the distinction is clear. Cross peaks in the lower part of this ROESY spectrum show exchange of peaks C1 and C2 with a peak under B2 and with the peak B1, respectively, and the peak D with the small shoulder just downfield of B2.

Thus, spinach ACP exists in at least two conformationally discrete forms in slow exchange. It is possible that related proteins that show a single set of resonances, such as *E. coli* ACP, do this more by virtue of a shift in time scales of interconversion than by elimination of one of the discrete conformational forms.[7] This possibility suggests caution in the indiscriminate application of a rigid model in the determination of protein structures from NMR data for ACPs as well as for other proteins.

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Alkali Metal Ion Complexation of Crown Ethers and Related Ligands Studied by Californium-252 Plasma Desorption Mass Spectrometry

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The potential for ^{252}Cf plasma desorption mass spectrometry (PDMS) to be used in the study of the relative complexation of crown ethers and other ligands with different alkali-metal cations has been investigated. In the PD spectra of different ligands after exposure to a mixture of alkali-metal salts (LiCl, NaOAc, and KOAc), it was found that the ratio of molecular ion abundances reflected the relative complexation trends.

Crown compounds and cryptands have many applications in analytical chemistry. Variable ring sizes as well as type, number, and position of the donor atoms in the ring permit a selective adaptation to specific cations. Monomeric cyclic polyethers are used mainly in the separation and determination of elements of the first and second main groups of the periodic table.[1] Several methods have been employed for the determination of the complexation constants of crown ethers, and these have been discussed in a number of reviews.[2]

^{252}Cf PDMS has previously been employed for the determination of molecular weights of large involatile molecules.[3] It has hitherto found extensive use in studies of peptides, proteins, and related compounds as well as porphyrins and related compounds;[5] its use for the characterization of polyethers has also been described.[6] Recently it has been shown[7] that fast atom bombardment mass spectrometry (FAB-MS) can be used in determination of the crown ether–alkali cation stability constants. Here we report the use of ^{252}Cf PDMS for analysis of crown ethers and their relative complexation with alkali-metal cations. The technique takes advantage of the nonsolubility of the studied compounds in aqueous solutions when these are fixed on a nitrocellulose matrix and thereby the possibility of including the cation by washing a sample with an alkali-metal salt solution.

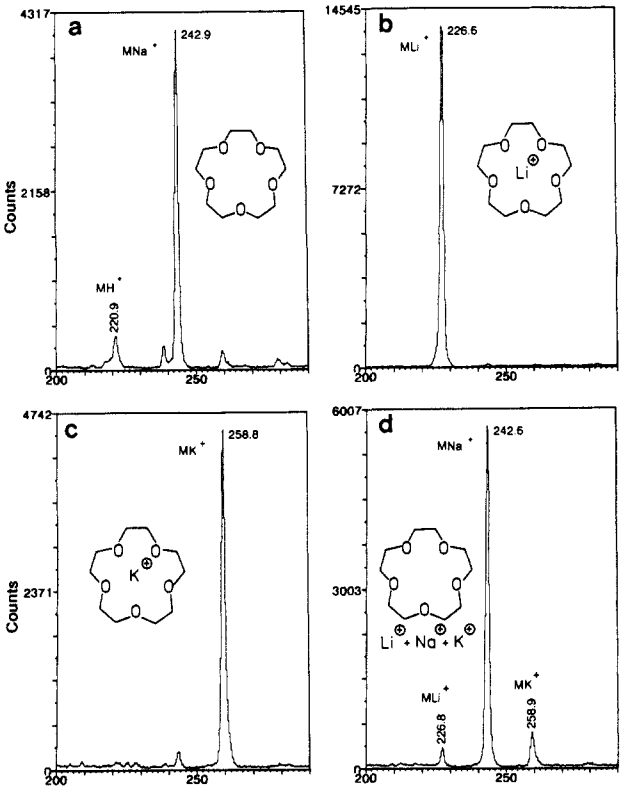
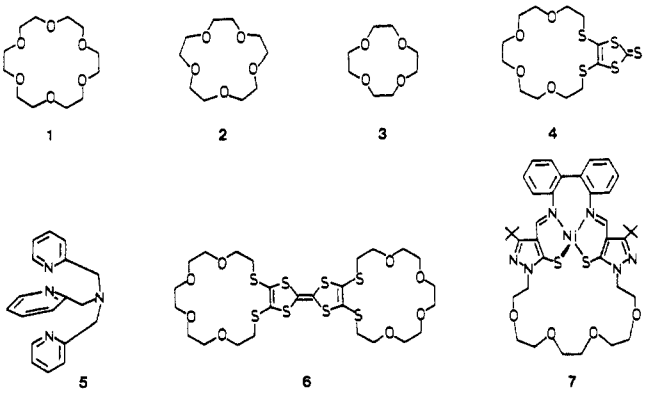


Figure 1. Positive-ion Pd spectra of 15-crown-5 (**2**): (a) The commercial sample dissolved in 100% TFA. (b) After complexation with a 0.1 M solution of LiCl. (c) After complexation with a 0.1 M solution of KOAc. (d) After complexation with a 0.1 M (1:1:1) solution of LiCl–NaOAc–KOAc.

The positive-ion ^{252}Cf Pd spectra of compounds **1–7** are summarized in Table I. All spectra were obtained[8] without attempts to remove the cations already present in the sample. For com-



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(8) The samples of compounds **1–7**[13] were applied on a nitrocellulose matrix prepared by electrospraying 25–50 μL of a 2μg/L solution of nitrocellulose (Bio-Rad Laboratories, Richmond, CA) in acetone onto an aluminized Mylar foil. The samples were dissolved in 100% trifluoroacetic acid (TFA) to a concentration of 1 μg/μL. Between 2 and 3 μL of this solution was slowly deposited on the nitrocellulose matrix with simultaneus evaporation of the solvent. For exchange of the cation or for determination of the relative complexation, 2 μL of a 0.1 M solution of LiCl, NaOAc, or KOAc or an equimolar mixture of all three was applied directly on the nitrocellulose-bound sample. After a few minutes (to allow the ion-exchange reaction), the matrix was dried by spinning of the target. The plasma desorption mass spectra were obtained on a Bio-ion Bin 10k plasma desorption instrument (Bio-ion AB, Uppsala, Sweden). The instrument and data handling procedures have been described earlier.[14] The spectra were accumulated for 500 000 fission events. A smooth background has been subtracted from all spectra.

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Table I

compd no.	av mol mass	PDMS[a]	unresolved av mol mass of mol ion (m/z)[b] LiCl	NaOAc	KOAc	rel mol ion ratio[c] Li^+	Na^+	K^+
1	264.3	282.3 ($M^+ + H_2O$), 287.2 ($M + Na^+$)	269.9	286.0	302.1	1.0	17.2	23.4
2	220.2	243.6 ($M + Na^+$), 221.6 (MH^+)	225.9	242.2	258.3	1.0	15.5	2.0
3	176.2	177.2 (MH^+), 199.6 ($M + Na^+$)	183.1	199.3	215.6	1.0	3.0	0.6
4	400.6	401.1 (MH^+), 424.0 ($M + Na^+$)	406.5	424.2	438.8	1.0	3.8	1.5
5	290.4	290.8 (MH^+), 313.2 ($M + Na^+$)	296.5	312.6	328.7	1.0	0.3	
6	737.1	737.5 (MH^+)		760.3	776.2	1.0	0.2	
7	775.6	775.3 (M^+)		798.4	814.7		1.0	2.7

[a] Ions observed. [b] Observed upon wahing with a 0.1 M solution of LiCl, NaOAc, or KOAc. [c] Upon washing with a solution of a 1:1:1 mixture of LiCl-NaOAc-KOAc.

pound **1**, $(M + H_2O)^+$ and $(M + Na)^+$ and no free molecular ion MH^+ were observed.[9] This shows that the cavity size is big enough to incorporate a water molecule and consequently MH^+ was not observed. Compound **2** showed a single peak corresponding to $(M + Na)^+$ and a very weak peak corresponding to MH^+ (Figure 1a). For all other compounds **3–7**, both $(MH)^+$ and $(M + Na)^+$ peaks were observed. This shows that commercial samples of these compounds already contain sodium ions, and therefore, precautions must be taken during the purification of such ligands in order to exclude the alkali-metal ions completely. Each of these samples was fixed on a nitrocellulose matrix and then separately washed with 0.1 M solutions of either lithium chloride, sodium acetate, or potassium acetate, respectively, followed by mass spectrometric analysis. In each case, the molecular ion showed complete complexation of the ligands with the respective cations (Li^+, Na^+, or K^+) (Table I, Figure 1b,c).

The next step was to investigate if the mass spectra were able to reflect the relative binding affinities of different alkali-metal ions, i.e., the specificity of different cavities of the ligands present on the matrix. Compounds **1–7** were washed, after application on the mass spectrometric target, with a 0.1 M solution of a mixture of all three cations (Li^+, Na^+, K^+) (Figure 1d). The absolute number of ions found for each molecular ion species was measured and the relative molecular ion ratio calculated and normalized relative to $(MLi)^+$ (Table I). From Table I, the following relative binding affinities can be established, e.g., for compound **1**, $K^+ > Na^+ \gg Li^+$; for compound **2**, $Na^+ \gg K^+ > Li^+$; and for compound **3**, $Na^+ > Li^+ > K^+$. These observations are consistent with results obtained with other methods used for measuring the complexation with alkali-metal ions.[10] For compound **5**, the relative affinity determined here was in agreement with that measured by calorimetry.[11]

Comparison of compounds **1** and **4** shows that, although the sizes of the rings are comparable,[12] the binding capacity for sodium and potassium relative to lithium ions is reduced for compound **4** relative to compound **1**. The reason for this is that the orbitals of the sulfur atoms present in the macrocyclic ring take up more space than the similar oxygen atoms in compound **1** and hence reduce the actual cavity size.[12] In compound **6**, the cavity size is too small to accommodate potassium, and preferential binding of a lithium ion is therefore observed. Compound **7** has a large cavity, and consequently, potassium is favored in this case.

This study clearly demonstrates that positive-ion ^{252}Cf PDMS reflects the relative binding trend of different alkali-metal ions with crown ethers and related ligands. Because of the simplicity and ease of interpretation, this technique therefore provides a simple, rapid, and qualitative determination of the relative complexation between different metal ions and crown ethers. It is a further advantage that only a very small amount of sample is necessary for such studies.

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Absolute Stereostructure of Swinholide A, a Potent Cytotoxic Macrolide from the Okinawan Marine Sponge *Theonella swinhoei*

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In search of new biologically active substances from marine organisms,[1] we have isolated a potent cytotoxic macrolide swinholide A[2] and five tridecapeptide lactones named theonellapeptolides Ia, Ib, Ic, Id, and Ie from the Okinawan marine sponge *Theonella swinhoei*, and we have recently elucidated the absolute stereostructures of those tridecapeptide lactones[3] and the plain structure of swinholide A (**1**) having a 44-membered dimeric dilactone skeleton.[4]

The atomic array in the structure of swinholide A (**1**) is mostly like that of cytotoxic macrolide scytophycin C (**2**), which was isolated from the cultured terrestrial blue-green alga *Scytonema pseudohofmanni* and whose absolute configuration was determined on the basis of an X-ray crystallographic analysis by Prof. Moore and his group.[5] In order to clarify the stereochemical correlation between **1** and **2**, we have further investigated the stereostructure of **1** and have elucidated its absolute configuration by means of

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