

Detection of Al(III) and Ga(III) Complexes with Morin by Electrospray Ionization Mass Spectrometry

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The Al(III) and Ga(III) complexes formed by morin (M) in aqueous solution were investigated by means of electrospray ionization mass spectrometry (ESI-MS). In the full scan mass spectra, Al:M showed 1:2 and 2:3 stoichiometric ratios. When (*S*)-*N*-acetylserine methyl ester (Ser), as a partial mimic of the serine residue in silk, was added to Al:M and Ga:M complexes in aqueous solution, the mass spectra of Ser:Al:M showed 1:1:1 and 1:1:2 stoichiometric ratios. The patterns of the mass spectra of Ga:M and Ser:Ga:M complexes were similar to those for the corresponding Al(III) complexes. Calculated heats of formation of potential structures of the complexes, with and without bound water, were obtained using semiempirical PM3 calculations.

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Introduction

The yellow flavonoid, morin (M), is a major component of the heartwood of the plant *Maclura cochinchinensis* (Lour.) Corner (family Moraceae).¹ In Thailand, especially in the Northeast, aqueous extracts of the wood of this plant are used for the dyeing of silk.² The dye extract, which has morin (3,5,7,2',4'-pentahydroxyflavone) (Fig. 1(a)) as a major component together with quercetin, imparts a beautiful yellow color to the silk. However, the use of this natural dye mixture is often linked to poor fastness properties and thus metal-based mordants are used to increase fastness (*e.g.*, wash fastness) properties. One such mordant used by villagers in Northeast Thailand is alum.² Alum is widely used as a mordant for dyeing silk fibers with polyphenolic dyes to obtain a brighter color rendition and an improved wash-fast finish.³⁻⁶ However, problems with wash fastness are still encountered and this aspect needs to be improved. In order to start to do this, detailed information on the nature of the major dye component-mordant complexing is needed together with an understanding of the molecular basis of the dye-mordant-silk interaction. From this basis, interactions with other dye components could start to be assessed, leading possibly to the incorporation of auxiliary ligands or bridging ligands to mediate more effective dyeing of the silk and hence overcome some of the natural dye fastness problems.

A traditional analytical method (UV-visible absorption spectroscopy) has been used to elucidate structural information on the Al(III)-flavonoid complex or complexes formed in solution.⁷⁻¹⁴ It was found that flavonoids form complexes with Al(III) with various Al:flavonoid stoichiometries such as 2:1, 1:1 and 1:2, either through the 4-*keto* and neighboring hydroxyl (3-OH, 5-OH, or both) groups or through adjacent hydroxyl groups on the B ring (*i.e.*, 3'-OH and 4'-OH). In addition, the

formation of complexes between alum with morin in aqueous solution with and without control of pH have been studied by UV-visible spectroscopy.¹⁵ The emergence of electrospray ionization mass spectrometry (ESI-MS)¹⁶⁻²² in recent years has greatly enhanced the application of mass spectrometry in the analysis of complexes including Al(III):flavonoid complexes. However there have been no ESI-MS studies with flavonoid aglycones, which also incorporate an extra complexing site in the form of the 2'-hydroxyl group, as is the case with morin.

We have been investigating the complexing of morin with Al(III) in methanolic aqueous solutions and undertaking comparative studies with the related Ga(III) ion. One aspect of this investigation has involved the use of ESI-MS. The two stable isotopes of Ga (⁷¹Ga (39.9%) and ⁶⁹Ga (60.1%)) were expected to assist with the identification of metal ion-containing complex peaks by ESI-MS. This study was also extended to the first preliminary assessment of potential model interactions of the metal ion-flavonoid complexes with an analogue of the silk component, serine, through ESI-MS, and associated molecular modeling. Serine is a significant component of silk fibroin protein^{23,24} (together with alanine and glycine, and, to a lesser extent, tyrosine) and is one possible site for Al(III) mordant complexing in view of the primary hydroxyl group in its side chain. A partial mimic of the serine residue, (*S*)-*N*-acetylserine methyl ester (Ser, Fig. 1(b)), was thus used in these initial complexing studies by ESI-MS.

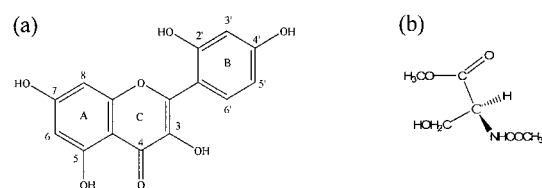


Fig. 1 (a) Chemical structure of morin (M) and (b) (*S*)-*N*-acetylserine methyl ester (Ser).

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The aims of this work were thus to investigate the complexing of Al(III) and Ga(III) with morin through ESI-MS and complementary molecular modeling, and to begin to evaluate potential interactions of the complexes with silk through a mimic of one of the silk amino acid components. The results are presented in this paper.

Experimental

Reagents and chemicals

Morin (M_w 302.24) was purchased from Sigma. Aluminum nitrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) (M_w 375.14) 99.997% and gallium nitrate ($\text{Ga}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$) (M_w 255.74, Anh.) 99.999% were obtained from Aldrich. Alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) (M_w 474.38) was obtained from Univar. HPLC grade methanol was obtained from Unichrom. Milli-Q water was used in the preparation of all solutions. (*S*)-*N*-Acetylserine methyl ester (M_w 161.16) was obtained from Bachem. All materials were used without further purification.

Sample preparation

These stock solutions were prepared: 1.0 mM morin in methanol, 1.0 mM alum in water, 1.0 mM aluminum nitrate in water, 1.0 mM gallium nitrate in water, and 1.0 mM (*S*)-*N*-acetylserine methyl ester in methanol. The 1.0 mM solution of morin was mixed separately with solutions of alum, aluminum nitrate and gallium nitrate in volumetric flasks (10 mL) and the volume was adjusted with water in each case to give a 3:2 mole ratio; the final concentration of the sample was 100 μM . Pre-mixed solutions of morin-aluminum nitrate and morin-gallium nitrate were each added to separate solutions of (*S*)-*N*-acetylserine methyl ester in volumetric flasks (10 mL) and the volume adjusted with water to give a 3:2:3 mole ratio in each case. The pH values of the mixture solutions were measured with a pH meter.

Electrospray ionization mass spectrometry (positive ion mode)

A Thermo Finnigan LTQ quadrupole ion trap (QIT) instrument equipped with an electrospray ionization (ESI) source was used. The flow-rate of the solutions was set at 10 $\mu\text{L min}^{-1}$. The heated capillary temperature was kept at 275°C. The ESI spray voltage was set at +4.5 kV. The injection time was set at 50 ms. The other instrumental parameters were tuned to optimize the relative abundance of the Al(III):M or Ga(III):M complex. All spectra were obtained in the positive mode. The scan mode was positive and the isolation width for multistage tandem mass spectrometry (MS^n) was 1.0 – 6.0 Da.

High-resolution mass spectrometry

High-resolution electrospray ionization mass spectrometry for the determination of the accurate masses of the complexes was performed on a QTOF Ultima instrument fitted with a lockspray source. Samples were loop injected (10 μL) using 50% aqueous acetonitrile (v/v). The mass scale was calibrated with polyethylene glycol (PEG) and masses were compared with a leucine enkephalin lock mass (m/z 566.2771).

Computational modeling

For computational modeling, PC Spartan Pro (Wavefunction, Irvine, CA) was used. Lowest energy conformers of morin and the serine ester were determined by molecular modeling using MMFF94 force fields²⁵ and the resulting structures were used for the appropriate Al(III) or Ga(III) complex minimum energy structure utilizing the semiempirical PM3 program.²⁶ The PM3

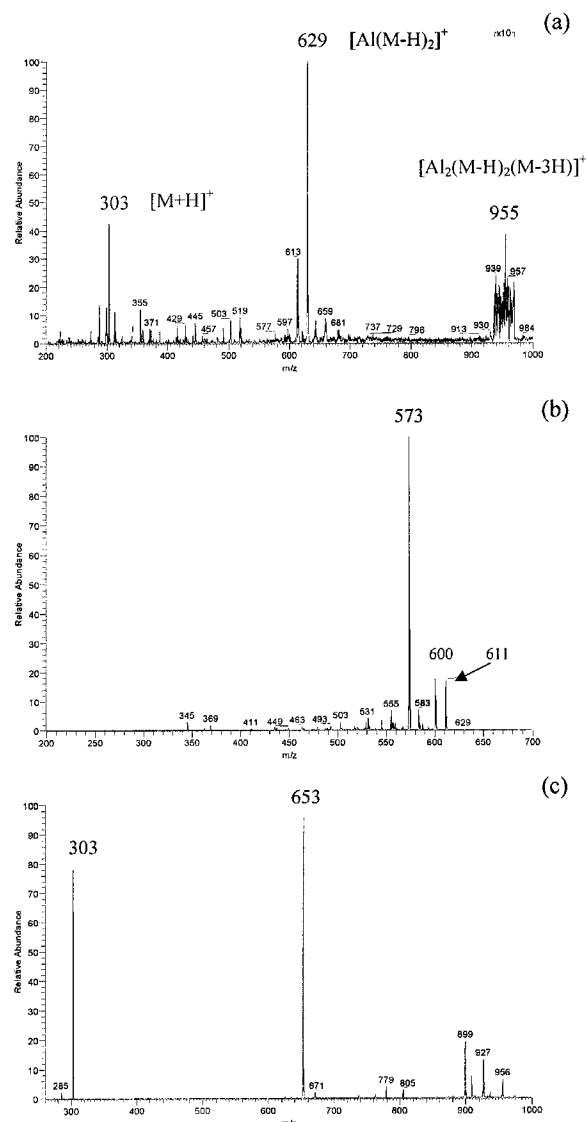


Fig. 2 (a) Full scan mass spectrum of Al:M complex, (b) MS^2 spectrum of the m/z 629 ion in Fig. 2(a), (c) MS^2 spectrum of the m/z 955 ion in Fig. 2(a).

program was also used for calculating the approximate heats of formation of the probable structures of the complexes.

Results and Discussion

Al(III) and Ga(III)* ions were used to form complexes with morin (Fig. 1(a)) in methanolic aqueous solution. (*S*)-*N*-Acetylserine methyl ester (Fig. 1(b)), as a potential auxiliary ligand, was mixed with aluminum-morin and gallium-morin solutions. The positive scan mode was used for all the electrospray ionization mass spectrometric experiments. In order to elucidate the structure of the complexes and the fragmentation mechanism of the aluminium- and gallium-morin complexes, MS^n was performed to produce abundant fragments.

Al:M and Ser:Al:M complexes

The full-scan ESI mass spectrum of the Al and M complexing

*In the Results and Discussion section, for simplicity, aluminum (or Al) and gallium (or Ga) refer to Al(III) and Ga(III), respectively.

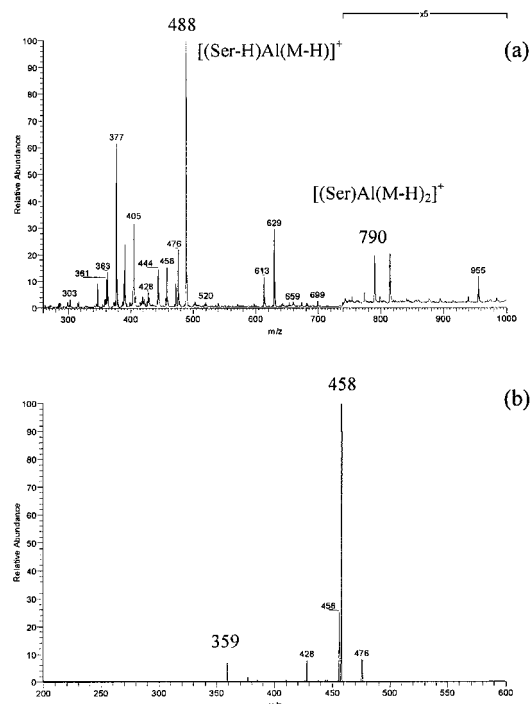


Fig. 3 (a) Full scan mass spectrum of Ser:Al:M complex, (b) MS² spectrum of the m/z 488 ion in (a).

is shown in Fig. 2(a). In this spectrum, two Al:M complexes were found, with peaks at m/z 629 and 955. On the basis of molecular mass and assuming a single positive charge, the complexes were assigned as $[\text{Al}(\text{M-H})_2]^+$ and $[\text{Al}_2(\text{M-H})_2(\text{M-3H})]^+$, respectively. In order to obtain more structural information on the Al:M complexes, further analyses using MSⁿ were performed. Three ions at m/z 611, 600 and 573 were present in the MS² spectrum of m/z 629 (Fig. 2(b)). The ion at m/z 611 is from the neutral loss of H_2O (–18 Da), while the ion at m/z 600 corresponds to the neutral loss of CHO (–29 Da). The most intense fragment ion (m/z 573) resulted from the neutral loss of $\text{C}_3\text{H}_4\text{O}$ (–56 Da). Figure 2(c) shows the MS² spectrum of m/z 955, in which it was found that the most intense fragment ion (m/z 653) resulted from the neutral loss of $\text{C}_{15}\text{H}_{10}\text{O}_7$ (morin; 302 Da). It is of interest to note, however, that the stoichiometric ratio for the Al:M complex in aqueous methanolic solution at pH 4.5 was determined as 1:1 from UV-Vis spectrophotometric studies (molar ratio method), while without control of pH (range 4.0–5.5), a 3:2 Al:M complex predominated¹⁵ (molar ratio method). The nature of the solvent and pH can influence the stoichiometry of the Al:M complex and in methanol both a 1:1 and a 1:2 complex was observed, while in acidic methanol the main complex stoichiometry was 1:1.¹⁴ The pH of the solution will influence the degree of ionization of the acidic hydroxyl groups¹⁹ present in morin and hence the nature of the complexing. In the ESI-MS work, the pH of the Al:M solution was 4.0, but during the electrospray ionization process itself this pH may change.²⁷ This change in pH could be one of the factors, together with the transformation from the aqueous to the gas phase, influencing the difference in the preferred complex stoichiometry observed.

The full-scan ESI mass spectrum of the Ser:Al:M solution is shown in Fig. 3(a). In this spectrum, two Ser:Al:M complexes were detected. The ions for these complexes appeared at m/z 488 and 790, consistent with $[(\text{Ser-H})\text{Al}(\text{M-H})]^+$ and

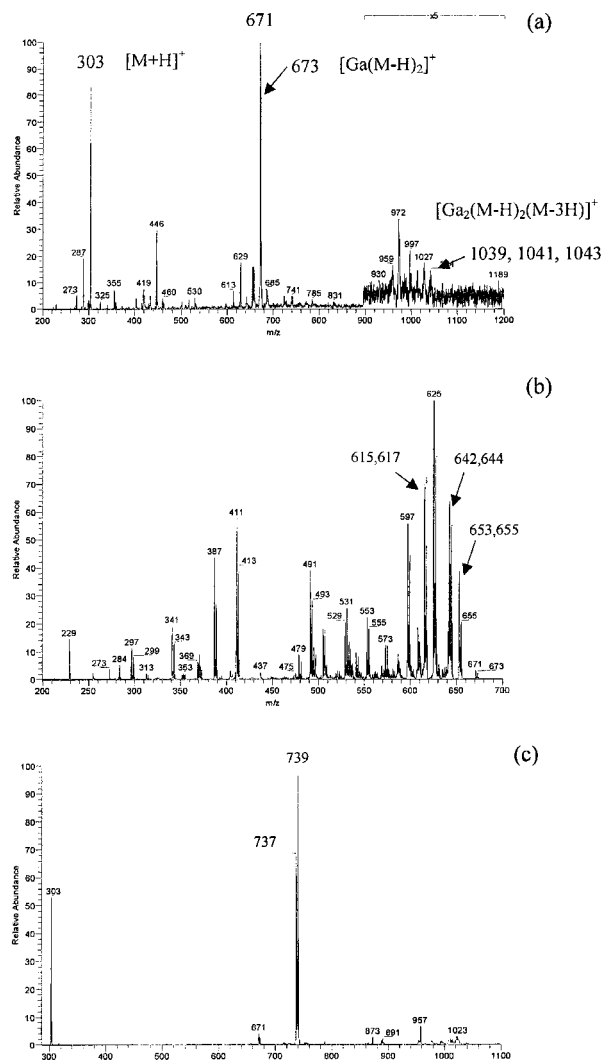
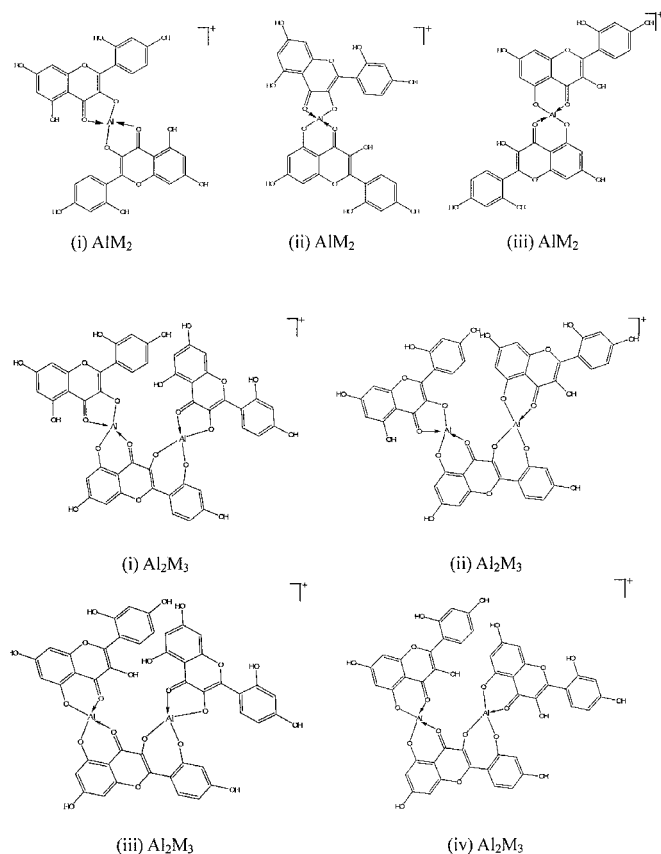


Fig. 4 (a) Full scan mass spectrum of Ga:M complex, (b) MS² spectrum of the m/z 671 ion in (a), (c) MS² spectrum of the m/z 1041 ion in (b).

$[\text{SerAl}(\text{M-H})_2]^+$, respectively. MSⁿ was also used to study these complexes and the results provided further structural support. Ions at m/z 458 and 359 were present in the MS² spectrum of m/z 488 (Fig. 3(b)). The m/z 458 ion is formed from neutral loss of CH_2O (–30 Da) and the signal at m/z 359 corresponds to the ion $[\text{Al}(\text{OCH}_3)(\text{M-H})]^+$. The MS³ spectrum of the m/z 488 ion ($488 > 458$) indicated that the m/z 426 ion resulted from loss of CH_3OH from m/z 488. In addition, an MSⁿ experiment was employed on the m/z 426 fragment ion (MS⁴ spectrum of m/z 488 ($488 > 458 > 426$)). In this last spectrum, the m/z 426 ion cleaved to produce the m/z 398 ion as a result of the neutral loss of CO (–28 Da). In the MS² spectrum of m/z 790, ions at m/z 629 and 488 were present. The ion at m/z 629 most likely resulted from neutral loss of *N*-acetylserine methyl ester (–161 Da) and the m/z 488 ion from the neutral loss of morin (–302 Da).

Ga:M and Ser:Ga:M complexes

In order to gain further corroboration for the presence of the Al:M and Ser:Al:M complexes, the analogous Ga complexes were assessed. The full-scan ESI mass spectrum of the Ga:M solution is shown in Fig. 4(a). In this spectrum, two clear Ga:M complexes were detected with isotopic signature ions at m/z

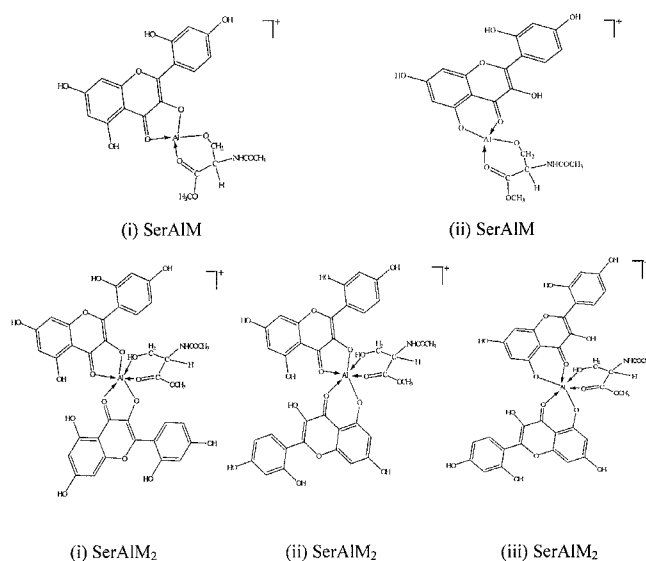
Fig. 5 Proposed structures of AlM_2 and Al_2M_3 complexes.

671, 673 and m/z 1039, 1041, 1043. High resolution ESI-MS substantiated these complex assignments as $[\text{Ga}(\text{M}-\text{H})_2]^+$ and $[\text{Ga}_2(\text{M}-\text{H})_2(\text{M}-3\text{H})]^+$, respectively. The MS^2 spectrum of m/z 672 (Fig. 4(b)) indicated fragment ions for the neutral loss of H_2O (–18 Da), CHO (–29 Da) and $\text{C}_3\text{H}_4\text{O}$ (–56 Da) at m/z 653, 655, m/z 642, 644 and m/z 615, 617, respectively. The same fragmentation pattern was observed with the corresponding $\text{Al}:\text{M}$ complex. In addition, in the MS^2 spectrum of m/z 1041 (Fig. 4(c)), it was found that the most intense fragment ion (m/z 739) resulted from the neutral loss of $\text{C}_{15}\text{H}_{10}\text{O}_7$ (morin).

In the full-scan ESI mass spectrum of the $\text{Ser}:\text{Ga}:\text{M}$ solution, two $\text{Ser}:\text{Ga}:\text{M}$ complexes were apparent from the isotopic ion peaks at m/z 530, 532 and m/z 831, 833. The high resolution ESI-MS analysis indicated these complexes were most likely to have the compositions $[(\text{Ser}-\text{H})\text{Ga}(\text{M}-\text{H})]^+$ and $[\text{SerGa}(\text{M}-\text{H})_2]^+$, respectively. Signals for ions at m/z 500, 502 and 401, 403 were present in the MS^2 spectrum of m/z 531. The m/z 500, 502 ions are most likely derived from the neutral loss of CH_2O (–30 Da), and the ions at m/z 401, 403 from the neutral loss of $\text{C}_5\text{H}_7\text{NO}_3$ from the serine analog component resulting in $[\text{Ga}(\text{OCH}_3)(\text{M}-\text{H})]^+$. Ions at m/z 671, 673 and 530, 532 were present in the MS^2 spectrum of m/z 833. The gallium-containing ions at m/z 671, 673 ion are likely to arise from neutral loss of *N*-acetylserine methyl ester (–161 Da) and the m/z 530, 532 ions from the neutral loss of morin (–302 Da).

Our results demonstrated that the preferred chelates for both $\text{Al}(\text{III})$ and $\text{Ga}(\text{III})$ with morin are of stoichiometries $\text{Al}:\text{M}$ (or $\text{Ga}:\text{M}$) 1:2 and 2:3. The stoichiometries of the $\text{Ser}:\text{Al}:\text{M}$ and $\text{Ser}:\text{Ga}:\text{M}$ complexes were both 1:1:1 and 1:1:2.

Possible structures of the 1:2 and 2:3 complexes of $\text{Al}:\text{M}$ and the 1:1:1 and 1:1:2 complexes of $\text{Ser}:\text{Al}:\text{M}$ are shown in Figs. 5

Fig. 6 Proposed structures of SerAlM and SerAlM_2 complexes.

and 6, respectively. The proposed structures are based on the observed m/z ratios of the ions in the ESI mass spectra and also the same pattern for the corresponding Ga complexes, together with acceptable accurate mass determinations (error values $\Delta < 5.0$ ppm) on the ions from the AlM_2 , GaM_2 , SerAlM , SerGaM , and SerGaM_2 complexes (Table 1).

Computational modeling

Molecular modeling was carried out on $\text{Al}:\text{M}$, $\text{Ga}:\text{M}$, $\text{Ser}:\text{Al}:\text{M}$ and $\text{Ser}:\text{Al}:\text{M}$ complexes using MMFF94 force fields for the ligands; then semiempirical PM3 calculations were made in a preliminary attempt to determine potential structures of the complexes, both in aqueous solution and in ESI-MS. Various binding modes in the complexes were modeled; it should be noted, however, that these calculations refer to complexes in a vacuum and solvent effects are not included. Water molecules bound to aluminum *via* oxygen were added to obtain an octahedral environment in the complex ions, which is most likely for the $\text{Al}(\text{III})$ complexes in aqueous solution. On electrospray ionization, no bound water is likely to be retained. Table 2 shows calculated heats of formation values of $\text{Al}:\text{M}$, $\text{Ga}:\text{M}$, $\text{Ser}:\text{Al}:\text{M}$ and $\text{Ser}:\text{Ga}:\text{M}$ complexes with and without water ligands. In the case of $\text{Al}:\text{M}$ with water molecules, the opportunity exists to form 3 different dimer structures, with structure (i) (Table 2, $\text{Al}:\text{M}$) involving 5-membered chelate rings having the largest negative heat of formation; structure (ii) was preferred on the basis of the calculated heat of formation with the corresponding Ga complex (Table 2); the larger $\text{Ga}(\text{III})$ ion²⁸ is better accommodated in the two 6-membered rings in structure (iii). With the $\text{Al}:\text{M}$ and $\text{Ga}:\text{M}$ 2:3 complexes with bound water, there was very little difference among these three structures in terms of heats of formation, although with gallium a preference for the corresponding structure type (i) was seen. In the case of $\text{Al}:\text{M}$ or $\text{Ga}:\text{M}$ complexes without bound water, the calculated heats of formation of the Al complex structures (i), (ii), and (iii) are very similar (Table 2, Fig. 5), while for the Ga complexes structures analogous to (iii) (for the $\text{Ga}:\text{M}$ 1:1 complex) and (ii) (for the $\text{Ga}:\text{M}$ 2:3 complex) had the largest negative heats of formation (Table 2). The computer-modeled Al -based structures with the serine analogue as a co-ligand are shown in Fig. 6, with the corresponding calculated heats of

Table 1 Exact mass measurements (ESI) and elemental compositions for the ions with a charge of +1 from the AlM₂, GaM₂, SerAlM, SerGaM, and SerGaM₂ complexes

Complex	Ion	<i>m/z</i>	Elemental composition	Theoretical value	Experimental value	Δ , ppm
AlM ₂	[Al(M-H) ₂] ⁺	629	C ₃₀ H ₁₈ O ₁₄ Al	629.0512	629.0539	4.3
GaM ₂	[Ga(M-H) ₂] ⁺	671	C ₃₀ H ₁₈ O ₁₄ ⁶⁹ Ga	670.9952	670.9952	-0.1
SerAlM	[(Ser-H)Al(M-H)] ⁺	488	C ₂₁ H ₁₉ O ₁₁ NAl	488.0774	488.0795	4.4
SerGaM	[(Ser-H)Ga(M-H)] ⁺	530	C ₂₁ H ₁₉ O ₁₁ N ⁶⁹ Ga	530.0229	530.0214	2.8
SerGaM ₂	[SerGa(M-H) ₂] ⁺	832	C ₃₆ H ₂₉ O ₁₈ N ⁶⁹ Ga	832.0640	832.0616	-2.9

Table 2 Calculated heats of formation of complexes

Complex	Ratio	Heat of formation/kcal mol ⁻¹	
		With H ₂ O	Without H ₂ O
Al:M	(i) 1:2	-548.8	-407.6
	(ii) 1:2	-540.0	-407.4
	(iii) 1:2	-543.0	-407.3
	(i) 2:3	-977.7	-713.5
	(ii) 2:3	-976.4	-714.4
	(iii) 2:3	-978.5	-713.3
Ser:Al:M	(iv) 2:3	-976.1	-713.5
	(i) 1:1:1	-472.1	-347.3
	(ii) 1:1:1	-473.9	-346.4
	(i) 1:1:2	—	-590.2
	(ii) 1:1:2	—	-593.5
	(iii) 1:1:2	—	-586.1
Ga:M	(i) 1:2	-561.9	-383.7
	(ii) 1:2	-566.0	-391.4
	(iii) 1:2	-569.7	-399.5
	(i) 2:3	-1057.4	-700.7
	(ii) 2:3	-1050.4	-770.1
	(iii) 2:3	-1052.1	-707.7
Ser:Ga:M	(iv) 2:3	-1066.7	-713.7
	(i) 1:1:1	-527.2	-369.3
	(ii) 1:1:1	-532.6	-376.4
	(i) 1:1:2	—	-642.9
	(ii) 1:1:2	—	-649.2
	(iii) 1:1:2	—	-653.9

formation given in Table 2. These results indicated structures (i) or (ii) for the 1:1:1 complex (Ser:Al:M) with the serine ester binding to Al *via* the serine side chain hydroxyl group and the ester carbonyl oxygen. In the analogous Ga complexes with the serine ligand present, the same type of serine ligand binding was indicated in the 1:1:1 complex, although favoring structure (i) over (ii). With the 1:1:2 complexes, a preference for structure (ii) and for structure (iii) were indicated with Al and Ga, respectively (Table 2); with these complexes, though, the serine hydroxyl group was involved rather than the corresponding alkoxide.

Conclusion

Although different from the stoichiometry observed in aqueous methanolic solution, electrospray ionization mass spectra of Al:M and Ga:M complexes showed 1:2 stoichiometry for the major complex formed in each case, possibly due to changes in pH and the medium during the ionization process. Calculated heats of formation were reasonable for both these charged complexes and tentatively indicated possible structures for these

complexes in the gas phase. The stoichiometries of the Ser:Al:M and Ser:Ga:M complexes observed mass spectrometrically were 1:1:1 and 1:1:2, and molecular modeling was consistent with the probable involvement of the primary hydroxyl group in (*S*)-*N*-acetylserine methyl ester in the complexing process to Al(III) and Ga(III). These water-excluded structures may also possibly have some relevance to those formed in part on interaction with silk.

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