One and two dimensional $^1$H and $^{13}$C high resolution NMR investigation of lariat ethers and their alkali metal ionic complexes: A more tangible evidence for the presence of less common C–H⋯O hydrogen bonds

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Abstract

The possible existence of less common hydrogen bonds in three lariat ethers and their alkali-metal ionic complexes have been investigated with one- and two-dimensional (1D and 2D) proton and carbon-13 high resolution liquid state NMR spectroscopy. The occurrence of hydrogen-bonding induced by the addition of metal ions has been identified with the observation of indirect dipolar coupling between the coupling partners involved in the hydrogen-bonding. The addition of metal ions, moreover, causes appreciable change of chemical shift of several protons and carbons. The chemical shift change depends on the ion radius, larger ions causing smaller change. Moreover, the change of chemical shift is in coincidence with the occurrence of hydrogen-bonding. The values of the coupling constants have been obtained for each of these hydrogen bonds and were used for evaluating the hydrogen-bond strength. An intriguing and surprising observation is that a C–H⋯O hydrogen bond identified in solution by this work was not found in the previous study with X-ray diffraction or other methods.

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1. Introduction

The structural and chemical similarities between lariat crown ethers and a number of biologically important enzymes make this type of compounds a favored model for biochemists as well as chemists. In particular, the presence of hydrogen-bonding in crown ethers (lariat) bound with metal ions has been an interesting and vigorously pursued subject because of its implications to protein structure [1] and the molecular mechanism of enzymatic reaction [2]. Previous studies [1,2] using X-ray and other methods such as binding constant determination and picrate extraction efficiency suggest the existence of hydrogen bonding between an aromatic hydrogen on the side arm and a nitrogen or oxygen on the crown macro-ring. These hydrogen bonds are believed to be analogous to those responsible for catalysis mechanism of some enzymes. While these studies are enlightening and offer interesting insights into this type of less common hydrogen-bonds, the most essential signature of hydrogen bonding, i.e., the overlap of wave-functions of the bonding partners, however, has not been directly confirmed. Moreover, even when a hard evidence for hydrogen-bonding is found in the solid state via, e.g., X-ray diffraction, it does not necessarily mean that the same hydrogen-bonding still occurs in the solution state reminiscent of biological/physiological environments. On the other hand, it is also possible, although seemingly rare, that more hydrogen bonds exist in solution state than in solid state. Therefore two important aspects are worth being addressed: (1) what should be attributed to be the direct and unambiguous evidence of this type of less common hydrogen bonds and (2) how to directly observe the hydrogen-bonds in the solution state which is closer to physiological conditions? In deed, we will present a case where hydrogen bonding was observed in the solution state but not reported in the crystalline state.
NMR has been employed to study hydrogen bonds in solutions and solids for a long time, the development of which has recently been reviewed by Limbach [3]. The prowess of NMR to identify and characterize the presence of H-bonds in various systems has been well demonstrated [3]. Furthermore, there has been a large body of literature on applying both liquid state and solid state NMR spectroscopy to the study of crown ethers, as reviewed by Buchanan [4]. We note that, however, all those studies focused on much stronger interactions such as chemical shift, three-bond J couplings, direct dipolar coupling and quadrupolar coupling. Besides, in all of those studies, only one-dimensional NMR spectroscopic methods were used, a number of interesting and important fine details about the systems being not revealed. Because of the weak magnitude of C–H–O hydrogen-bonds (thus the associated J-couplings) in this type of compounds, however, none has ever explored this topic with NMR. On the other hand, although much theoretical work has been devoted to the understanding and characterizing this type of less common hydrogen bonds [5–22] and experimental evidence for its presence in DNA systems [23–30] and proteins [31–33] has been provided, some debates remain whether it should be categorized as a hydrogen bond [34–36].

In this work, we report the result of applying liquid state NMR spectroscopy to investigate this problem. Based on various one-dimensional and two-dimensional (1D and 2D) proton and carbon-13 liquid and solid state NMR studies, including COSY, LR-COSY, TOCSY, NOESY, HSQC and CPMAS spectroscopy, we have found that for the three ethers we studied, hydrogen bonds are unambiguously formed as a result of the addition of a metal ion (sodium or potassium). The prudential execution and analyses of the long-range correlation spectroscopy combined with regular correlation and NOESY enable us to identify unambiguously the existence of this type of less common hydrogen bond when the lariat ethers are complexed with alkali metal ions.

The major principle underlying this work is that the protons close to the H-bond acceptor (oxygen on the macroring) can be used as a probe to detect the perturbation of its wavefunction caused by the H-bond donor (if the H-bond is real). The parameters related to the hydrogen bonds such as the relevant J-coupling constants, the distances between hydrogen bonding partners and the torsion angles can be elucidated and verified with these NMR results. Because the extra complications involved with the interpretation of solid state NMR spectra of these systems, only the solution NMR results are reported in this paper. The solid state NMR results will be presented separately.

2. Experimental section

The selected lariat ethers are: N,N’-Dibenzyl)-4,13-diaza-18-crown-6 (1), N,N’-Bis(1-naphthylmethyl)-4,13-diaza-18-crown-6 (2) and N,N’-Bis(9-anthryl-methyl)-4,13-diaza-18-crown-6 (3), as shown in Table 1. Two alkali metal ions, potassium and sodium, were chosen to be added to the ethers. Their molecular formulae are also shown in Table 1. Given in Table 1 were also the schemes for numbering protons on the macro ring and on the side-arms, which are followed when tabulating chemical shifts and other parameters obtained in this work.

2.1. Synthesis of lariat ethers

The synthesis of the lariat ethers was conducted by following the previously published procedures of Gatto et al. [37] for compound 1, Gatto and Gokel [38] for compound 2 and Kubo et al. [39] for compound 3. The yields were 29%, 90% and 90% for compounds 1–3, respectively.

2.2. Sample preparation

Sodium and potassium ions were added to the solutions by adding NaI, KI to the lariat ethers. NaI and KI were purchased from Sigma-Aldrich without further purification before use. The solvent was a mixture of CDC13 and TMS, or of CDC13, CD3CN or CD3OD as NMR chemical shift references. The measured samples were as follows: (1) The ethers were dissolved in CDCl3 to measure 1D 13C and various proton NMR spectra. (2) The three ethers were added with NaI with molar ratio of 1:1 and then added with CD3CN and CDCl3 (molar ratio 1:1) to measure 1D 13C and various proton NMR spectra. (3) The three ethers were added with KI with molar ratio of 1:1 and then added with CD3OD and CDCl3 (molar ratio 1:1) to measure 1D 13C and various proton NMR spectra. All solvents were purified to prevent possible acidic impurities.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Lariat ethers used in this work</th>
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<tr>
<td><img src="chart.png" alt="Diagram" /></td>
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<td>N,N’-Dibenzyl)-4,13-diaza-18-crown-6</td>
</tr>
<tr>
<td>2</td>
<td>N,N’-Bis(1-naphthylmethyl)-4,13-diaza-18-crown-6</td>
</tr>
<tr>
<td>3</td>
<td>N,N’-Bis(9-anthryl-methyl)-4,13-diaza-18-crown-6</td>
</tr>
</tbody>
</table>
2.3. NMR spectroscopy

Both one and two dimensional proton NMR spectroscopy were used for studying the pure and ion-bound ethers. The experiments were performed on both Varian VXR 300 MHz NMR Spectrometer and Varian Inova 500 MHz NMR Spectrometer.

For 1D $^1$H experiments, single 90° pulse (7 and 5.6 $\mu$s on 300 and 500 MHz NMR spectrometers, respectively) was used for excitation; the typical spectral width was 12 ppm, recycle delay 2 s and the number of transients 32.

For 1D $^{13}$C experiments, single 90° pulse (11 and 8 $\mu$s on 300 and 500 MHz NMR spectrometers, respectively) was used for excitation; the typical spectral width was 220 ppm, recycle delay 10 s and the number of transients 4000 (on the 300 MHz NMR spectrometer) and 1000 (on the 500 MHz NMR spectrometer).

A number of 2D experiments including homonuclear $^1$H COSY, TOCSY, LR-COSY, NOESY and heteronuclear $^1$H–$^{13}$C HSQC were carried out. For COSY, TOCSY and NOESY, usually a 256 (t1)×1024(t2) data matrix was used in acquisition and it then was expanded to 2048(t1)×2048(t2) by linear prediction and/or zero-filling for better resolution. The time increment in the first dimension was set to 300 $\mu$s (for 500 MHz NMR spectrometer) and 180 $\mu$s (for 500 MHz NMR spectrometer). For NOESY spectra, 20–500 $\mu$s of mixing times were used. For all these 2D spectra, 32 transients were accumulated.

For LR-COSY spectra, a 512 (t1)×1024(t2) data matrix was used with first dimension time increment the same as COSY measurements; the extra delays used were from 80 to 120 ms. The data matrix was also zero-filled to 2048 (t1)×2048(t2) before Fourier transform. The number of transients accumulated was set to 128 to ensure sufficient signal-to-noise ratio.

For $^1$H–$^{13}$C HSQC spectra, a 512 (t1)×1024(t2) data matrix was used. The time increment in the $^{13}$C dimension was set to 100 $\mu$s (on the 300 MHz NMR spectrometer) and 40 $\mu$s (on the 500 MHz NMR spectrometer). The number of transients was 128.

Recycle delay of 2 s was the same for all proton 1D and 2D experiments and 10 s for all $^{13}$C experiments. All experiments were performed at room temperature.

3. Results and discussions

Based on the 1D $^1$H, 1D $^{13}$C, 2D COSY, TOCSY, NOESY and $^1$H–$^{13}$C HSQC spectra acquired on NMR spectrometers at two different magnetic fields, we were able to successfully give a complete assignment of the NMR spectral peaks of the $^1$H and $^{13}$C spins in the three ethers and their corresponding alkali metal ionic complexes. The chemical shifts were tabulated and are shown in Tables 2 and 3 for $^1$H and $^{13}$C, respectively.

3.1. 1D proton and carbon-13 spectra

The 1D $^1$H and $^{13}$C spectra of pure ethers and their sodium and potassium complexes were recorded and each peak was assigned. Evident changes of both the proton and $^{13}$C spectra
upon complexation were observed for almost all spectral peaks. It is noted that most changes of the protons and $^{13}$Cs on the macroring are to lowfield with few exceptions. It is understandable because the metal ions are electron-pulling, deshielding the nuclei. For the protons on the sidearms, however, more are shifted to the lowfield (for 1 and 3 but not for 2), whereas for $^{13}$C, all but the first 3 $^{13}$Cs are shifted to lowfield. This means that the electronic structure on both the macroring and sidearm is perturbed by complexation but the macroring nuclei are more shielded while the side-arm nuclei are less shielded. This fact implies a picture of the electrons being ‘pushed’ to the sidearm from the macroring as a result of adding alkali metal ions to the ethers. This picture is helpful in understanding the formation of weak H-bonds between a sidearm proton and an oxygen or nitrogen on the macroring.

For all compounds, the largest relative change occurs on protons and $^{13}$Cs at $b$, $c$, and $d$ sites. The spectral change on the sidearm protons or $^{13}$Cs is less appreciable than that on macroring. This is reasonable since the metal ions are placed within the macroring. The change of the sidearm protons or $^{13}$Cs, although smaller, indicates that complexation also causes global change of the molecular structure. It is also noted that the protons (or the carbon bonded with it) corresponding to the H-bond donor (e.g., the No. 9 proton or carbon of 2-Na or 2-K) show largest changes in chemical shift as shown in Tables 2 and 3. The strongest cross peaks (implying largest J-couplings) in LR-COSY were also observed for those protons and the proton $b$ (or $c$) on the macroring (see later).

On the other hand, the difference between sodium and potassium complexes is also clearly shown although it is much less pronounced than between complexes and pure ethers.

The major features are the same for all the three complexes but the size of the sidearm (differing in the number of aromatic rings) does have observable effect on the proton and $^{13}$C chemical shifts as seen in the spectra and Tables 2 and 3.

Approximately the chemical shift change of proton $b$, $c$, and $d$ is proportional to the number of aromatic rings on the sidearm. The change of proton $c$ is most pronounced. This is not surprising if we recognize that the proton $c$ has strongest crosspeak with the sidearm proton that is the H-bond donor (the shortest distance between the metal ions and proton $c$, as shown in the X-ray result, might also be a factor). However, the largest change of $^{13}$C spectra varies depending on the number of sidearms and the type of the complexed ions, as shown in Table 3 (first 4 columns).

Moreover, the change upon addition of sodium ions is larger than that upon addition of potassium ions. This is an interesting observation because it implies that ion radius has important effect on the shift: smaller ion radius causes larger change in chemical shift of protons in the molecule.

### 3.2 Conventional 2D homonuclear and heteronuclear spectra

The unambiguous evidence for the existence of the possible weak hydrogen bond as indicated by the J-coupling between a...
sidearm proton and macroring proton (proton c), however, is not sufficient merely from 1D proton or $^{13}$C spectra. Furthermore, for all these compounds, conventional $^1$H COSY, TOCSY and $^1$H–$^{13}$C HSQC spectra do not show cross peaks caused by hydrogen bonds either. An example of the HSQC spectra is shown in Fig. 1 (more can be found in Supporting Information).

![Fig. 1. HSQC spectra of 3 (a), its sodium complex (b) and its potassium complex (c). The changes observed as a result of complexation are observable but cannot indicate the existence of the weak hydrogen bonding.](image)

$^1$H NOESY shows that the distance between the sidearm protons and the protons b, c on the macroring are less than 4 Å. A number of examples of NOESY spectra for the three complexes are shown in Fig. 2. While the close distances suggest the favorable condition for H-bonding, in NMR, the distance constraint alone, however, cannot be regarded as the sufficient evidence for the occurrence of the H-bond. To some extent, to suggest the existence of hydrogen-bond based on the short distances observed by NOESY is similar to X-ray diffraction, the inadequacy of NOESY criterion implying that extra caution should be taken in claiming the existence of hydrogen bonds based on X-ray data alone.

![Fig. 2. $^1$H NOESY of sample 2 (a) and its potassium complex (b) with mixing time of 200 ms. Strong cross peaks between sidearm protons and macroring protons indicate that the short spatial distance between each pair of the protons. However, most of these coupling pairs do not mean hydrogen bonds between one of the protons and the oxygen close to the other.](image)

3.3. 2D proton LR-COSY spectra

The unambiguous evidence comes from the long-range COSY which has the advantage of detecting weak J-coupling
by using an extra evolution period in the indirect dimension of proton correlation spectra.

The LR-COSY spectra of the three complexes are shown in Figs. 3, 4, and 5, respectively. Evident cross peaks between the sidearm proton(s) and micro-ring proton(s) are clearly shown for all these compounds. However, not all those short-distance separated protons as observed in NOESY show hydrogen-bonding cross peaks. Understandably, the most pronounced cross peaks arise from the macroring protons $b$, $c$ and the sidearm proton that is the H-bond donor.

All the H-bonds deduced from X-ray crystallography were observed in LR-COSY. In addition, surprisingly, one more H-bond (i.e., from the sodium complex of compound 1) was detected in LR-COSY that was not reported in X-ray study [1,2].

No cross peaks were observed for pure ethers. This means that the H-bonds are induced by complexation. This is consistent with the X-ray results.

3.4. Evaluation of the hydrogen-bond strength

The J-coupling constants were measured precisely from the LR-COSY spectra based on the approach proposed previously [40–42] and the strongest J-couplings are summarized in Table 4. One example of the structures of the complexes is shown in Fig. 6, indicating the positions of the hydrogen bonds and the J-coupling partners. For those weaker cross peaks caused by smaller J-couplings, line broadening effects such as from relaxation and magnetic field inhomogeneity may cause much larger errors in evaluation of J-couplings. Therefore, only the largest splittings from J-coupling are reported in this work. The measurement errors may also contribute to the difference of the J-coupling values in different magnetic fields because the long-range COSY cross peaks are generally noisy, particularly for those caused by weak J-couplings.

It is clear that these values are normally in the range of a few hertz. This should not be surprising considering the weak
magnitude of hydrogen bonding. Although much efforts have been devoted to understanding the properties of CH⋯O bonds [5–22], no quantitative formula analogous to Karplus equation describing three-bond J-coupling has been proposed. While it is not feasible at this moment to deduce the precise strength of the hydrogen bonds caused by metal ionic complexation, the J-coupling constant of the bonding partners can be used as a good indicator for the strength of the bonds. By comparing those protons with largest J-couplings with the distances evaluated from NOE relaxation rates, it was found that the distances between those protons indicate that the CH⋯O bonds are approximately linear, in good agreement with the theoretical results of Houk et al. [13] drawn from calculations on simpler systems.

These values are comparable to the results of Meadows et al. [1,2] and an approximate, proportional relation between the hydrogen bond strength and J-coupling constant can be obtained. The real H-bond strength in liquid state, however, may be weaker than that listed in Table 4 because the crystal packing effect may cause an increase in the hydrogen bonding strength.

3.5. Comparison with X-ray results

Table 5 lists the less common H-bonds found in these compounds by X-ray and solution NMR. First, it is worth to note that the there is one H-bond that was observed in compound 1 by LR-COSY was not suggested based on X-ray crystallographic data. It is not clear whether this discrepancy is because that the solution structure differs from crystal structure. The NMR result, however, is unambiguous about the existence of this H-bond. To our best knowledge, this phenomenon was not reported before. Considering the physiological or biological environment is more similar to solution rather than to

<table>
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<tr>
<th>Sample</th>
<th>J (Hz)</th>
<th>Estimated H-bond strength (kJ/mol)</th>
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<tbody>
<tr>
<td>1-Na</td>
<td>2.8(b−6)</td>
<td>~3.0</td>
</tr>
<tr>
<td>1-K</td>
<td>3.2(b−6)</td>
<td>~3.0</td>
</tr>
<tr>
<td>2-Na</td>
<td>3.4(b, e−9)</td>
<td>~3.2</td>
</tr>
<tr>
<td>2-K</td>
<td>3.8(b, e−9)</td>
<td>~3.3</td>
</tr>
<tr>
<td>3-Na</td>
<td>3.9(e−13)</td>
<td>~3.5</td>
</tr>
<tr>
<td>3-K</td>
<td>4.3(e−13)</td>
<td>~3.5</td>
</tr>
</tbody>
</table>

a The symbols given in the parentheses identify the coupled protons numbered according to Table 1.
b Data estimated from Ref. [2].
Table 5
Comparison between the X-ray and NMR results on H-bonding in the lariat ether complexes (○ means H-bonds were observed and × means no H-bond was detected)

<table>
<thead>
<tr>
<th>Compound</th>
<th>NMR</th>
<th>X-ray</th>
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<tbody>
<tr>
<td>1</td>
<td>○</td>
<td>×</td>
</tr>
<tr>
<td>1-Na</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>1-K</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>×</td>
<td>○</td>
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<tr>
<td>2-Na</td>
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<tr>
<td>2-K</td>
<td>○</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>×</td>
<td>○</td>
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<tr>
<td>3-Na</td>
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<tr>
<td>3-K</td>
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crystalline state, the NMR result should be taken into account when the hydrogen bonds are used for interpreting, e.g., enzymatic catalysis.

In addition, the protons on the two sidearms of 1-K and 2-K show significant differences in the X-ray result [1]. From the spectra shown in Figs. 3 and 4, these protons, however, do not show any difference in NMR spectra of solutions.

Recent interest in the less common hydrogen-bonding in biological molecules has been growing. A number of groups investigated the presence and effects of C–H⋯O bonding in DNA systems [23–30] and in proteins [31–33]. Use of this type of hydrogen-bonding in NMR determination of proteins has also been proposed and demonstrated by a number of groups [43–47]. We believe this work offers an approach that does not require 15N or 17O isotopic enrichment while acquiring unambiguous evidence for detection of weaker H-bonds.

Further studies include quantum chemistry calculations of the wavefunctions, hydrogen-bonding strength so that the mechanism and implication of the hydrogen-bonding of this type can be revealed in full detail.

4. Conclusions

In light of the fundamental understanding of hydrogen bond formation that electronic wavefunction overlap between the bonding partners, we have shown in this work that 1D and 2D proton NMR spectroscopy can be employed to provide most direct, definite and accurate evaluation on the existence of the less common hydrogen bond C–H⋯O in lariat ethers and their metal complexes. It is also shown clearly that the relatively short spatial distance does not necessarily mean hydrogen-bonding as indicated by NOESY spectra. Compared with other techniques used in other publications, NMR method is technically sophisticated and offers results that are more irrefutable. There exists a straightforward correspondence between the presence of hydrogen bonding and cross peaks in proton homonuclear correlation spectra. The occurrence of C–H⋯O hydrogen bonding in metal complexes of lariat ethers thus has been verified. The fact that we have observed the cross peaks in the correlation spectra of Na complex of compound 1 reminds us to scrutinize the conclusion in previous publications that C–H⋯O hydrogen bonding claimed not to exist in that compound. To resolve this problem from a different, interesting perspective, however, we are preparing 15N enriched samples for 15N/1H heteronuclear correlation spectroscopy. The related results will be presented elsewhere. Further work underway in this lab includes the documentation of proton (and 13C, 15N) spectra with different ion strengths and the correlation between J-coupling constant and hydrogen-bond strength.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.bpc.2006.01.002.

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