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Gas-Phase reactivity of glycosides and methyl-glycosides with Cu^+ , Ag^+ and Pb^{2+} ions by FAB-MS/MS.

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Abstract

The analytical potential of the complexation of three isomeric monosaccharides (D-glucose, D-galactose, D-fructose) and two methyl-glycosides (O-methyl- α -D-glucose and O-methyl- β -D-glucose) by three metal ions, Ag^+ , Cu^+ , and Pb^{2+} , has been investigated by Fast Atom Bombardment (FAB) ionization and tandem mass spectrometry. Our results have shown that the unimolecular reactivity of Ag^+ complexes allows the characterization of the C(4) stereochemistry of the pyranose ring, whereas a distinction between D-glucose and D-fructose is not achieved. On the other hand, each of the three $[\text{Cu} + \text{monosaccharide}]^+$ complexes exhibits specific dissociation patterns. We have also observed that Pb^{2+} ions induce the richest reactivity and are of particular interest the identification of the three isomers. Finally, this study has demonstrated that the stereochemistry of the anomeric center of O-methyl-D-glucose is easily determined by reaction with Ag^+ and Pb^{2+} ions. Several mechanisms are proposed to account for the main fragmentations of cationized glucose.

Keywords: mass spectrometry, Fast Atom Bombardment, cationization, silver, copper, lead, glycoside, gas-phase, unimolecular reactivity

This work is dedicated to Dr. Jean-Claude Promé on the occasion of his 65th birthday.

Introduction

The bio-inorganic chemistry of metal ions is currently one of the most fascinating field of investigation. Depending on both concentration and structural context in biosystems, properties of metal ions differ drastically. If we consider the case of silver, copper and lead, each of them exhibits a different behavioural pattern. Silver for example, due to (i) strong germicidal properties, (ii) development of antibiotic resistance in many diseases, and (iii) increase of new strains of bacteria and viruses, currently arouses the interest.^{1,2} However, it turns out that silver may be toxic when taken in large doses. For instance, excess deposition of silver in internal organs causes argyria³, a cosmetic trouble by which the skin turns gray or bluish gray. This decoloration is permanent and there is no treatment for it. Furthermore, silver salts may be dangerous, such as AgNO_3 , which is toxic for aquatic organisms.

Copper is one of the metallic elements which are essential to human health^{4,5}. Copper combines with certain proteins to produce enzymes that act as catalysts in numerous bodily functions. Copper is widely distributed in plants and animals, and its redox chemistry is involved in a variety of oxidation processes. Copper deficiency may cause severe conditions such as anemia, coronary heart diseases or bone fragility. Conversely, high levels of copper in humans may also be toxic. Wilson's disease⁶, an inborn error of metabolism, causes toxic amounts of copper to accumulate in the liver. The copper is then slowly released into other parts of the body, especially the brain, which can lead to brain damage.

With the development of industrial activities, anthropogenic emissions of lead now exceed natural emissions. As a matter of fact, lead is used in storage batteries, alloys, ceramics, paints, but also for atomic radiation and X-ray protection. Therefore, lead appears to be useful, but also toxic. For instance, alkylleads are rapidly metabolized and

readily cross the blood-brain barrier. These physicochemical properties make the central nervous system the main site of their toxic action⁷. Lead also causes damages to organs including the liver, kidneys and heart while Pb^{2+} ions, a large-scale environment pollutant, are known to obstruct heme biosynthesis, to inhibit several zinc enzymes, and to interact with nucleic acids and transfer RNA, affecting protein synthesis.^{8,9}

In the environment, the bioavailability and toxicity of such metals are governed by the ability of organic molecules to bind metal ions. This ability depends on many parameters of natural media, such as pH, ionic strength of aqueous media, or metal/ligand ratio. In order to properly assess the influence of each of these parameters, a preliminary step consists in describing the gas-phase complexation of metallic cations by natural organic compounds, and the present work aims to study the reactivity of the three aforementioned metals: silver, copper and lead.

Among the organic compounds found in nature, polysaccharides are the most abundant and are essential for both vegetal and animal life. They may be easily converted in a large variety of monosaccharides, which, in turn, may complex metal ions due to their numerous hydroxyl groups.

The reactivity between carbohydrates and metal ions have been widely studied in solution^{10,11}, because of the involvement of metal ion-saccharide interactions in key biological processes. Several papers have also reported the gas-phase mass spectrometric study of the complexation by glycosides of alkali, alkaline earth and transition metal cations. All these studies showed that gas-phase metal ion chemistry is a powerful tool in the stereochemical differentiation^{12,13,14,15,16,17} or linkage position determination^{18,19,20,21,22} of saccharides.

Apart from this analytical point of view, mass spectrometry, notably Fast Atom Bombardment (FAB) ionization and MS/MS experiments, is also of particular interest to study in a first step the "intrinsic" reactivity of metallic species, in order to

understand in a final step, what really happens in natural media. In this context, the present paper reports the FAB-MS/MS study of the gas-phase reactivity of the three metal ions Ag^+ , Cu^+ and Pb^{2+} , with five monosaccharides: D-glucose (**1**), D-galactose (**2**), D-fructose (**3**), O-methyl- α -D-glucose (**4 α**) and O-methyl- β -D-glucose (**4 β**), presented here in their pyranose form (Scheme 1).

<Scheme 1>

Experimental

FAB mass spectra were recorded on a tandem ZAB-HSQ mass spectrometer (Fisons Instrument) of BEqQ configuration (B and E represent the magnetic and the electrostatic sectors, q, an r.f. only quadrupole collision cell, and Q, the mass selective quadrupole). The FAB ion source was used as follows: accelerating voltage: 8 kV, neutral xenon beam 7 kV and neutral current of $\sim 10 \mu\text{A}$. Monosaccharides were dissolved in a few drops of a mixture of glycerol and a saturated aqueous metallic (lead, silver or copper) salt solution. Note that except for O-methyl- α -D-glucose (**4 α**) and O-methyl- β -D-glucose (**4 β**), dissolving the monosaccharide in water leads to the loss of stereochemistry of the anomeric center. Few microliters of the resulting mixture were then transferred onto the stainless steel FAB tip. Solutions of Ag^+ , Cu^+ and Pb^{2+} were prepared by dissolving AgNO_3 , CuCl_2 and $\text{Pb}(\text{NO}_3)_2$, in distilled water, respectively. Cu^+ ions are likely generated from Cu^{2+} by oxidation/reduction processes as has been previously postulated²³. Mass calibration was achieved by using sodium iodide. Glycerol was chosen as matrix. Thioglycerol was also tested but didn't lead to any significant improvement in signal to noise ratio. Copper, silver and lead present several natural isotopes: 69% ^{63}Cu / 31% ^{65}Cu , 51% ^{107}Ag / 49% ^{109}Ag and 1.4% ^{204}Pb / 24.1% ^{206}Pb / 22.1% ^{207}Pb / 52.4% ^{208}Pb , respectively. Unless otherwise noted, results

presented thereafter refer to the study of organometallic complexes involving for each metal, the most abundant isotope.

Unimolecular chemistry of organometallic species of interest, which takes place in the second Field-Free Region (2nd FFR) of the mass spectrometer, located just after the magnet, has been studied by Mass analyzed Ion Kinetic Energy (MIKE) spectroscopy. This technique consists in focusing the parent ion magnetically into the 2nd FFR, and detecting its spontaneous fragmentation products by scanning the electrostatic analyzer, E. The MIKE spectra were recorded at a resolving power of ~1000. For Collisionally Activated Decomposition (CAD) MS/MS spectra, the pressure of argon in the collision cell was adjusted so that the main beam signal was reduced by approximately 30%. Metallic salts and monosaccharides are commercially available from Aldrich and Fluka and were used without any further purification.

Results and discussion

Positive ion FAB spectra of cationized glycosides

Silver cationization

Figure 1a exhibits the FAB-MS spectrum of an aqueous mixture of D-glucose/silver nitrate/glycerol. It is worth mentioning that this spectrum does not give prominent protonated ions but principally cationized ions whatever the monosaccharide studied. The Ag⁺-containing ions are identified by the presence of ion pairs 2 u apart with approximately similar abundance (51% and 49%). In the first region of the spectrum (below m/z 100), fragment ions of protonated glycerol are detected. Then, we observed abundant doublets at m/z 287/289 and m/z 199/201 corresponding to the ¹⁰⁷Ag and ¹⁰⁹Ag isotopes of silver-cationized D-glucose (**1**) and glycerol, respectively. Isotopic triplets at m/z 215/217/219, m/z 231/233/235 and m/z 276/278/280 are attributed to the

ions Ag_2H^+ , Ag_2OH^+ and Ag_2NO_3^+ , respectively, and the quadruplet at m/z 321/323/325/327 to Ag_3^+ ions. It is important to note that the FAB-MS spectra for each glycoside (**1**, **2** and **3**) lead to rather similar ions, but with different intensities. These results are consistent with those obtained by Berjeaud et *al.* at the time of the study of stereochemically controlled decomposition of silver-cationized methyl-glycosides. Hence, aldoses and ketose can't be distinguished by means of their positive ion mass spectra.

An additional ion of interest at m/z 407/409/411 is only detected for methyl-glycosides (**4 α** and **4 β**) and agrees with an ion containing two silver ions $[\text{2Ag} + \text{monosaccharide} - \text{H}]^+$. This ion is more abundant for O-methyl- β -D-glucose than for O-methyl- α -D-glucose.

Copper cationization

Figure 1b shows the FAB-MS spectrum of an aqueous mixture of D-glucose/ CuCl_2 /glycerol. For all the monosaccharides, the molecular ion region is characterized by many peaks corresponding to fragment ions of both protonated glycerol and protonated monosaccharide, such as $[\text{monosaccharide} + \text{H} - 3\text{H}_2\text{O}]^+$ (m/z 127), $[\text{monosaccharide} + \text{H} - 2\text{H}_2\text{O}]^+$ (m/z 145), $[\text{monosaccharide} + \text{H} - \text{H}_2\text{O}]^+$ (m/z 163), and exclusively for the methyl-glycosides (**4**) $[\text{monosaccharide} + \text{H} - \text{CH}_3\text{OH}]^+$ (m/z 163). As in the case of silver, Cu-containing ions are discernible by a doublet separated by 2 mass units. In the FAB spectrum, we detected doublets of interest at m/z 243/245 and m/z 155/157 corresponding to the ^{63}Cu and ^{65}Cu isotopes of copper-cationized D-glucose (**1**) and glycerol, respectively. A couple of weak ions are also observed for all the monosaccharides: $[\text{2Cu} + \text{monosaccharide} - \text{H} + 2\text{H}_2\text{O}]^+$ (at m/z 341/343/345) and $[\text{Cu} + 2\text{monosaccharide}]^+$ (at m/z 423/425). In the particular case of

D-galactose (**2**) and D-fructose (**3**), a significant $[\text{Cu} + \text{monosaccharide} - \text{H}_2\text{O}]^+$ ion at m/z 225/227 is observed.

Some particular ions are detected in the FAB spectra of both methyl-glycosides (**4 α** and **4 β**), which correspond to $[\text{Cu} + \text{monosaccharide} - \text{CH}_3\text{OH}]^+$ (at m/z 225/227), $[\text{Cu} + \text{monosaccharide} + \text{H}_2\text{O}]^+$ (at m/z 275/277), $[2\text{Cu} + \text{monosaccharide} - \text{H} + 2\text{H}_2\text{O}]^+$ (at m/z 355/357/359) and $[\text{Cu} + 2\text{monosaccharide}]^+$ (at m/z 451/453). Finally, the high mass region (above m/z 400) presents many complex species containing one or more molecules of glycerol and copper atoms. In summary, as in the case of silvered complexes, positive ion FAB spectra don't permit aldoses to be distinguished from ketoses.

Lead cationization

Figure 1c presents the m/z 100-510 range of a FAB-MS spectrum of an aqueous mixture of D-glucose/lead nitrate/glycerol. For all the monosaccharides, the FAB mass spectrum may be divided in three distinct regions: (i) below m/z 200, (ii) between m/z 200 and m/z 510, and (iii) above m/z 510.

The low mass region (i) presents fragment ions of both protonated glycerol and protonated monosaccharide, as already mentioned for copper cationization.

In the intermediate region (ii) (see Figure 1c), we can see a series of triplets, characteristic of ions containing a single lead atom, species involving the less abundant lead isotope (^{204}Pb) being very small indeed absent. The triplet at m/z 206/207/208 corresponds to bare lead ion Pb^+ . The triplet at m/z 225 is attributed to PbOH^+ . The region is dominated by a very intense adduct at m/z 297/298/299, identified as $[\text{Pb} + \text{glycerol} - \text{H}]^+$. Interaction of Pb^{2+} ions with monosaccharides leads to an abundant adduct $[\text{Pb} + \text{monosaccharide} - \text{H}]^+$, (at m/z 385/386/387 for D-glucose, Figure 1c), together with a very weak $[\text{Pb} + \text{monosaccharide} - \text{H} - \text{H}_2\text{O}]^+$ species. In the particular

case of D-glucose (**1**) and D-galactose (**2**), a significant ion at m/z 325/326/327, which probably corresponds to $[\text{Pb} + \text{monosaccharide} - \text{H} - \text{C}_2\text{H}_4\text{O}_2]^+$, is also observed. This loss of 60 mass units has already been observed in previous FAB²⁵ or electrospray studies.²⁰ The region (ii) also presents three species due to reactions between lead and glycerol, at mass to charge ratios 249/250/251, 265/266/267 ($[\text{Pb} + 2\text{glycerol} - \text{H} - \text{CH}_3\text{OH}]^+$ and 389/390/391 ($[\text{Pb} + 2\text{glycerol} - \text{H}]^+$). Therefore, we don't observe in the particular case of lead, $[\text{metal} + \text{glycoside}]^+$ ions. During the complexation process, a deprotonation systematically takes place and unlike copper, lead retains its oxidation state.

The high mass region (iii) (not shown in Figure 1c) is relatively complex and difficult to interpret. It shows many species with low but significant intensities, containing one, two or three lead atoms. Most of these ions arise from gas-phase reactions between lead and glycerol, since they are still observed without or when varying the glycoside. Moreover, some are isobaric with $[\text{Pb} + \text{monosaccharide} + \text{glycerol} - \text{H}]^+$ species. Therefore, we can't assert that these latter ions are formed. However, the fact that addition of D-glucose, D-galactose or D-fructose to the $\text{Pb}(\text{NO}_3)_2/\text{glycerol}/\text{H}_2\text{O}$ mixture results in an increase in the intensity of the m/z 479 peak, suggests that the $[\text{Pb} + \text{monosaccharide} + \text{glycerol} - \text{H}]^+$ complexes might also be generated. Finally, note that complexes involving one lead atom and two glycoside units are not observed.

To summarize, our experiments show that potentially toxic metal ions Pb^{2+} complex with monosaccharides by displacing one acidic proton and therefore retain their oxidation state. Furthermore, the positive ion FAB spectra allow the aldoses (**1** and **2**) and the ketose (**3**) to be distinguished. The nature of bonding of Pb^{2+} with polyfunctional molecules in the gas-phase is not well described, but this behaviour is in agreement with what was observed by Saraswathi *et al.* in their study concerning the

interaction of Pb^{2+} ions with nucleobase-substituted polyethers²⁴. Nevertheless, unlike these authors, we don't observe metal chelated species involving counter-ions.

Metastable decompositions of cationized glycosides

This section presents the MIKE spectra of the following complexes: $[\text{Ag} + \text{monosaccharide}]^+$, $[\text{Cu} + \text{monosaccharide}]^+$, and $[\text{Pb} + \text{monosaccharide} - \text{H}]^+$. For these species, experiments have been carried out on adducts containing the most abundant metal isotope, i.e. ^{107}Ag , ^{63}Cu and ^{208}Pb . CAD spectra have been also systematically recorded for each combination of metal and glycoside, but are not presented here since they don't provide any additional information.

$[\text{Ag} + \text{glycoside}]^+$

The MIKE spectrum of the ^{107}Ag adduct of D-glucose (**1**) is given in Figure 2a. The corresponding spectra of other glycosides are available upon request to the authors. Unimolecular decompositions of the silver-cationized glycosides, lead to rather similar fragment ions, but with different intensities. MIKE spectra of $[\text{Ag} + \text{monosaccharide}]^+$ ion of **1**, **2** and **3** (m/z 287) is characterized by dehydrogenation (m/z 285) and dehydration (m/z 269) processes (see Table 1). The species corresponding to the loss of H_2 is the most abundant ion in the case of **1** and **3**, whereas the elimination of H_2O appears to be predominant for D-galactose (**2**). Note that careful examination of positive ion FAB spectra (see Figure 1a for example) show that $[\text{Ag} + \text{glycoside} - \text{H}_2]^+$ species are not likely to be generated during the FAB process. Two observations support this conclusion: (i) lack of m/z 285 on the spectra, and (ii) correct relative intensities of the doublet m/z 287/289 (51%/49%), suggesting that $[\text{Ag} + \text{glycoside} - \text{H}_2]^+$ are not present on spectra.

In summary, for all the saccharides, the major fragmentations systematically involved losses of simple neutral molecules such as hydrogen or water. It must be also reminded

that in protic solvents, aldoses exist predominantly in the form of cyclic pyranose hemiacetals. Thus, the sample solutions deposited onto the FAB tip should essentially contain pyranose structures. Assuming that these are pyranoses desorbed into the gas-phase, the decompositions observed allow the C(4) stereochemistry of the pyranose ring to be characterized. On the other hand, use of silver salt does not allow a clear-cut distinction between D-glucose and D-fructose.

[Cu + glycoside]⁺

Relative intensities of fragment ions observed during metastable dissociation of the five [Cu + monosaccharide]⁺ complexes are reported in Table 2. From this Table, we can see that their MIKE spectra are significantly different from those obtained for the silver-glycosides adducts. Similar losses are observed (dehydrogenation, dehydration), but copper cationization induces additional species. Within the time scale of the experiment, fragmentation of the various complexes studied produces exclusively Cu⁺-containing fragment ions, the metal never being eliminated. Metastable-ion fragmentation pathways common to all copper cationized monosaccharides are losses of H₂, 16 u and H₂O. To illustrate this reactivity, the MIKE spectrum of the [⁶³Cu + D-glucose]⁺ (m/z 243) is given in Figure 2b and is characterized by a very abundant dehydration (m/z 225), by intense losses of hydrogen (m/z 241), of 16 mass units corresponding to [Cu + D-glucose - O]⁺ (m/z 227) and of 2H₂O (m/z 207). Two main differences between the decomposition pathways of [Cu + **1**]⁺ and [Cu + **3**]⁺ can be observed: (i) removal of 16 u from [Cu + **1**]⁺ is a minor process but becomes the major reaction channel for [Cu + **3**]⁺, and (ii) loss of two molecules of water is completely suppressed for [Cu + **3**]⁺. As mentioned earlier, we may deduce from positive ion spectra that [Cu + saccharide - H₂]⁺ are very probably not generated during the FAB process, and consequently m/z 243 ions correspond to the single [⁶³Cu + monosaccharide]⁺ species.

The main fragmentation of the three glycosides is different for each copper complex, that is dehydration for $[\text{Cu} + \mathbf{1}]^+$, dehydrogenation for $[\text{Cu} + \mathbf{3}]^+$ and finally loss of 16 mass units for $[\text{Cu} + \mathbf{3}]^+$. Hence, from our results, it turns out that glycosides **1**, **2** and **3** exhibit specific dissociation patterns (Table 2) and are easily distinguished.

$[\text{Pb} + \text{glycoside} - \text{H}]^+$

Table 3 summarizes the abundances of ions generated by metastable dissociation of the five $[\text{Pb} + \text{monosaccharide} - \text{H}]^+$ complexes. Several fragmentations have been already observed with copper and silver, namely loss of H_2 and H_2O , but comparison between Tables 1, 2 and 3 displays interesting differences. Thus, elimination of molecular hydrogen, which was predominant with silver and abundant with copper, is only a minor process with lead. Loss of water is observed but with minor extent for **1** and **2**. We have seen previously that elimination of two molecules of water is specifically observed for $[\text{Cu} + \mathbf{1}]^+$ and $[\text{Cu} + \mathbf{2}]^+$ complexes, that is, with aldoses. With lead, the reactivity is completely different since this fragmentation occurs only for $[\text{Pb} + \text{D-fructose} - \text{H}]^+$ ions.

Pb^{2+} ions retain their oxidation state when binding the glycosides, and therefore induce a different reactivity. Indeed, metastable decomposition of $[\text{Pb} + \text{monosaccharide} - \text{H}]^+$ complexes leads to the formation of many unsaturated organic molecules involving one or several carbon and oxygen atoms. As illustrated by Figure 2c, loss of $\text{C}_2\text{H}_4\text{O}_2$ is the major dissociation channel observed for D-glucose (**1**) but also for D-galactose (**2**), and occurs for **3**. Other organic neutrals such as CH_2O , $\text{C}_3\text{H}_6\text{O}_3$ are also generated. Such processes have been already observed, on the one hand, under FAB conditions by Smith et al.²⁵, who studied the unimolecular chemistry of $[\text{Ni}(\text{diaminopropane}/\text{monosaccharide}), - \text{H}]^+$ complexes, and on the other hand, for Zn(II) and Cu(II) complexes of glycosides under electrospray conditions.²²

Our data also show a clear distinction in the dissociation ions for each of the complexes of D-glucose, D-galactose and D-fructose (Table 3). A predominant dehydration and a specific loss of formaldehyde and a second molecule of water characterize the latter one. Elimination of $C_2H_4O_2$ by a cross-ring cleavage is clearly overwhelming for $[Pb + \mathbf{1} - H]^+$ and $[Pb + \mathbf{2} - H]^+$ (base peak). Moreover, these two adducts are differentiated by a loss of $C_3H_6O_3$ mostly encountered for $[Pb + \mathbf{2} - H]^+$. Finally, note that like Ag and Cu, lead is not eliminated during dissociation.

In conclusion, our results clearly show that Pb^{2+} ions induce the richest reactivity and are of particular interest from the analytical point of view.

Metastable decompositions of cationized methyl-glycosides

Unimolecular decomposition of complexes $[^{109}Ag + \mathbf{4}\alpha]^+$ and $[^{109}Ag + \mathbf{4}\beta]^+$ are presented in Figure 3. The principal fragmentation of $[Ag + \mathbf{4}\alpha]^+$ species (m/z 303) is loss of methanol (m/z 271), and in a minor extent, dehydration (m/z 285) and elimination of silver hydride (m/z 193). The unimolecular reactivity of $[Ag + \mathbf{4}\beta]^+$ species is totally different, characterized by two dissociation channels: predominant loss of AgH (m/z 193), and a significant loss of CH_3OH . Therefore, complexation by Ag^+ cations as already observed by Berjeaud et al.¹⁷, results in a clear-cut characterization of the anomeric center. Distinction between D-glucose and O-methyl- α -D-glucose is also straightforward.

Unimolecular decomposition of complexes $[Cu + \mathbf{4}\alpha]^+$ and $[Cu + \mathbf{4}\beta]^+$ involving ^{63}Cu isotope (m/z 257), are presented in Figure 3. Careful examination of positive ion FAB spectra shows that $[Cu + \mathbf{4} - H_2]^+$ species are not likely to be generated during the FAB process. However, the MIKE spectrum of $[^{63}Cu + O\text{-methyl-D-glucose}]^+$ (m/z 257) shows a specific loss of methanol (m/z 225), and therefore are significantly different from that of $[Cu + D\text{-glucose}]^+$. Nevertheless, distinction between $\mathbf{4}\alpha$ and $\mathbf{4}\beta$ isomers is

not as straightforward as with silver cations (Table 2). Note also the presence of an intense peak at m/z 227.5, probably due to a metastable dissociation occurring in the first field free region.

Finally, we can see from MIKE spectra of $[\text{Pb} + \mathbf{4}\alpha - \text{H}]^+$ and $[\text{Pb} + \mathbf{4}\beta - \text{H}]^+$ complexes (m/z 401), that stereochemistry of the anomeric center is easily determined by reaction with Pb^{2+} ions. Common fragmentations for the two anomers are loss of methanol (base peak), elimination of H_2 and of a $\text{C}_3\text{H}_6\text{O}_2$ fragment. Several dissociation channels are almost specifically observed for O-methyl- α -D-glucose, such as loss of water (m/z 383), water and methanol (m/z 351), and also elimination of carbohydrates $\text{C}_2\text{H}_4\text{O}_2$ and $\text{C}_4\text{H}_8\text{O}_3$. Finally, comparison of these six spectra shows that once again, Pb^{2+} ions induce the richest reactivity.

Unimolecular reactivity of metal/glucose complexes

D-glucose is one of the most abundant monosaccharides found in nature. It may be generated from the degradation of various polysaccharides encountered in both vegetal and animal organisms, such as cellulose or starch. Due to its high abundance in the environment but also to its involvement in key-biochemical processes, it is of particular interest to properly describe the intrinsic interaction between this polyfunctional molecule and metallic cations. In this purpose, MS/MS experiments provide useful information, and the present section aims to propose several dissociation mechanisms of cationized glucose, based on the origins of the neutral losses. Within this context, comparison with results obtained for O-methyl-D-glucose (**4**) may be useful for some dissociation channels. This section is divided in two main parts. The first part deals with the unimolecular reactivity of $[\text{Metal} + \text{D-glucose}]^+$ complexes, with $\text{M}=\text{Ag}$ and Cu , whereas the second will be specifically devoted to the interaction between glucose and Pb^{2+} ions.

Unimolecular reactivity of silver and copper associated glucose.

Studying the unimolecular reactivity of cationized glucose is to first locate the most favored coordination site of the cation. Metallic species may, in principle, attach to any of the electron-rich centers of glucose, including the hemiacetal oxygen. However, trends can be deduced from several theoretical investigations about the gas-phase interaction between metallic cations and monosaccharides. Cerda *et al.* have carried out experiments and calculations concerning the complexation of Na^+ ions with a series of pentoses and hexoses.¹⁹ Given that in protic solvent, glucose exists exclusively in the pyranose form²⁶, and assuming that these pyranoses are desorbed into the gas-phase, these authors noticed that whatever the conformation of the pyranose ring and the stereochemistry of the anomeric center (α or β), structures with monodentate coordination of Na^+ are higher in energy than multidentate conformers. Furthermore, the most stable geometries obtained at the HF/6-31G(d) level are tri- or tetradentate $[\text{Na} + \text{D-glucose}]^+$ complexes, with sodium ion ($r = 0.97 \text{ \AA}^{27}$) bound to the hydroxymethyl group, the hemiacetal oxygen, and at least one additional hydroxyl group (Scheme 2).

<Scheme 2>

Similar results have been obtained by Zheng *et al.*, who carried out calculations about the interaction between Ca^{2+} ions ($r = 0.99 \text{ \AA}^{27}$) and β -D-glucose.²⁸ Thus, despite a different ground state electronic configuration, Cu^+ ions, given their size (0.96 \AA^{27}) should follow the same coordination scheme, as well as silver cations, which are slightly larger (1.26 \AA^{27}). Note that for the clarity of the upcoming schemes, only the interactions between the metal on one hand, and the hydroxymethyl group and the hemiacetal oxygen on the other hand, will be represented.

Loss of H_2 . Elimination of H_2 from $[M + D\text{-glucose}]^+$ complexes is an important process for both silver and copper. One may reasonably assume that the anomeric center, which is the most active site of monosaccharides, is involved in this dissociation. To account for this fragmentation, we propose two possible 1,2-elimination mechanisms (Scheme 3).

<Scheme 3>

The first one involves hydrogens borne by two adjacent carbon atoms, namely C(1) and C(2). This mechanism should prevail over similar mechanisms because the resulting double bond can be delocalized by mesomeric effects implying the lone pair of three oxygen atoms (2 hydroxyl groups and the hemiacetal oxygen). The second one involves the C(1) hydrogen together with the hydrogen the anomeric hydroxyl, and results in the formation of a $[M + \delta\text{-gluconolactone}]^+$ species. Experiments with labelled compounds have to be performed to confirm or weaken the proposed mechanisms.

Loss of water. The second important dissociation channel observed for $[Ag + D\text{-glucose}]^+$ and $[Cu + D\text{-glucose}]^+$ complexes is dehydration. Rigorously, this fragmentation may involve any of the hydroxyl groups. Nevertheless, comparison of results obtained for D-glucose with those of O-methyl-D-glucose (**4**), indicates that the anomeric hydroxyl is systematically eliminated. As a matter of fact, when the anomeric hydroxyl is changed for a methoxy group, loss of water is no longer observed for silver (Table 1), and is replaced by formation of methanol. Consequently, in the case of silver-cationized glucose, loss of water implies almost exclusively the anomeric hydroxyl. A simple mechanism, given in Scheme 4, might account for this fragmentation.

<Scheme 4>

This mechanism is supported by experiments with a series of deuterium-labelled methylglucopyranosides¹⁷, which demonstrated that the hydrogen atom eliminated together with the methoxy group, is probably that of the C(2) hydroxyl group.

Examination of Table 2 shows that this mechanism is also predominant for [Cu + D-glucose]⁺ complexes. However, the presence on MIKE spectra of cationized α -O-methyl-D-glucose (**4 α**) and β -O-methyl-D-glucose (**4 β**), of a peak corresponding to an elimination of water, demonstrate that other hydroxyl groups are also expelled. Finally, note that for copper, subsequent losses of two molecules of water are observed (m/z 207, Figure 2b), that might be explained by the formation of a cationized dienic species (Scheme 5).

<Scheme 5>

Loss of metal hydride. Table 1 and 2 show that Ag⁺ and Cu⁺ ions react with O-methyl-D-glucose (**4**) by hydride abstraction, leading to the elimination of a MH fragment. Previous studies with O-methyl-D-glucose-1-d₁, indicate that the C(1) hydrogen atom is exclusively eliminated.¹⁷ Their findings further suggest that this fragmentation clearly depends on the C(1) stereochemistry, and is particularly important for the β anomer. Elimination of the metal moiety from [Metal + O-methyl-D-glucose]⁺ complexes certainly implies structures for which the metal is weakly bonded to the glycoside, and consequently a coordination scheme different from that presented in Scheme 2. Therefore, this different way of fragmentation seems to indicate that [Metal + O-methyl-D-glucose]⁺ ions detected in positive ion mass spectra probably correspond to a mixture of structures. A possible mechanism, involving a ¹C₄ conformation of the pyranose ring, might account for this stereoselective dissociation (Scheme 6).

<Scheme 6>

Attachment of the metal below the plane of the pyranose ring might be favored because of hydrogen bonds hindering the upper face of the ring. The metallic cation then might interact with the C(2) hydroxyl, leading to a monodentate structure, and finally expel the C(1) hydrogen.

Loss of 16 mass units. An interesting dissociation channel, only observed for [Cu + D-glucose]⁺ complexes, is elimination of a 16 mass units fragment, that we attribute to an oxygen atom. This fragmentation has already been noticed by Madhusudanan *et al.*, during the study of the collision-induced decomposition of cationized thioglycosides.¹⁸ Such a decomposition requires a considerable rearrangement within the complex. At the point of our study, we are not able to propose a mechanism. Additional experiments with deoxyglucoses and labelled glucoses are necessary in order to give a reliable mechanism.

Unimolecular reactivity of lead/glucose complex.

We have seen previously that the gas-phase reactivity of Pb²⁺ ions towards D-glucose is different from that of Ag⁺ and Cu⁺. The reaction is indeed characterized by the removal of an acidic proton, and leads to [Pb + D-glucose - H]⁺ species. According to several theoretical studies about the relative gas-phase acidities of glucopyranose^{29,30,31,32}, the anomeric hydroxyl is found to be the most acidic. A complete theoretical study about the interaction of Pb²⁺ ions with D-glucose is currently under progress³³ and is beyond the scope of the present paper, but preliminary semi-empirical calculations with the PM3 method³⁴ suggest that Pb²⁺ ions do not deprotonate the anomeric hydroxyl, but preferentially the less acidic hydroxymethyl group, and also interacts with the hemiacetal oxygen and an other hydroxyl groups. Therefore, it turns out that deprotonation of the hydroxymethyl group results in a better solvation of the metal by

the ligands due to the free rotation of the C(5)-C(6) bond, and that the best coordination site of Pb^{2+} ions is similar to that of silver and copper cations.

As mentioned earlier, $[\text{Pb} + \text{D-glucose} - \text{H}]^+$ complexes dissociate according to four channels, dehydrogenation, dehydration and loss of $\text{C}_2\text{H}_4\text{O}_2$ and $\text{C}_3\text{H}_6\text{O}_3$ molecules.

Loss of H_2 is a minor process and may be explained by a mechanism equivalent to those depicted in Scheme 3.

Elimination of water is also a minor but significant process. Comparison with results obtained for methyl-glycosides **4 α** and **4 β** (Table 3) show that the anomeric hydroxyl group is preferentially eliminated by a mechanism, which could be similar to the mechanism presented in Scheme 4. But like copper, other hydroxyl groups are involved in the fragmentation.

Fragmentations characteristic of Pb^{2+} ions reactivity are elimination of organic molecules of general formula $\text{C}_n\text{H}_{2n}\text{O}_n$. These processes involve the cleavage of the pyranose ring and are observed neither with silver nor with copper. Thus, formation of the $\text{C}_2\text{H}_4\text{O}_2$ corresponds to the predominant dissociation of $[\text{Pb} + \text{D-glucose} - \text{H}]^+$ complex. Once again, a careful comparison with results obtained for O-methyl-D-glucose provides useful information about the carbon atoms eliminated. As a matter of fact, when the anomeric hydroxyl is replaced by a methoxy group, loss of $\text{C}_2\text{H}_4\text{O}_2$ is almost totally changed for $\text{C}_3\text{H}_6\text{O}_2$, thus pointing out that the anomeric center is once again involved in the fragmentation. One may reasonably assume that the second carbon center that is lost can be assigned to C(2), because elimination of a $\text{C}_2\text{H}_4\text{O}_2$ fragment involving C(1) and not C(2), would require a considerable rearrangement in the ion structure during the dissociation. Hence, the following mechanism may be proposed to account for experimental results (Scheme 7).

<Scheme 7>

Similarly, careful examination of Table 3 indicates that the anomeric center is also present in the $C_3H_6O_3$ loss suggesting that this fragment might include C(1), C(2) and C(3) centers.

Finally, one of the most striking features of our results is that the unimolecular chemistry of $[Pb + D\text{-glucose} - H]^+$ and $[Pb + O\text{-methyl-}\beta\text{-D-glucose} - H]^+$ complexes are remarkably similar (Table 3). This would mean that the observed reactivity would rather involve the β anomer of D-glucose.

Conclusion

The present FAB-MS/MS study shows that the complexation process of Pb^{2+} ion with monosaccharides is clearly different from that of Cu^+ and Ag^+ ions. The former remains formally divalent and forms $[Pb + \text{monosaccharide} - H]^+$ species, while the latter ions lead to $[\text{Metal} + \text{monosaccharide}]^+$ complexes. Metastable decomposition studies have shown that these three metallic cations are of potential interest in the structural characterization of isomeric glycosides. For example, each metal allows D-glucose and D-galactose to be distinguished. Differentiation between D-fructose and D-glucose is also clearly achieved with copper and lead. Furthermore, experiences carried out with α and β anomers of O-methyl-D-glucose, demonstrate that the stereochemistry of the anomeric center is easily characterized by reaction with Ag^+ or Pb^{2+} ions. Two principal ways of fragmentation are typically observed: (i) loss of functional groups such as water, methanol or formaldehyde, (ii) cross-ring cleavages leading to the elimination of $C_nH_{2n}O_n$ molecules, which are specifically encountered with Pb^{2+} ions. According to our results, but also to those of other groups, all these fragmentations involve the anomeric center. Several mechanisms are proposed to account for the main

fragmentations of cationized glucose. Finally, the unusual loss of an oxygen atom from $[\text{Cu} + \text{monosaccharide}]^+$ complexes requires a detailed mechanistic study involving a series of labelled glycosides.

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Table caption

Table 1. Abundance of the principal ions in the MIKE spectra of the [Ag + monosaccharide]⁺ ions of 1-4^a.

Table 2. Abundance of the principal ions in the MIKE spectra of the [Cu + monosaccharide]⁺ ions of 1-4^a.

Table 3. Abundance of the main fragment ions in the MIKE spectra of the [Pb + monosaccharide - H]⁺ ions of 1-4^a.

Figure caption

Figure 1. Positive ion FAB spectra of glycerol/H₂O/D-glucose solution mixed with AgNO₃ (1a), CuCl₂ (1b) and Pb(NO₃)₂ (1c).

Figure 2. MIKE spectra of [Ag + D-glucose]⁺ (2a), [Cu + D-glucose]⁺ (2b) and [Pb + D-glucose - H]⁺ (2c) complexes

Figure 3. Metastable decomposition of [M + O-methyl-D-glucose]⁺ (M= Ag, Cu) and [Pb + O-methyl-D-glucose - H]⁺ complexes. See text for details.

Scheme caption

Scheme 1. Glycosides (1,2,3) and methyl-glycosides (4 α , 4 β) studied

Scheme 2. Privileged coordination of metallic cations to D-glucose.

Scheme 3. Mechanisms accounting for the loss of molecular hydrogen from [M + D-glucose]⁺ complex (M= Ag, Cu)

Scheme 4. Dehydration process from D-glucose cationized by Ag⁺ or Cu⁺ ions.

Scheme 5. Successive elimination of two molecules of water from D-glucose cationized by Cu⁺ ion.

Scheme 6. Loss of metal hydride from $[M + \text{O-methyl-}\beta\text{-D-glucose}]^+$ complex (M= Ag, Cu).

Scheme 7. Loss of $\text{C}_2\text{H}_4\text{O}_2$ from $[\text{Pb} + \text{D-glucose} - \text{H}]^+$ complex.

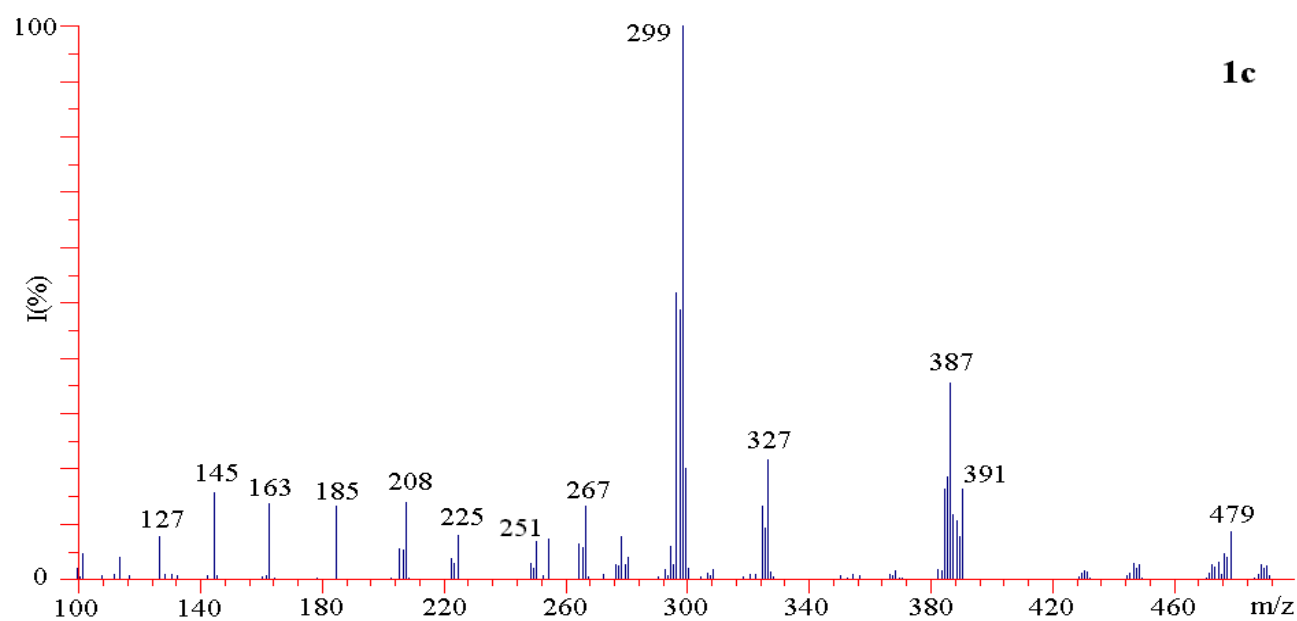
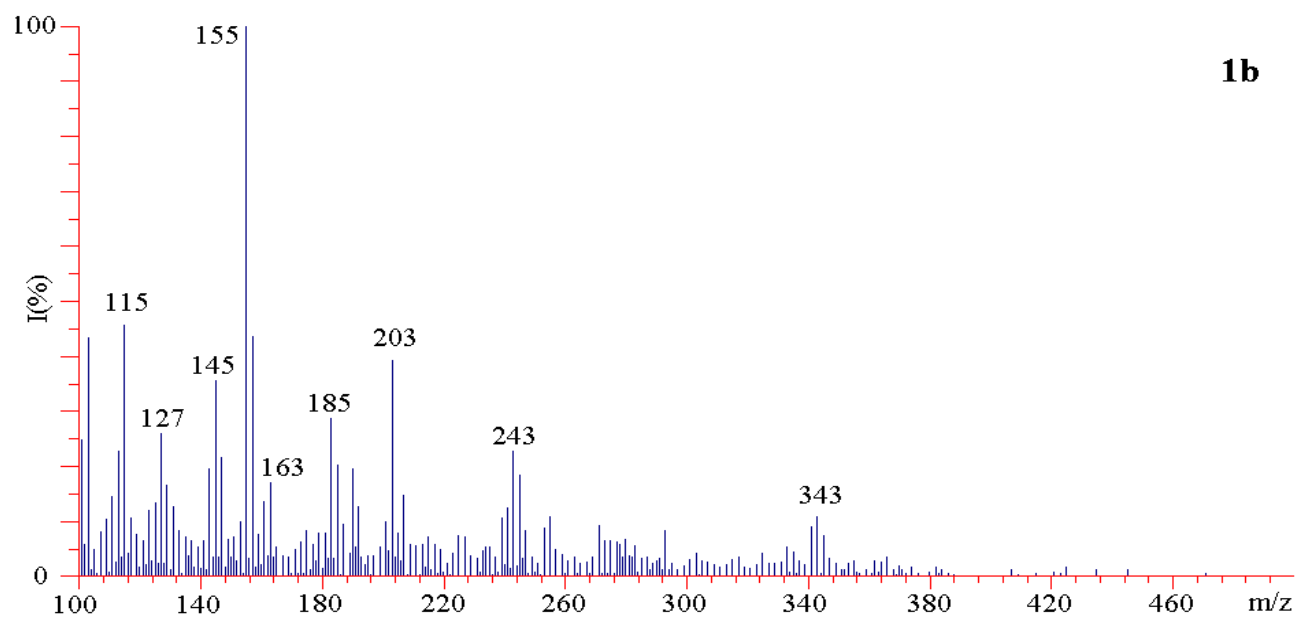
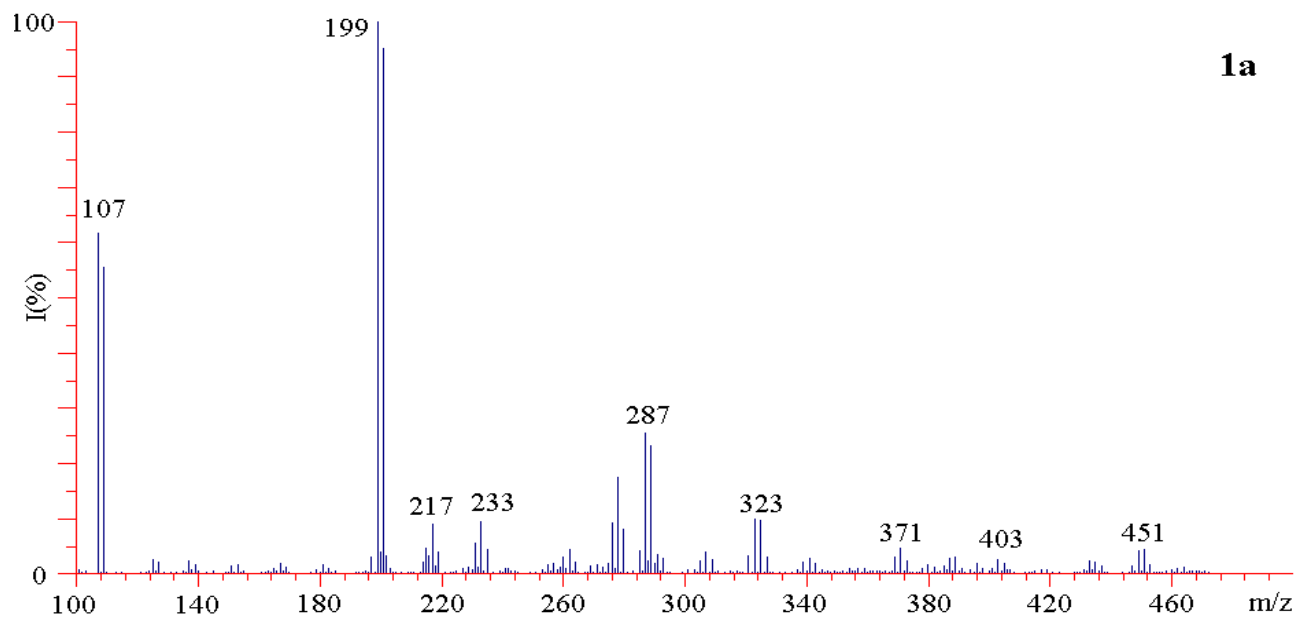


Figure 1

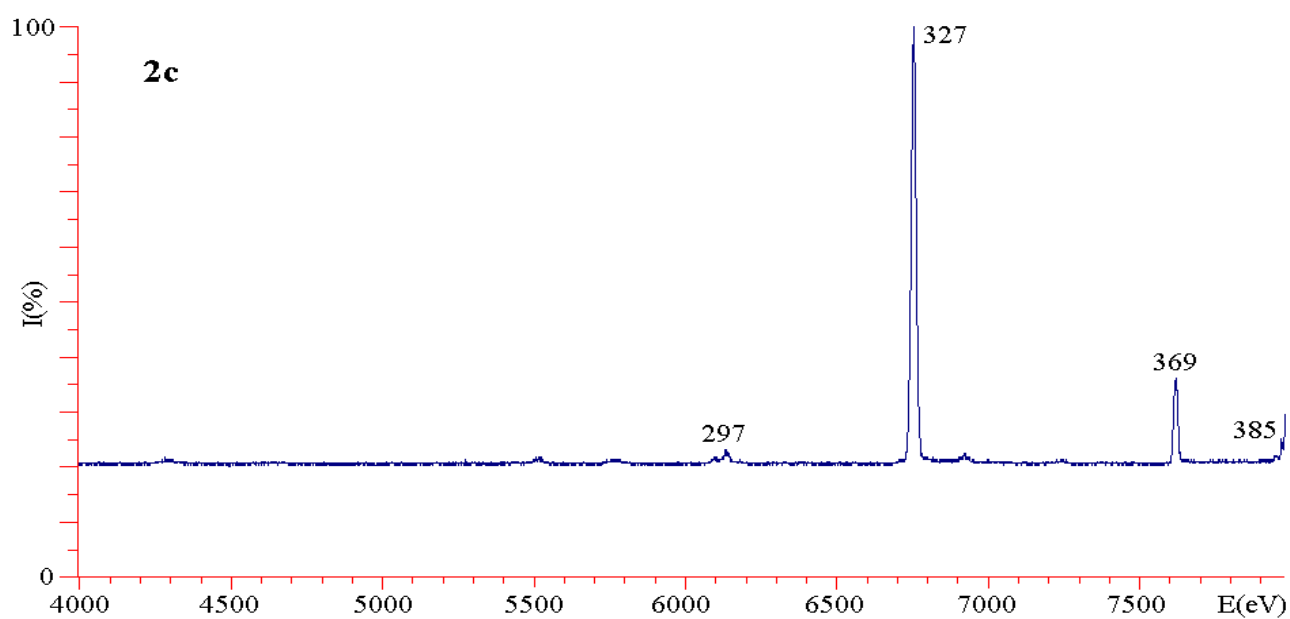
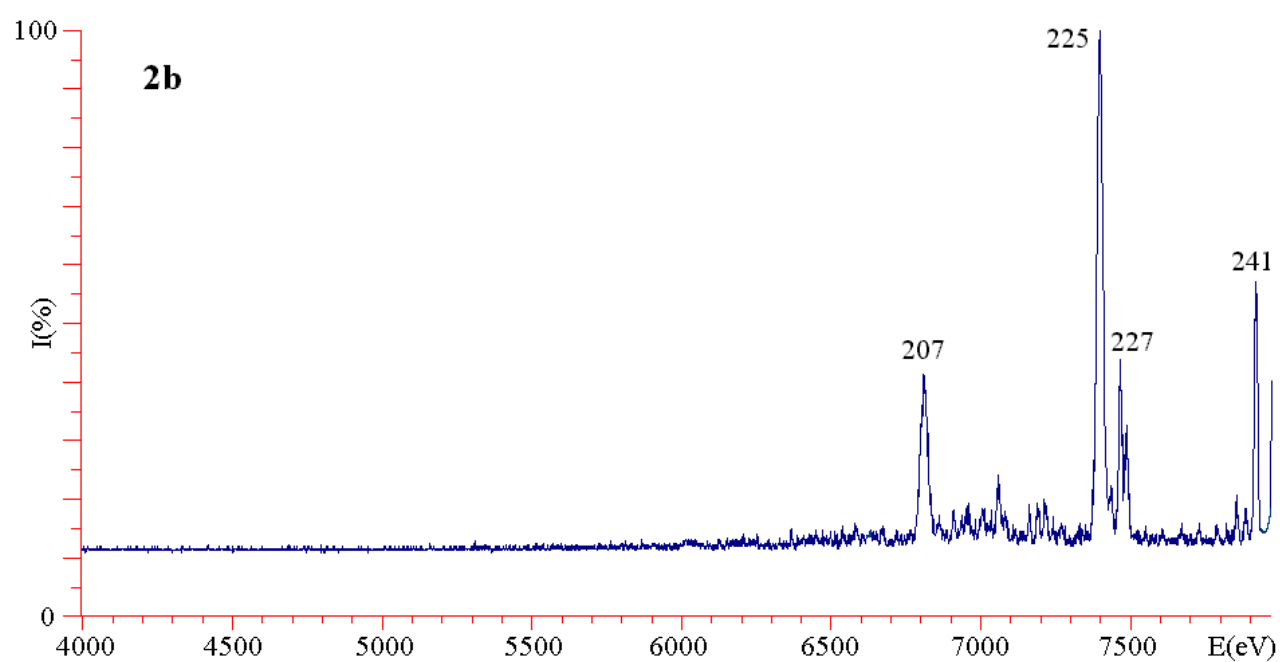
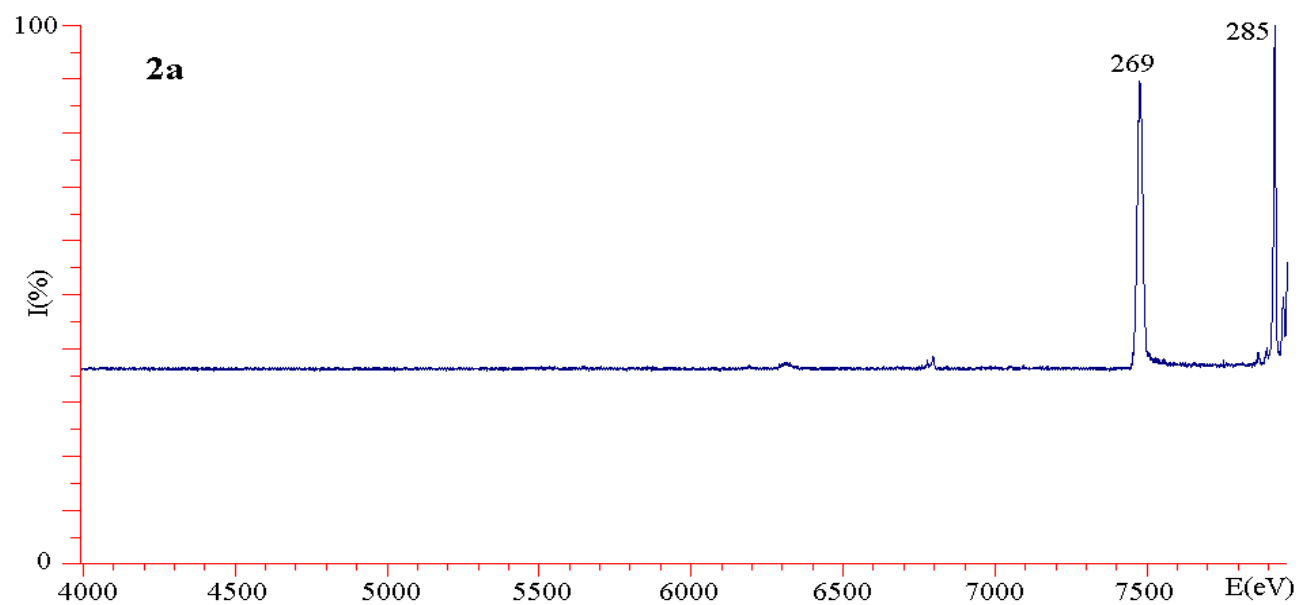
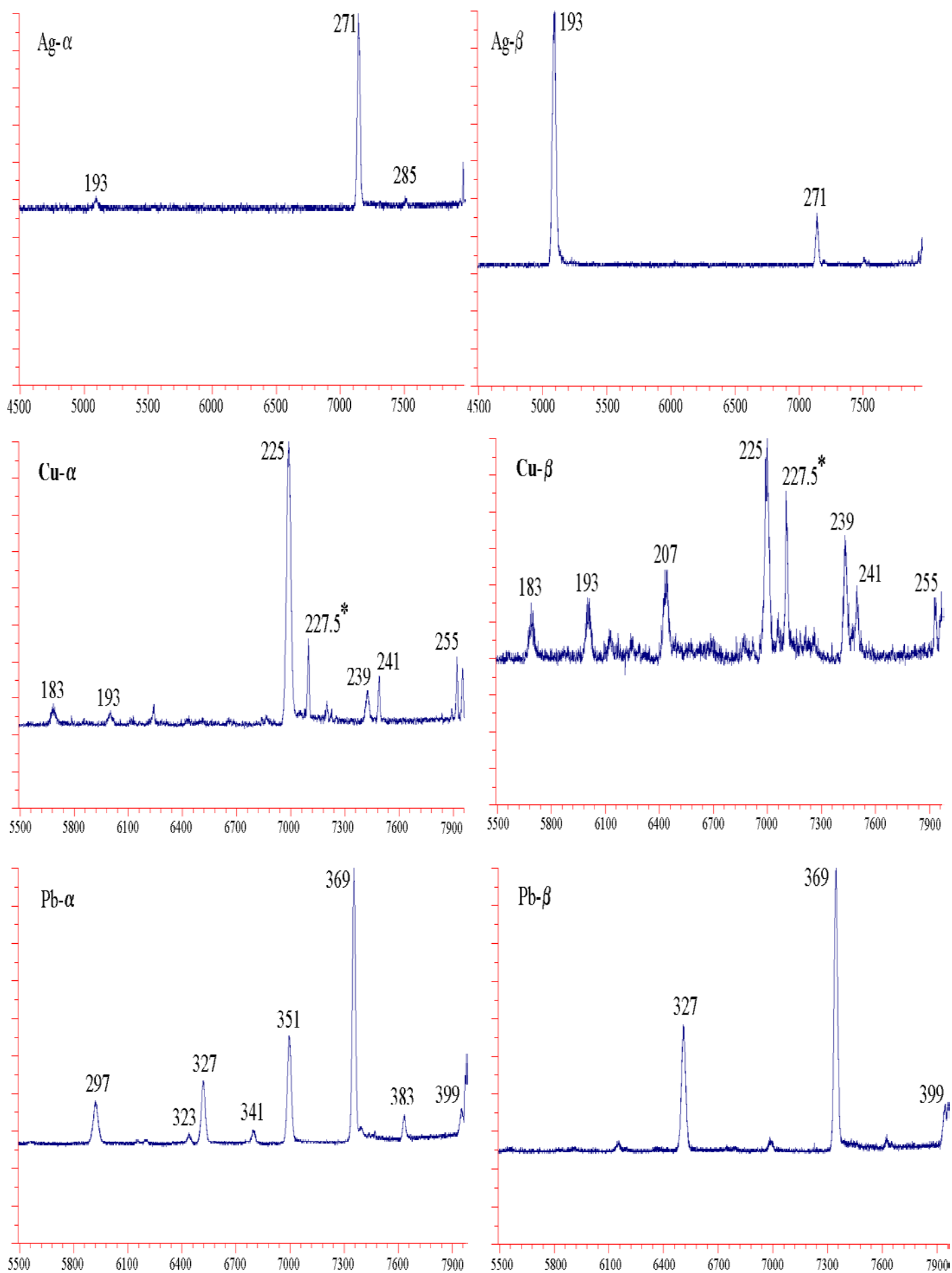
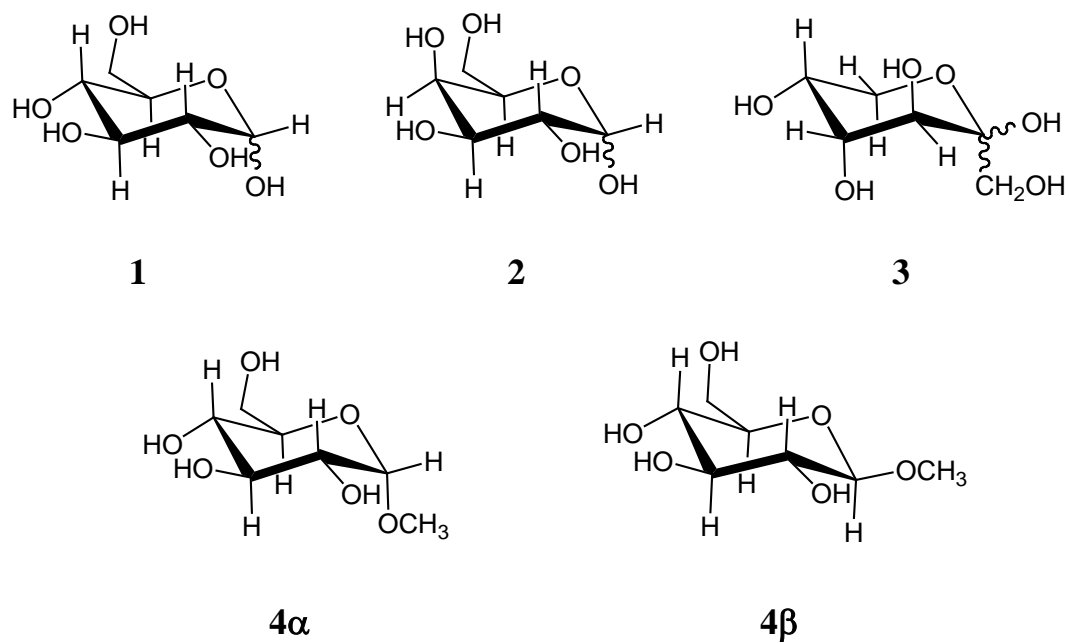


Figure 2

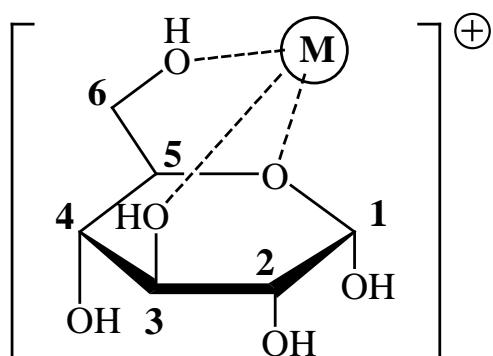
Figure 3



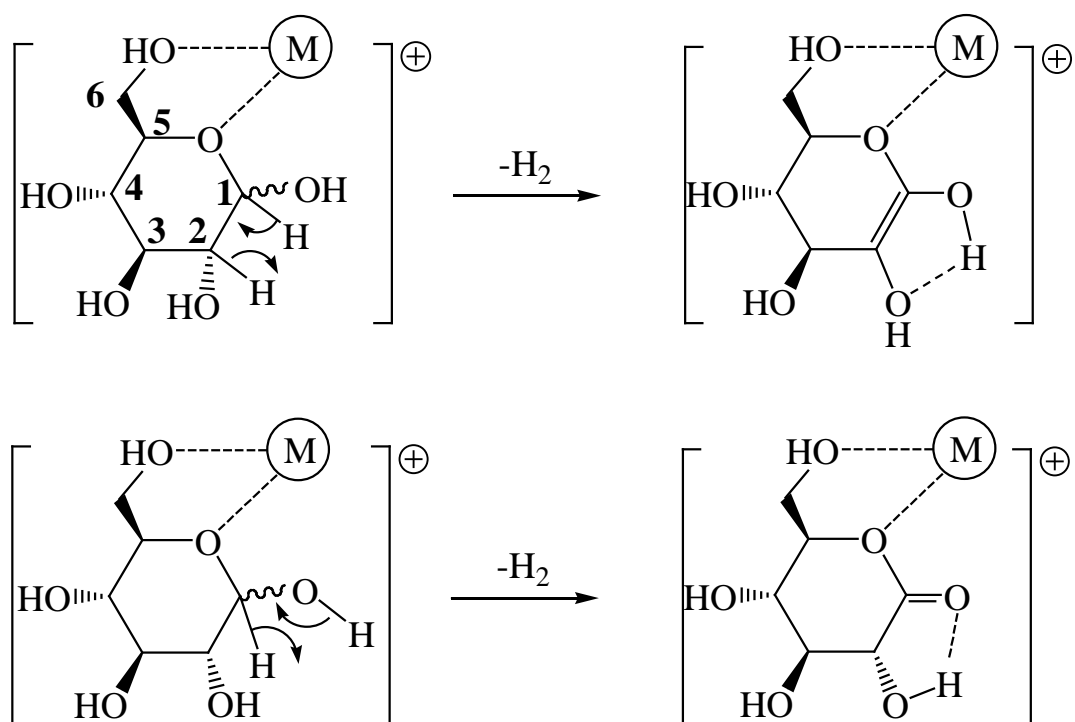
Scheme 1



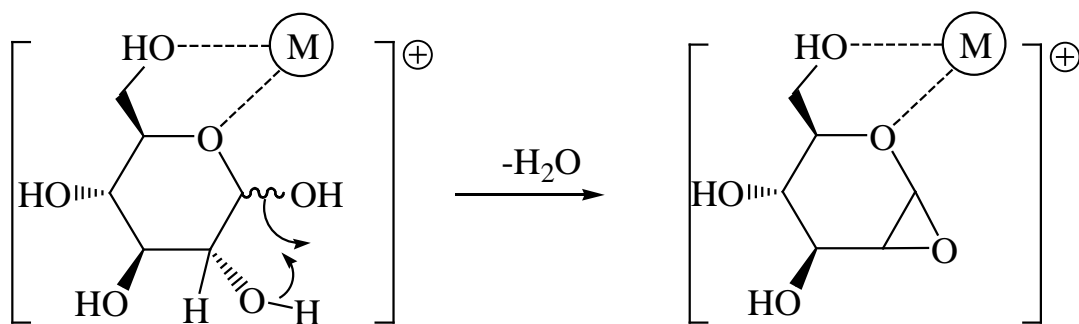
Scheme 2



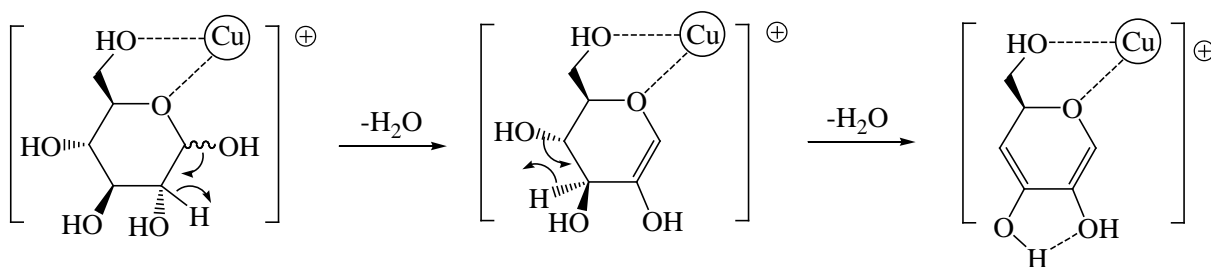
Scheme 3



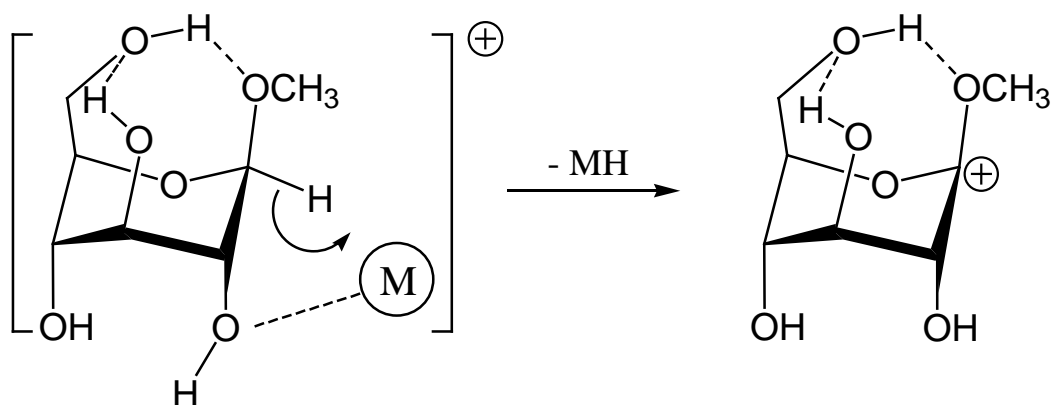
Scheme 4



Scheme 5



Scheme 6



Scheme 7

