Arsenic, Antimony and Bismuth Complexation by L-Cysteine in Water

G. ALONZO, N. BERTAZZI and M. CONSIGLIO

Istituto di Chimica Generale, Università di Palermo, Via Archirafi 26, 90123 Palermo, Italy

Received November 9, 1983

Widely different types of inorganic arsenic, antimony and bismuth derivatives have been used in the treatment of human diseases, especially when protozoal infections are concerned. Their bacteriostatic and parasiticidal action is well established. Arsenic and bismuth derivatives have formerly been used extensively in the treatment of syphilis, and it is also well known since the beginning of this century that antimony potassium tartrate and other water soluble antimony compounds are highly effective against leismaniosis and certain helminthiases [1].

The pharmacological effect of these elements against micro-organisms appears to derive mainly from the formation of insoluble complexes with sulphydryl groups of cell enzymes, thus hindering specific vital functions. The same behaviour accounts for the toxic side effects elicited by the same drugs [1].

In the light of the above considerations, it is surprising that the interaction of these elements with relevant bio-molecules, including cysteine, has been scarcely investigated. We report here on the synthesis in water of the L-cysteine complexes $As(cysH)_3$, $Sb(cysH)_3 \cdot H_2O$ and $Bi(cysH)_3 \cdot H_2O$ (cysH denotes a monoanionic form of L-cysteine) and hypotheses on their structure based on infrared evidence. A previous report concerns the reaction of $SbCl_3$ or antimony potassium tartrate with L-cysteine, but the white solids obtained have not been further characterized [2].

The solid complexes have been obtained as follows:

$As(cysH)_3$

As_2O_3 was suspended in water, solubilized with NaOH, and the excess basicity neutralized with HCl. On adding a solution of L-cysteine (BDH Biochemical, commercial product) a white precipitate was immediately obtained which was filtered out, washed with water and dried *in vacuo* over $CaCl_2$. M.p.: dec. 200 °C. *Anal.* Found (Calcd. for $As(C_3H_6NO_2S)_3$): C, 24.97(24.83); H, 4.30(4.17); N, 9.71(9.65).

$Sb(cysH)_3 \cdot H_2O$

The compound was obtained as a white solid by mixing aqueous solutions of SbF_3 or antimony potassium tartrate and L-cysteine. M.p.: 198–200 °C. *Anal.* Found (Calcd. for $Sb(C_3H_6NO_2S)_3 \cdot H_2O$): C, 20.89(21.61); H, 3.73(4.03); N, 8.49(8.40).

$Bi(cysH)_3 \cdot H_2O$

L-cysteine in slight excess was added to a suspension of $Bi_2(CO_3)_3$ in water, and the reaction mixture stirred for one hour at room temperature. The yellow product was collected, washed with water and dried under reduced pressure. M.p.: 194–196 °C. *Anal.* Found (Calcd. for $Bi(C_3H_6NO_2S)_3 \cdot H_2O$): C, 18.18 (18.40); H, 3.22(3.83); N, 6.96(7.15).

Aqueous solutions of L-cysteine have been also found to react slowly with M_2O_3 (M = As, Sb, Bi) to give the above tris-derivatives. The possibility of forming phenylantimony(III) and phenylbismuth(III) cysteinates has been also investigated using PhSbO or $[Me_4N][PhSbCl_3]$ and $[Me_4N][PhBiCl_3]$ as starting materials. Probably because of the ready hydrolysis of $PhSb^{III}$ and $PhBi^{III}$ moieties and the insolubility of L-cysteine in organic solvents, we were unable to isolate identifiable compounds. Several products have been obtained under different experimental conditions, showing infrared evidence that cysteine did react through the deprotonated sulphur atom.

The complexes are all air-stable powders and the antimony and bismuth derivatives do not loose water when heated at 100 °C overnight.

Mass spectra of the complexes have been recorded on a Jeol JMS-01SG-2 double-focusing spectrometer at an exciting voltage of 70 eV (100 μA) and at different probe temperatures up to 300 °C. Spectra of L-cysteine have been also obtained. The latter show a fragmentation pattern under electron impact sensibly dependent on the temperature. At 120 °C the spectrum consists mainly of fragments rationalizable as the decomposition of cysteine (m/e = 121) and cystine (m/e = 240). However, the molecular peaks relative to these species are absent, and the most intense peak is found at m/e = 166 (corresponding, for example, to [cystine–$CH(NH_2)COOH$]). With masses lower than 121 the fragments corresponding to the loss of CH_2SH, COOH, and SH are noticeable. The other important fragments deriving from cystine correspond to the loss of $2NH_2$, COOH, and NH(COOH). A few peaks at m/e higher than 240, up 304, have been also found.

The mass spectra of the arsenic complex show peaks of m/e 121 and 120, corresponding to cysteine and cysH, respectively. Other relevant peaks concern the arsenic-containing species AsO, AsS_n and $As(CH_3)_n$ (n = 1, 2, 3). The molecular peak is absent, and the base peak is [As(cysH)–HCOOH]. In the spectra of the antimony derivative the base peak occurs in the lower m/e region together with

0020-1693/84/$3.00

TABLE I. Relevant Infrared Absorptions (cm^{-1}).

L-cysteine[a]	$As(cysH)_3$	$Sb(cysH)_3 \cdot H_2O$	$Bi(cysH)_3 \cdot H_2O$	Assignments
		3520m	3580w,sh	
		3420w,br	3530m	ν(OH)
		3330w,br	3350mw,br	
3160sh	3150s	3130sh	3160ms	ν(NH)
2540mw				ν(SH)
1610vs	1625s	1630s,sh[b]	1630sh[b]	$\delta(NH_3^+)$
			1615s	
1580s	1590vs,br	1600vs,br	1590s,br	$\nu(COO^-)$
1555m[c]	1555s	1560sh	1560m	$\delta(NH_3^+)$?
1510vs	1505s	1485s,br	1490vs	$\delta(NH_3^+)$
1425s	1422s	1422s	1425s	$\delta(CH_2)$
	1412ms			
1395s	1400s	1390ms,br	1380s	$\nu(COO^-)$
	420m			ν(AsS)
	355mw			

[a]Monoclinic form; see Refs. [3, 4] for assignments. [b]$\delta(H_2O)$ is probably contributing. [c]A similar band which appears as a branch of the main absorptions around 1600 cm^{-1} is reported, unassigned, also in Refs. [3, 4].

many fragments of cysteine. The antimony-containing species observed are SbS and [Sb(cysH)–HCOOH]. The mass spectra of the bismuth complex do not show peaks at m/e above *ca.* 160.

The fragmentation observed with the arsenic and antimony derivatives is indicative of sulphur-bonded cysteine. Noticeable is the preferential loss of the carboxylic group with respect to the amino group.

All the obtained cysteinates are poorly soluble in water in a large pH range around neutrality (approx. from pH 4 to 9); clear solutions are obtained in very acidic or alkaline media, but in the latter case a slow precipitation of hydrolytic-derivatives of the element is observed. The very low solubility in water, as well as in common organic solvents, precluded the solution study of present cysteinates, and information on metal–ligand bonding has been inferred from i.r. spectra. These have been recorded on nujol and HCBD mulls in the 4000–200 cm^{-1} range using a Perkin-Elmer 580 spectrophotometer. Relevant bands are reported in Table I.

The i.r. spectra of the complexes do not show the band due to ν(SH), present in the spectrum of solid cysteine as a medium weak absorption at 2540 cm^{-1} (Table I; see also [3, 4]), indicating the coordination to a deprotonated sulphydryl group. This is not unexpected and agrees with structural results on several cysteine complexes [3, 5–9] showing that of the three possible coordination sites of the ligand, the sulphur atom always appears bound to the metal. The expected ν(MS) absorptions have been identified in the case of the arsenic compound, and assignments are supported by similar ν(AsS) frequencies observed for thioarsenite esters, $As(SR)_3$ [10]. In the spectra of antimony and bismuth derivatives, both intensity and resolution of low energy bands appeared to be reduced, preventing any confident assignment. We were also unable to locate a ν(CS) vibration, which could have been of value in establishing more firmly the presence of M–S bonds, probably being extremely weak as found in cysteine itself.

In the 1700–1400 cm^{-1} and 3500–3100 cm^{-1} regions, spectra resemble that of the free aminoacid in the zwitterionic form $HSCH_2CH(NH_3^+)CO_2^-$ [3, 4] and that of $CH_3HgSCH_2CH(NH_3)Co_2^- \cdot H_2O$ [3], rather than spectra of cysteinates where the carboxylate, the amino, or both groups, are engaged with the metal [4, 8, 9]. In particular a coordinated carboxylate can be excluded from the frequency of the asymmetric COO-stretching, and ν(NH) observed for the arsenic compound appears typical of a NH_3^+ group. In the spectra of antimony and bismuth derivatives, features above 3300 cm^{-1} have been assigned to O–H stretchings of solvent water.

Considering the insolubility of the complexes and i.r. evidences, a tentative structure can be figured out in which each element is bound to the tail of three aminoacids through the sulphur atom, whereas the ammonium and the carboxylate groups interact mainly intramolecularly through hydrogen bond formation. In this res-

pect, the crystallization water of antimony and bismuth derivatives is presumably playing an important stabilizing role participating to the hydrogen bond systems.

Since the antitumor activity of derivatives of arsenic, antimony and bismuth has received very little attention, and considering that bismuth is the least toxic among these elements, the compound $Bi(cysH)_3 \cdot H_2O$ has been tested for activity toward lymphocytic leukemia P 388 in mice, in accordance with U.S. National Cancer Institute standard protocols for primary screening. The compound exhibited no activity, while showing lethal toxicity at the relatively high doses of 25 mg pro Kg.

Acknowledgements

Financial support from CNR (Rome) is acknowledged.

References

1 See for example: a) S. C. Harvey, in 'The Pharmaceutical Basis of Therapeutics', 5th edn., chp. 46, L. S. Goodman and A. Gilman, eds., Macmillan Publishing Co. Inc. 1975; b) G. O. Doak and C. D. Freedman, in 'Medicinal Chemistry', 3rd edn., part I, chp. 25, A. Burger ed., Wiley–Interscience 1970.
2 S. O. Wandiga, *J. Chem. Soc. Dalton*, 1894 (1975).
3 N. J. Taylor, Y. S. Wong, P. C. Chieh and A. J. Carty, *J. Chem. Soc. Dalton*, 438 (1975).
4 C. Madec, J. Lauransan and C. Garrigou-Lagrange, *Can. J. Spectrosc., 25*, 47 (1980).
5 H. Shindo and T. L. Brown, *J. Am. Chem. Soc., 87*, 1904 (1965).
6 C. A. McAuliffe and S. G. Murray, *Inorg. Chim. Acta Rev.*, 103 (1972).
7 A. J. Carty in 'Organometals and Organometalloids – Occurrence and Fate in the Environment', F. E. Brinkman and J. M. Bellama, ACS Symposium Series, *82*, 339 (1978).
8 P. Llopiz and J.-C. Maire, *Bull. Soc. Chim. Fr.*, 457 (1979).
9 C.-D. Hager, F. Huber, R. Barbieri and A. Silvestri, *Z. Anorg. Allg. Chem., 471*, 194 (1980).
10 T. B. Brill and N. C. Campbell, *Inorg. Chem., 12*, 1884 (1973).