1).

The extraction strenght of the dicarboxylic ligands for Ca^{++} and Ba^{++} is comparable to that of structurally related 3,6-dioxaoctanedioic diamides.[12] As confirmed by ion extraction studies,[18] the latter ligands appeared to be superior complexing agents for alkaline earth metal ions relative to the macrocyclic analogues.

Moreover, the ion extraction ability of the esters (ethyl and benzyl in Table 3) is inferior to that of the acids, so that it appears that the carboxyl groups do inhance the stability of complexes in methylene chloride.

This could imply a pseudocyclization *via* H-bonding formation either between the convergent carboxyl groups[19] or *via* interposed water molecules, as observed in antibiotics belonging to the nigericin group.[20] The picrate anions could also be linked by H-bonding to the carboxyl group.[21]

In conclusion, being highly selective for divalent cations, these ligands are especially attractive in view of their use as carriers in investigations concerning biological systems, which are presently under study.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-100 spectrometer (Fourier transform) and the chemical shifts were measured in ppm from TMS. UV spectra were measured on a Jasco Uvidec 505 spectrophotometer. Metal ion concentrations in water were obtained by atomic absorption measurements using a Perkin-Elmer model 303 instrument.

Titration method. Alkali and alkaline earth metal salts, dried under vacuum overnight, were added in a 5-fold excess to a 0.1 M solution of ligand in anhydrous CD_3OD and the ^{1}H and ^{13}C NMR spectra were recorded.

The stoichiometry of binding was determined by the mole-ratio method: a weighted quantity of ligand was dissolved in 1.0 ml of CD_3OD to give a 0.1 M solution. The solution containing the salt was added (1 equivalent at one time) by a 10 µl syringe so that volume changes could be neglected up to a $[M]/[L] = 3$ molar ratio and the ^{1}H NMR spectra were recorded.

Ligand solubility. Ligand partition coefficients between methylene chloride and water ($P_e = [L_{aq}] / [L_{org}]$) were determined by equilibration of the two phases as in the extraction procedure (initial concentration: $7.5 \cdot 10^{-2}$ M in the organic phase). The following values were obtained: Phe-1-O 1.37; Phe-2-O $4.2 \cdot 10^{-2}$ Phe-3-O $2.6 \cdot 10^{-2}$; Phe-4-O $2.4 \cdot 10^{-2}$; Phe-2-O-EE and Phe-4-O-EB $1.01 \cdot 10^{-2}$.

Picrate extraction procedure. The method was essentially that of Frensdorff,[8] modified by using aqueous solutions of metal picrates, previously prepared and recrystallized ($1.5 \cdot 10^{-2}$ M). Ligands were dissolved in methylene chloride ($7.5 \cdot 10^{-2}$ M). Equal volumes of the two solutions (1 ml) in stoppered centrifuge tubes were mixed with a small bar (5 mm long) on a megnetic stirrer for two h to ensure complete equilibration. Centrifugation was carried out to obtain complete phase separation. The extractions were conducted at 25±1 °C. Picrate concentrations were determined in both phases: the direct value and that obtained by difference from the aqueous phase usually agreed well. The aqueous ($1.45 \cdot 10^{4}\ cm^{-1}\ M^{-1}$ at $\lambda_{max} = 354$ nm) and the CH_2Cl_2 extinction coefficient ($1.8 \cdot 10^{4}\ cm^{-1}\ M^{-1}$ at $\lambda_{max} = 370$ nm) were obtained by Frensdorff.[8]

The extraction constants K_e were evaluated from the eq. 1 for alkali and alkaline earth metal picrates.[12]

$$M^{z+}(aq) + zPic^{-}(aq) + nL(org) \overset{K_e}{\rightleftharpoons} ML_nPic(org) \quad (1)$$

$$K_e = \frac{[ML_nPic_z]_{org}}{[M^{z+}]_{aq}[Pic^{-}]_{aq}^{z}[L]_{org}^{n}}$$

$[L]_{org}^{n}$ = ligand concentration in the organic phase (n = 1, 2); $[M^{z+}]_{aq}$ = metal concentration in the aqueous phase (z = 1, 2); $[Pic^{-}]_{aq}$ = anion concentration in the aqueous phase; $[ML_nPic_z]_{org}$ = complex concentration in the organic phase; n = complex stoichiometry. Activity coefficient for all ions were taken to be unity.

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