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Definitive identification of cysteine and glutathione complexes of bismuth by mass spectrometry: assessing the biochemical fate of bismuth pharmaceutical agents

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Solutions containing $BiCl_3$, bismuth subsalicylate or $Bi(NO_3)_3$ with L-cysteine, DL-homocysteine, D-methionine or glutathione have been examined by electrospray mass spectrometry. Prominent peaks are assigned to bismuth complexes of these biomolecules and provide insight towards understanding the bioactivity of bismuth compounds.

Despite a long history of medicinal applications for many bismuth compounds, the mechanisms of bioactivity are not understood.[1–4] The thiophilicity of bismuth[5] has prompted speculation that sulfur-based biomolecules represent the primary target for pharmaceuticals such as 'colloidal bismuth subcitrate' (CBS) and 'bismuth subsalicylate' (BSS). Bismuth complexes of such ligands have been characterised by ^{13}C NMR spectroscopy[6–11] and X-ray absorption spectroscopy,[12] but structural assignments and formulations are speculative, as the compounds are difficult to isolate. Electrospray mass spectral (ESI-MS) data provide definitive assignments for fragments and molecular species arising from solutions of such systems,[13] as demonstrated below (summarised in Table 1) for solutions containing $BiCl_3$, BSS or $Bi(NO_3)_3$ with L-cysteine (CYS), DL-homocysteine (HCYS) and glutathione (GSH).†

Irrespective of reaction stoichiometry (1:1, 1:3, 1:5), reaction mixtures involving CYS show prominent peaks at *m*/*z* 449 and 328 (Fig. 1), that are assigned to the monocationic bismuth complexes **1** and **2**,‡ respectively. Formation of the bisthiolate complex (**1**) is consistent with previously reported solid state structures for aminothiolate derivatives.[14] Cation **2** can be described as a bismuth complex containing the dianionic conjugate base of CYS and is modelled on the crystallographically characterised dimethyl derivative, penicillaminatobismuth chloride.[15] In addition, tandem mass spectra of *m*/*z* 445 (**1**) gave 328 (**2**), and *m*/*z* 328 (**2**) gave *m*/*z* 241 (BiS^+) and 209 (Bi^+).

ESI-MS of reaction mixtures containing $BiCl_3$ or BSS and HCYS in aqueous solution, shown in Fig. 2, contain peaks at *m*/*z* 818, 477 and 342, which correspond to the tethered dibismuth cation (**3**) as well as the bis- (**4**) and mono-thiolate (**5**) complexes, respectively. ESI-MS/MS of ion **3** (*m*/*z* 818) gives a fragment at *m*/*z* 342 (**5**), *m*/*z* 445 (**4**) fragments to give *m*/*z* 342 (**5**) and *m*/*z* 209 (Bi^+), and *m*/*z* 342 (**5**) gives 241 (BiS^+) and *m*/*z* 209 (Bi^+).

ESI-MS of solutions containing BSS or $Bi(NO_3)_3$ with GSH (Fig. 3) reveal analogous complexes to those observed for the bismuth–cysteine mixtures, in contrast to observations for the antimony–glutathione system.[16] These observations were also independent of stoichiometry (1:1 or 1:2). Peaks at *m*/*z* 821, 514 and 385 are assigned to **6**, **7**, and **8**, respectively. ESI-MS/

Table 1 Prominent cation peaks (*m*/*z*), relative peak intensities (%) and assignments observed for *in situ* ESI-MS of reaction mixtures containing bismuth salts with CYS, HCYS or GSH (pH 1–4)

m/*z*	Assignment	Relative Int. (%)	MS/MS *m*/*z*	Assignment
CYS				
449	**1**	45	328	**2**
328	**2**	100	241	BiS
			209	Bi
241	BiS	40		
209	Bi	10		
HCYS				
818	**3**	5	342	**5**
477	**4**	100	342	**5**
			209	Bi
342	**5**	55	241	BiS
			209	Bi
209	Bi	35		
GSH				
821	**6**	30	514	**4**
514	**7**	100	385	**5**
385	**8**	35	209	Bi
308	HGSH	90		

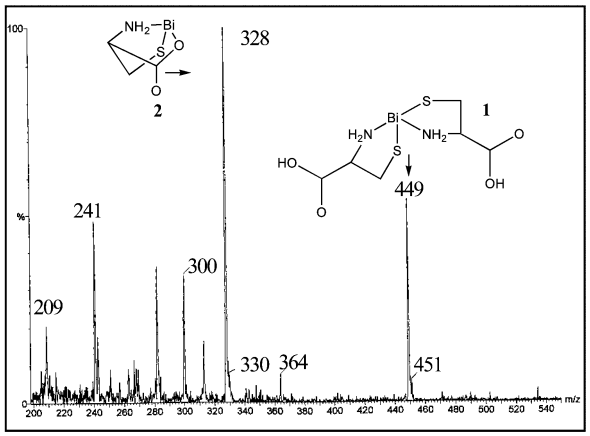

Fig. 1 A representative ESI-MS of a solution containing BSS and CYS.

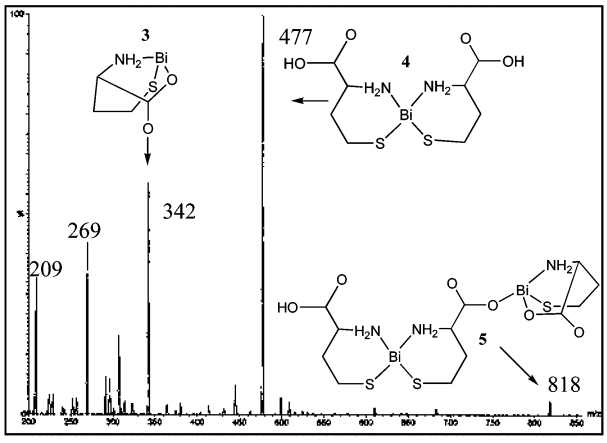

Fig. 2 A representative ESI-MS of a solution containing BSS and HCYS.

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MS of cation **6** gives fragments at *m*/*z* 514 (**7**), which releases glutamic acid to give *m*/*z* 385 (**8**). The same cations are observed using a MALDI source.§

Bismuth–methionine complexes could not be observed in solutions of $BiCl_3$, $Bi(NO_3)_3$ or BSS with D-methionine, despite previous reports of such complexes,[17] suggesting the importance of the thiolate anchor in the formation of bismuth complexes.

ESI-MS of reaction mixtures provide definitive identification data for bismuth complexes involving CYS, HCYS and GSH. The proposed formulations are important in the quest to understand the bioactivity of bismuth compounds,[15,18,19] and are consistent with the established series of tris- **9**, bis- **10** and mono- **11** thiolate complexes that have been previously isolated and comprehensively characterised.[14,20,21]

The interactions of bismuth with weakly donating functional groups of the hetero-bifunctional ligands in these complexes are made possible by the thermal and hydrolytic stability of the sulfur–bismuth bond which serves as an anchor for the ligand.[5,21] In this context, the observations described above provide support for the thiolation of bismuth as the primary biochemical fate of bismuth pharmaceuticals.

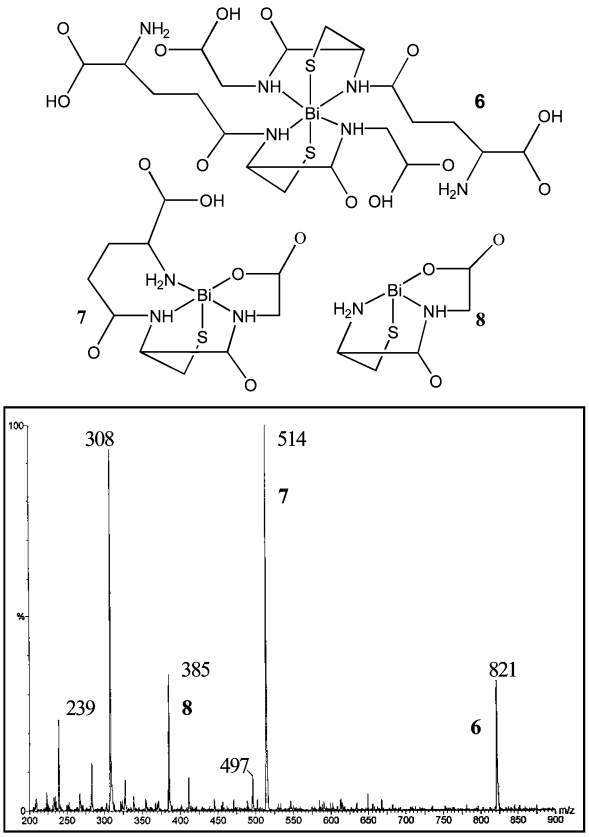
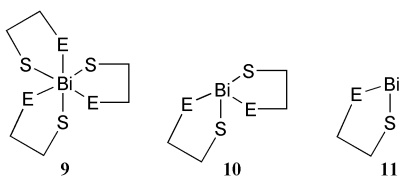


Fig. 3 A representative ESI-MS of a solution containing $Bi(NO_3)_3$ and GSH.



E = OC(O), OH, NR_2

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Notes and references

† Samples were injected directly at a flow rate of 5 μl min^{-1} into the electrospray source of a VG Micromass Quattro triple quadrupole mass spectrometer or an API 3000 PE Sciex triple quadrupole mass spectrometer, with a source temperature of 385 K and skimmer cone voltage of 50 V. MS/MS spectra were obtained using argon as a collision gas with a collision energy equal to 50 eV. The argon pressure was sufficient to reduce the intensity of the main beam by 1%. BSS (2.93 mmol) and CYS (13.5 mmol) [or $BiCl_3$ (3.52 mmol) and CYS (10.6 mmol) or CYS (3.49 mmol)] in distilled water (100 mL) stirred overnight at RT and suction filtered. BSS (3.88 mmol) and HCYS (3.91 mmol) or HCYS (6.05 mmol) [or $BiCl_3$ (3.52 mmol) and HCYS (10.6 mmol)] in distilled water (150 mL) stirred for 6 h and suction filtered. $Bi(NO_3)_3$ (3.63 mmol) and GSH (3.71 mmol) or GSH (7.52 mmol) [or BSS (3.20 mmol) and GSH (3.11 mmol)] in distilled water (150 mL) stirred for 2 h and suction filtered. BSS (3.01 mmol) and D-methionine (2.99 mmol) [or $BiCl_3$ (3.21 mmol) or $Bi(NO_3)_3$ (2.86 mmol) in distilled water, stirred for 3 h and suction filtered.

‡ Molecular drawings represent monocationic species and illustrate connectivity only; drawings of these complexes aimed at describing bonding features (*e.g.* Lewis) are not meaningful or are misleading.

§ MALDI MS was performed on a Micromass MALDI LR mass spectrometer using 0.01 g of α-cyano-4-hydroxycinnamic acid in a 50/50 mixture of acetonitrile and ethanol as the matrix.

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