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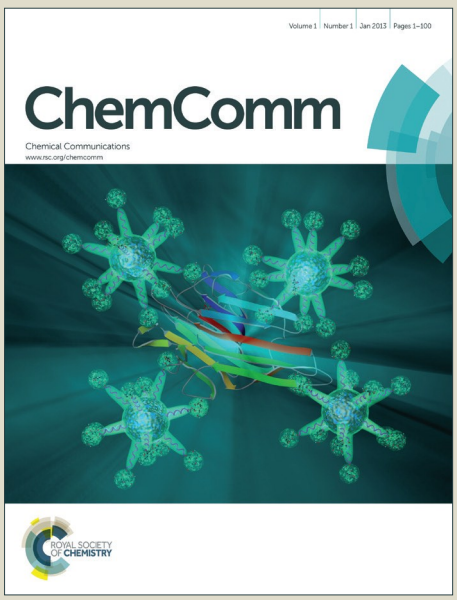

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Lewis Acid-Assisted Detection of Nerve Agents in Water

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The five-coordinate compound, Salen(^{t}Bu)Al(Ac), prepared *in situ* from Salen(^{t}Bu)AlBr and NH_4Ac, forms Lewis acid-base adducts in aqueous solution with the G-type nerve agents, Sarin and Soman, and the VX hydrolysis product, ethylmethylphosphonate (EMPA). The resulting compounds, [Salen(^{t}Bu)Al(NA)]$^+$[Ac]$^-$ (with NA = Sarin, Soman, and EMPA) are sufficiently stable to be identified by ESI-MS. Molecular ion peaks were detected for every compound with little or no fragmentation. The distinctive MS signatures for the [Salen(^{t}Bu)Al(NA)]$^+$ compounds provide a new technique for identifying nerve agents from aqueous solution. The energetics of the displacement of Ac$^-$ by the nerve agents to form [Salen(^{t}Bu)Al(NA)]$^+$[Ac]$^-$ were determined computationally.

Among the different types of Chemical Weapon Agents (CWAs) nerve agents (Fig. 1) are the most significant with respect to their toxicity, past use, military capacity and mode of action. Nerve agents are organophosphonate derivatives that attack the nervous system by irreversibly binding to the catalytic site of acetylcholinesterase (AChE), an enzyme that hydrolyzes the neurotransmitter acetylcholine.[1] Exposure to G-type nerve agents such as tabun (GA)), Sarin (GB), Soman (GD) and the V-type nerve agent, VX, can take place through inhalation, contact with the eyes or skin, and by ingestion. The G-type agents are not persistent and degrade, depending on conditions, within hours (at warm temperatures) to days (at cold temperatures). However, VX is highly persistent and the hydrolysis product, ethylmethylphosphonic acid (EMPA), is highly water-soluble and stable in environmental conditions.

Efforts to prevent chemical weapon attack or accidental releases prompted the Chemical Weapons Convention (CWC) to mandate that countries destroy all CWAs held in reserve.[2] However, the use of CWAs by terrorists or non- signatory countries is still a threat to civilian health and safety. The recent chemical weapon attack on civilians in Syria has confirmed this threat. LC/MS and GC/MS analyses confirmed the use of Sarin in Syria.[3]

One of the most difficult problems in responding to a CWA release is the decontamination of any affected objects and detection of any residual CWA or degradation products in complex environmental matrices.[4] VX is particularly problematic since it readily penetrates the skin and has a long-lived degradation product, S, 2-diisopropylaminoethylmethylphosphonothioic acid (EA2192), which has almost the same toxicity as the original VX. Nerve agents and simulants have been detected using techniques like ion mobility time-of-flight MS (IM(tof)MS)[5], ion mobility spectrometry[6] or LC-MS[7].

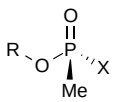
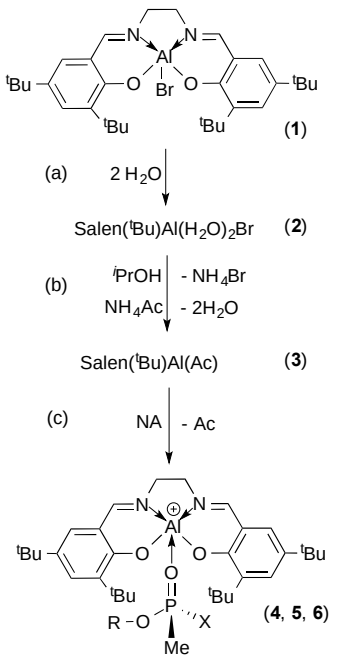


Fig. 1 General representation of organophosphate nerve agents: Sarin; R = $CHMe_2$, X = F (4), Soman; R = CH(Me)(^{t}Bu), X = F (5), and VX hydrosylate, EMPA, R = Et, X = OH (6).

The most commonly used method to detect nerve agents is GC/MS but the nerve agent degradation products like methylphosphonic acids must be derivatized before the analyses.[8, 9,10] Derivatization adds time, difficulty, and increases the potential for exposure to the nerve agents. Liquid chromatography coupled with mass spectrometry (LCMS) employing various ionization techniques has also been used to detect nerve agents.[11, 12] The most common derivatization methods include formation of trimethylsilyl (TMS) ethers or *tert*-butyldimethylsilyl (^{t}BDMS) ethers from acid,[13, 14] methylation with diazomethane,[15] and formation of an ion pair with trimethylphenyl ammonium hydroxide (TMPAH).[16] Most of these derivatives are unstable, moisture sensitive, or use carcinogenic reagents. LC/MS/MS and LC/ICP/MS have been used to detect G-agents, V- agents and their degradation products.[17] While these techniques (and others) are still in the development/optimization stage, hydrolysis products have been detected near 1 ppb.

Tetradentate salen(^{t}Bu)AlBr compounds dealkylate organophosphates (OP) such as VX and pesticides to produce salenAl(OP) compounds containing a covalent Al-O-P linkage (salen = N,N'-alkylene(or *o*-arylene)bis(3,5-di-*tert*-butylsalicylideneimine); when the alkylene is ethylene the compound is Salen(^{t}Bu)AlBr (**1**) with a capital "S"; Scheme 1).[18] For example, equimolar amounts of **1**, in non-aqueous solvents, dealkylated nerve agents, pesticides and their simulants under very mild conditions (dealkylation: Sarin = 24.5 % in 1.2 h Soman = 62.6 % in 6.2 hr; r).[19] The formation of transient ion-pairs, [salen(^{t}Bu)Al(NA)]Br, was implicit in the dealkylations and supported by NMR evidence.[20] In the present study it was found that Lewis basic nerve agents could displace the acetate anion (Ac) from Salen(^{t}Bu)Al(Ac) (**3**) to form the new compound, [Salen(^{t}Bu)Al(NA)]$^+$ which could be use to identify nerve agent by electrospray mass spectrometry (ESI-MS; NA = Sarin (**4**), Soman (**5**), EMPA (**6**)).



Scheme 1. Sequence of reactions (a-c) leading to formation of Lewis acid-base compounds between Salen(^{t}Bu)AlBr (**1**) (which forms the ion pair, [Salen(^{t}Bu)Al(H_2O)$_2$]Br (**2**) in water) and the nerve agents Sarin, R = $CHMe_2$, X = F (**4**)(Soman, R = $CH(Me)CMe_3$, X = F (**5**)and VX hydrosylate, EMPA, R = Et, X = OH (**6**)). See Table S1 for experimental and characterization details for **1-6.**

The ESI-MS of **1** in isopropanol was obtained as a baseline for comparison to the nerve agent derivatives. The positive ionization spectrum had m/z values attributed to [Salen(^{t}Bu)Al]$^+$ (517) from loss of bromide and [Salen(^{t}Bu)Al(^{i}PA)]$^+$ (577) with coordinated solvent (Fig. S1). These two peaks indicated that the compound readily loses bromide and that compounds with coordinated Lewis bases could be detected. The assignment for m/z 1051 probably corresponds to [{Salen(^{t}Bu)Al}$_2$O]$^+$ with a bridging oxide, a known structural motif for SalenAl compounds.[21] In the negative ionization mode the largest peak in the spectrum corresponded to [Salen(^{t}Bu)Al(^{i}PA)(H_2O)]Br (m/z 675) which indicated that the compound and coordinated solvent remained intact under the ESI-MS(+ and -) conditions (Fig. S2).

It has been established that Lewis basic solvents such as H_2O, MeOH, and THF displace the anion from SalenAlX (X = Br$^-$ and OTs$^-$, for example) to form solvated six-coordinate derivatives such as compound **2** in Scheme 1.[22] In the present study the bromide anion in **2** was exchanged for acetate through a salt elimination reaction. The ESI-MS(+) spectrum detected **3** (m/z 577) without water, in addition to an acetate-bridged compound, [{Salen(^{t}Bu)Al}$_2$Ac]$^+$ (m/z 1093) (Fig. S3). In negative ionization mode the largest peak for **3** was [Salen(^{t}Bu)Al(Ac)$_2$]$^-$ (m/z 635) (Fig. S4) and fragments corresponding to compounds containing bromide: [Salen(^{t}Bu)AlBr(Ac)]$^-$ m/z 657 and [{Salen(^{t}Bu)Al}$_2$Br(Ac)$_2$]$^-$, m/z 1233 (Fig. S4). A related salen acetate compound is known.[23] Salcen(^{t}Bu)AlAc, formed by combination of Salcen(^{t}Bu)AlMe with acetic acid has been structurally characterized (salcen, with a 1,2-diamino cyclohexane group is very similar to salen, with an ethylenediamine group).

Aqueous solutions of **3** were combined with micromolar quantities of Sarin (0.02 mM, 2.8ppm), Soman (0.02 mM, 3.6ppm) and EMPA (0.8 mM, 99ppm) to form the Lewis acid-base adducts, [Salen(^{t}Bu)Al(NA)]$^+$ (Scheme 1c). In positive ionization mode parent peaks were observed for the compounds containing Sarin (*m/z* 657, **4**) and Soman (*m/z* 699, **5**) (Figs S5 and S6). The peaks are weaker than that for **3** (m/z 577) due to the lower concentrations used, but easily identified. Moreover, the peak at *m/z* 517 could correspond to loss of Soman from **5** or loss of Ac from **3**. For **5**, the MS/MS spectrum of *m/z* 699 has a peak at *m/z* 615, which is likely due to loss of dimethyl butene (Fig. S7). The positive ionization spectrum of **6** has a parent peak at *m/z* 641 and another at *m/z* 1157 corresponding to dimeric, EMPA-bridged [{Salen(^{t}Bu)Al}$_2$(EMPA)]$^+$ (Fig. S8). Such dimeric compounds are known for SalenAl combinations with phosphonic and phosphinic acids, several of which have been structurally characterized.[24] Acetate adducts are observed at *m/z* 577 and *m/z* 1093. In the negative ionization spectrum (Fig S9), EMPA adducts were observed at *m/z* 699 for [Salen(^{t}Bu)Al(EMPA)(Ac)]$^-$ and *m/z* 763 for [Salen(^{t}Bu)Al(EMPA)$_2$]$^-$. No interferences from the ammonium or bromide were evident in the results.

Table 1 contains the computationally determined free energies for binding two types of Salen-aluminum cations with acetate (Eq. i) and nerve agents (Eq. ii). The calculations incorporate the influence of hydration energy in the formation of **4**, **5**, and **6**. The ΔG for Ac binding with **1** (-33 kcal/mol) is greater than the ΔG values for the nerve agents, which implies that the equilibrium should favor coordination of acetate over the nerve agents. However, the nerve agents are, by design, hydrophobic so that they can readily penetrate human skin. In contrast, the acetate anion is hydrophilic, meaning that the nerve agent will segregate out of the aqueous phase to coordinate to the more hydrophobic **3**. The displacement of Ac from the complex (Eq. iii) would be facilitated by coordination of the nerve agent to **3**, forming a six-coordinate intermediate. This compound has precedent in the X-ray structure of Salen(^{t}Bu)Al(OMe)(MeOH) where the Al is in a distorted O_h geometry.[25]

[Salen(^{t}Bu)Al(H_2O)$_2$]$^+$ + [Ac]$^-$ → Salen(^{t}Bu)Al(Ac) …… (i)

Salen(^{t}Bu)Al(Ac) + NA ↔ Salen(^{t}Bu)Al(Ac)(NA) ……(ii)

Salen(^{t}Bu)Al(Ac)(NA) → [Salen(^{t}Bu)Al(NA)]$^+$ + Ac(aq)$^-$ …..(iii)

Table 1. Computational determination of ΔG values (kcal/mol) for the combination of SalenAl cations with Ac$^-$ (a), water (b) and (c), G-type nerve agents (d-g), and EMPA (h). The X and R groups correspond to substitutions in Figure 1. The compound,

[SalenAl]$^+$does not have ^{t}Bu groups while in [Salen(Me)Al]$^+$ the ^{t}Bu groups were replaced with Me to make the computations manageable. Details of the computational analyses are given in the Supporting Information.

	Compound		L=Salen	L=Salen(Me)
a.	LAl(Ac)		-33.0	-31.5
b.	$[LAl(H_2O)]^+$		-19.8	---
c.	$[LAl(H_2O)_2]^+$		-26.0	---
	$[LAl(P(O)(Me)(OR)(X)]^+$			
	R	X		
d.	Me	F	-20.7	-19.6
e.	$CHMe_2$	F	---	-24.3
f.	CH(Me)^{i}Pr	F	---	-19.9
g.	CH(Me)^{t}Bu	F	---	-19.9
h.	Et	OH	-24.3	---

Conclusions

G-type nerve agents are difficult to analyze by LCMS due to low ionization efficiency. Analyzing for nerve agents after coordination to [Salen(^{t}Bu)Al]$^+$ has several advantages: 1) reduction of low mass noise by moving the ion to higher mass (e.g. Soman, MW 140.1, shifted to *m/z* 657 with SalenAl), 2) ability to detect nerve agents and degradation compounds without any additional water-sensitive derivatization, and 3) additional sample preparation options based on extraction of the Lewis acid complex. Replacement of the ^{t}Bu groups on the Salen ligand with electron-withdrawing or more hydrophilic groups could be used to increase the binding strength to particular compounds of interest and adjust the hydrophobicity of the complex. Further work is still necessary to identify the Lewis Acid/Base adducts in solution phase to determine their thermodynamic properties.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, synthesis of Salen(^{t}Bu)AlBr and derivative compounds, experimental conditions for the mass spectroscopic analyses, and computational details. See DOI: 10.1039/c000000x/. Note: Nerve agents are extremely toxic. Hence studies on nerve agents must be carried out only in specially designed laboratories. The present study was conducted on site at Edgewood Chemical and Biological Center by trained personnel using applicable safety precautions.

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