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Mercury(II) Complexes of Methionine

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Investigations of the complexation of methionine with mercury(II) chloride, acetate, and nitrate are described. With $HgCl_2$, the complex $(C_5H_{10}O_2NS)_3Hg_3(HCl)_3HgCl_4$ was obtained whereas the complex $(C_5H_{10}O_2NS)_2Hg$ was obtained from methionine directly with $Hg(OAc)_2$ and from lithium methioninate by reaction with either $Hg(OAc)_2$ or $HgCl_2$. ^1H- and ^{13}C-NMR spectral studies are reported, proton coupling for methionine is reported for the first time, and relative rotamer populations are compared to those of *S*-methylcysteine. In acidic aqueous solution, mercury bonding is localized to the sulphur of methionine which appears to adopt an extended chain configuration.

The nature of complexation of the amino acid, methionine, $CH_3SCH_2CH_2CH(NH_2)COOH$ (Hmt), with a number of transition and non-transition metal ions has been studied in the solid state by infrared spectroscopy by McAuliffe, Quagliano and Vallarino.[1] Methionine appears to behave as an anionic ligand (mt) and generally forms neutral complexes. $M^{II}mt_2$ and $M^{III}mt_3$ in which the metal attains its usual higher coordination number by linking with the N atom of the $-NH_2$ group and with one or both of the O atoms of the $-COO^-$ group. In these complexes the S atom of the $-SCH_3$ group is still available for coordination, as shown by the formation of mixed-metal complexes with Ag(I).[1] From studies in solution by ^1H-NMR spectroscopy, Li and Manning [2] concluded that the sulphur is not involved in coordination in methionine complexes of zinc, cadmium, and mercury. In a later study utilizing potentiometry, Lenz and Martell [3] concurred with the bidendate nature of methionine, *S*-methyl-L-cysteine, and ethionine, and they concluded that there is little or no involvement of substituted mercapto groups in chelation. More recently, Natusch and Porter [4,5] have provided evidence from ^1H-NMR spectral studies that mercury(II) bonds to the sulphur of methionine in 1 M HNO_3.

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As part of a study to investigate the nature of mercurial complexation with molecules of biochemical interest in order to obtain a better understanding of biotransformation and organomercurial toxicology,[6-8] we undertook a reinvestigation of mercurial complexation with methionine. In our studies, we have tried to prepare and isolate mercurial complexes for structural characterization using the mildest possible *in vitro* conditions in relation to the *in vivo* state. For this reason, mercurial complexation was restricted to acidic media in this investigation.

DISCUSSION

Complexation. Preliminary experiments indicated that difficulties would be experienced in attempting to isolate mercurial complexes of methionine under acid conditions and that variable composition would be a feature of the products isolated. Systematic investigation was undertaken to determine the role of various solvents and acidic conditions in the complexation of methionine by mercury(II) chloride, nitrate, and acetate. Preparative work, summarized in Table 1, was first carried out, and then all products were analyzed at the same time as a group for mercury content by atomic absorption. The highly variable mercury content of the various products is evident from Table 1.

Table 1. Summary of reaction conditions and results for complexation of methionine with mercury(II) chloride.

Methionine (mmol)	Solvent 95 % EtOH (ml)	Initial state[a]	$HgCl_2$ (mmol)	Solvent 95 % EtOH (ml)	State after addition[b]	Conditions for precipitation[c,d]	Yield (g)	Hg content (%)
1	200	S	5	20	N,C	R	1.42	[e]
10	40	H[f]	5	20	N	[g]	0.84[h]	0.9
5	20	H[i]	2.5	10	N	R[j]	Fr.A, 0.12	4.1
							Fr.B, 0.21	2.1
5	10	H[k]	2.5	10	N,T	O	0.42	52.1
5	20[l]	H[m]	2.5	10	N,T	R	0.80	33.3
5	0[n]	H[o]	2.5	10	N,T	R	0.65	46.1
10	0[p]	H	5	20[q]	P[r]	Fr.A, O	Fr.A, 1.06	45.4
						Fr.B, R	Fr.B, 0.37	47.8

[a] S, suspension; H, hot solution. [b] N, no precipitation; C, clarified; T, tail-effect; P, precipitation. [c] R, refrigeration; O, standing overnight. [d] 95 % EtOH used for washing. [e] Gave excellent C, H, O, N, and S analysis for methionine. [f] Dissolution achieved by addition of just sufficient conc. HNO_3. [g] Hot syrup treated with acetonitrile until gum formed, then treated with acetone, heated to obtain solution followed by diethyl ether. Crystalline solid obtained at room temperature. [h] Shown by infrared spectra to be mainly methionine hydronitrate. [i] Dissolution achieved by minimal addition of trifluoroacetic acid (1.5 ml). [j] Treated with diethyl ether. [k] Dissolution effected by adding water (15 ml required) to nearly boiling suspension. [l] 1.5 ml HOAc added. [m] Dissolution achieved by adding water (2 ml) to hot suspension. [n] 10 ml HOAc. [o] Dissolution effected by adding water (1–2 ml) near boiling temperature of suspension. [p] 20 ml HOAc+ 3.0 ml H_2O. [q] Hot solution. [r] Initially, precipitate dissolved with stirring, later precipitate accumulated. At end, mixture treated with 10 ml EtOH and raised to b.p. to obtain solution.

The isolation of a mercury(II) chloride complex of methionine appears to require rather special conditions. When a large volume of 95 % ethanol is used to dissolve methionine (see example one of Table 1) before treatment with an ethanolic solution of $HgCl_2$, beautiful, long crystals were obtained which gave an excellent analysis for methionine. The superbly formed crystals of methionine obtained under these conditions contrast with the small and difficultly obtained plate and needle polymorphs of methionine.[9,10] If a much smaller volume of ethanol is used to dissolve methionine aided by the addition of minimal water (see example four of Table 1), then a mercury(II) chloride complex of methionine is obtained having the composition $(C_5H_{10}O_2NS)_3Hg_3(HCl)_3(HgCl_4)$. Examination of the complex by NMR is precluded by its insolubility in boiling water and by its degradation in dimethyl sulfoxide. X-Ray diffraction showed the product to be amorphous. By examining solutions of methionine in 1 M HNO_3 containing variable $Hg(NO_3)_2$ concentration, Natusch and Porter [4,5] deduced evidence for mercurial bonding to sulphur and concluded that complexes of the type $M(LH_2)_2$ were formed. No complexes have been isolated from these systems. McAuliffe *et al.*[1] have reported the isolation of a yellow, polymeric mercury complex of methionine $(Hg\ mt_2)_n$ which was obtained from the lithium salt of methionine on treatment with mercury(II) perchlorate.

In contrast to the behaviour of methionine with $HgCl_2$, treatment with mercury(II) acetate readily gave a mercury complex having the composition, $(C_5H_{10}O_2NS)_2Hg$. X-Ray diffraction showed the substance to be crystalline and identical with the mercury complex obtained by reaction of lithium methioninate with either mercury(II) chloride or acetate. Infrared spectra showed the absence of the acetate moiety and this absence was confirmed by ^{13}C-NMR spectra.

Investigation of the formation and isolation of mercurial complexes of methionine in aqueous ethanol using mercury(II) nitrate was unrewarding and frustrated by the formation of glassy residues from intractable syrups.

NMR spectral studies. Natusch and Porter [4,5] first reported ^1H-NMR evidence for the binding of Hg^{2+} to the isolated sulphide group of methionine. The evidence was reported simply in the form of proton chemical shift differences ($\Delta\nu$ at 60 MHz) between the metal complexes and the corresponding free ligands but, as yet, no chemical shift or coupling data have been reported for methionine itself. In examining an aqueous solution * of the bis(methioninato)-mercury(II) complex by ^1H- and ^{13}C-NMR spectroscopy, we found it necessary to investigate similarly acidic aqueous solutions of methionine. As a result of this work the ^{13}C-NMR chemical shifts have been determined (Table 2) for both methionine and bis(methioninato) mercury(II), and the proton coupling parameters (Table 3) have been determined for the methionine system following iteration and computer simulation (Fig. 1).

A comparison of the ^{13}C-NMR chemical shifts (Table 2) for methionine and the mercury complex reveals deshielding of approximately 5 ppm for the *S*-methyl and -methylene carbons, respectively, of the complex relative to

* Dissolution of the complex was achieved on addition of a few drops of trifluoroacetic acid to a suspension of the solid in 5.0 ml D_2O followed by gentle warming.

Table 2. ^{13}C NMR chemical shifts (ppm relative to TMS) for D_2O solutions (pD 2.2) of methionine (Hmt) and bis(methioninato)mercury(II) [$(mt)_2Hg$].

$$\overset{5}{CH_3}S-\overset{4}{CH_2}-\overset{3}{CH_2}-\overset{2}{CH}(ND_3^+)\overset{1}{COOD}$$

Solute	Conc.(M)	1	2	3	4	5
Hmt	0.2	173.4	53.4	30.1[a]	29.5[a]	14.8
$(mt)_2Hg$	0.2	172.9	53.4	29.7	34.6	19.7

[a] Tentative assignment.

Table 3. Proton chemical shift (ppm relative to external TMS) and coupling data (100 MHz) for methionine and bis(methioninato)mercury(II) in D_2O (pD 2.2)

$$CH_3S-\underset{2,3}{CH_2}-\overset{4,5}{CH_2}-\underset{1}{CH}(ND_3)COOD$$

Solute	Conc.(M)	CH_3	SCH_2	CH_2	CH
Hmt	0.2	2.67	3.22	2.82, 2.72	4.66
$(mt)_2Hg$	0.2	3.24	3.85	2.9	4.67
J_{Hmt}:	$J_{1,2}=J_{1,3}=0$ Hz				
	$J_{2,4}=J_{2,5}=J_{3,4}=J_{3,5}=7.28$ Hz				
	$J_{1,4}=6.03$, $J_{1,5}=7.10$ Hz				
	$J_{23}=-11.00$[a], $J_{4,5}=-14.88$ Hz				

[a] Assumed value.

those of methionine whereas there is no appreciable change in the values for the other carbon atoms. This evidence not only supports the conclusion of Natusch and Porter [5] that mercurial bonding is localized to the sulphide group, but it is also consistent with an extended chain configuration for the complex in solution in which the amino and carboxyl moieties are remote from the mercury atom.

An analysis of the relative rotamer populations for methionine was performed in the standard fashion [12–15] by assuming that the rotamer with the bulkiest substituents in the *trans* position (in this case CH_3SCH_2 or NH_3 and COOH) is sterically favoured. In this conformation, the sulphur atom is located proximate to the ammonium group which might afford a means for rotamer stabilization through dipolar attraction. Adopting values of 12.0 and 2.0 Hz for J_t and J_g, respectively, which have been suggested by Martin and Mathur [16] for cysteine, one obtains the rotamer populations $P_t=(J_{1,5}-J_g)/(J_t-J_g)=0.51$, $P_g=(J_{1,4}-J_g)/(J_t-J_g)=0.403$ and $P_h=1-(P_t+P_g)=0.09$. These relative rotamer populations support the above conformational hypothesis and suggest that the alternate rotamer g (Fig. 2) with *trans* bulky substituents

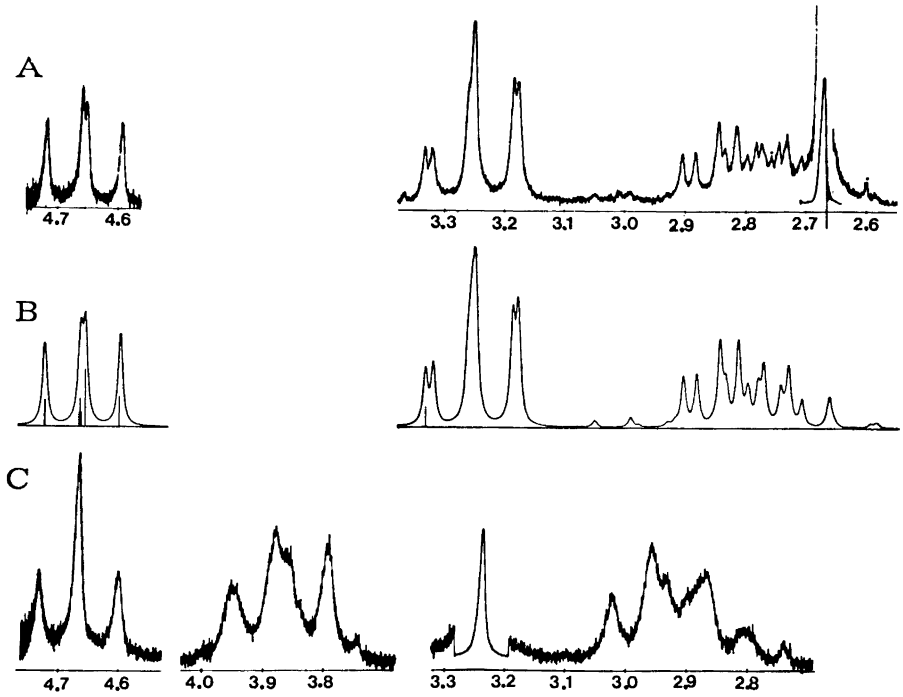

Fig. 1. ^{1}H-NMR spectra (100 HMz) in D_2O at pD 2.2. A: Methionine. B: Simulated spectrum for methionine (CH_3S- omitted). C: Bis-(methioninato)mercury(II).

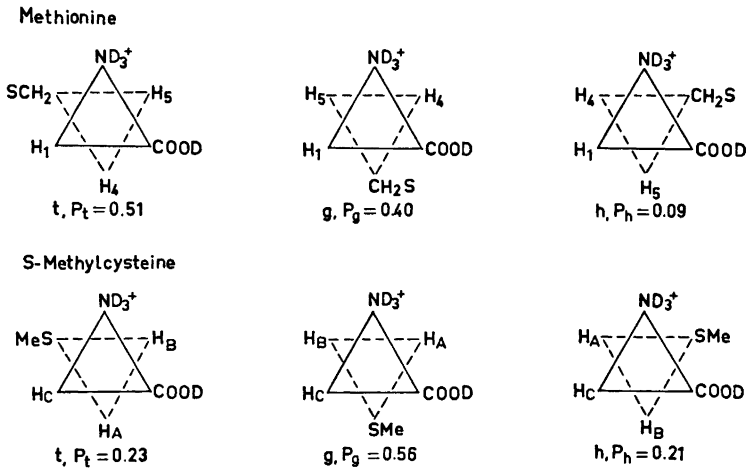

Fig. 2. Relative populations of the three staggered rotamers of methionine $[CH_3SCH_2CH_2CH(ND_3^+)CO_2D]$ and of *S*-methylcysteine $[CH_3SCH_2CH(ND_3^+)CO_2D]$ in D_2O at pD 2.2.

may be stabilized by intramolecular hydrogen bonding ($COOH\cdots CH_3SCH_2$). In addition, the excellent spectral matching (Fig. 1) serves to confirm the assumed equivalence of the *S*-methylene protons and the identical vicinal coupling between pairs of methylene protons.

It is interesting to compare the relative rotamer populations of methionine with those of *S*-methyl cysteine obtained under similar conditions (pD 2.2). For methionine the two rotamers (t and g) with bulky *trans* substituents are nearly equally populated and there is a very small fraction of the sterically hindered rotamer (h). In the case of *S*-methylcysteine, for which we have determined $J_{AC}=4.31$, $J_{BC}=7.64$, and $J_{AB}=-15.02$, the most highly populated rotamer is assumed to be that (g) in which COOH and SCH_3 are adjacent when hydrogen bonding in a six-membered ring is expected to exert a stabilizing influence. The population of this rotamer is more than twice that of the rotamer (t) having adjacent NH_3^+ and SCH_3 which is assumed to be stabilized by a more weakly dipolar attraction. In contrast to methionine, the most sterically hindered rotamer of *S*-methylcysteine is essentially as highly populated as the least favoured rotamer with *trans* bulky substituents. It appears that the forces of repulsion in the sterically hindered rotamer of *S*-methylcysteine are compensated by a combination of attractive forces between CH_3S and COOH (hydrogen bonding) and NH_3^+ (dipolar). It would appear that the reason that the sterically hindered rotamer of methionine is not as highly favoured as in *S*-methylcysteine is due to a combination of weaker hydrogen bonding in a seven-membered ring and the fact that a hydrogen-bonded SCH_3 group must be inclined away from the NH_3^+ group due to the presence of the extra methylene linkage resulting in reduced dipolar attraction. The sterically hindered rotamer of *S*-methylcysteine therefore appears to be unusually favoured by stabilizing forces.

The proton spectral features obtained for bis(methionineato)mercury(II) are shown in Fig. 1C. Unfortunately the broad nature of the bands makes it virtually impossible to find a unique set of coupling constants for the mercury complex because several combinations will give almost the same line shape. This situation arises because of the necessity in computer simulation of spectra to employ line widths related to experimental conditions, thus the derived line shapes do not allow one to discriminate between sets of possible parameters. It was possible, however, to conclude from these investigations of spectral matching that, unlike methionine, both methylene groups of the mercury complex possess non-equivalent protons.

EXPERIMENTAL

General methods. ^{13}C-NMR spectra were obtained at 27°C with a Varian XL-100 spectrometer following accumulation and Fourier transformation of signals in both proton decoupled and coupled modes. Frequencies were measured relative to dioxane and converted to the TMS scale ($\delta_{TMS}=\delta_{dioxane}+67.4$ ppm).[17] Proton spectra were obtained at 35°C at 100 MHz. Solutions were prepared from D_2O by acidification with trifluoroacetic acid, and pD values were obtained from the expression pD = scale reading + 0.4.[18]

X-Ray diffraction patterns were obtained using Guinier-Hägg cameras.

Atomic absorption measurements were performed with a Perkin-Elmer Model 303

spectrometer. Samples (approx. 10 mg) were weighed accurately, digested with warming in a little water treated with concentrated hydrochloric acid, and then made up to volume in 50 ml volumetric flasks. Standard samples containing 200, 100, 50, 25, and 12.5 ppm of mercury were prepared from mercury(II) chloride (BDH Analar), and calibration analyses were performed both before and after analyzing the series of samples of unknown mercury content.

Microanalyses. Microanalyses were performed by Alfred Bernhardt, Mikroanalytisches Laboratorium, 5251 Elbach über Engelskirchen, Fritz-Pregl Strasse 14 – 16, W. Germany. Appropriate separatory processes were employed to eliminate the interference of mercury with the C, H, and Cl determinations. Sulphur was analyzed by a reductive process.

C, H, and N analyses of the lithium and hydronitrate salts of methionine were performed by Dr. Lars Haraldson, Analytical Chemistry, University of Lund, Sweden.

Synthesis

Mercury(II) chloride complex of methionine. A suspension of D,L-methionine (0.745 g, 0.005 mol, Sigma 99.5 %) in nearly boiling ethanol (10 ml) was taken into solution by the addition of a minimum of water (~ 15 ml). To the magnetically stirred solution, a warm solution of mercury(II) chloride (0.681 g, 0.0025 mol, BDH Analar) in 95 % ethanol (5 ml) was slowly added. Although a "tail-effect" was seen during the addition, no precipitate formed immediately after the addition. On standing overnight at room temperature, the mixture produced a fine precipitate which had to be scraped from the flask. The solid (Fraction A, 0.416 g) was washed with 95 % ethanol and dried over NaOH *in vacuo*. X-Ray diffraction showed the product to be amorphous, and the solid was found to decompose above 115°. [Found: C 12.30; H 2.17; O 6.47; N 2.96; Hg 53.35; Cl 16.49; S 6.40. $(C_5H_{10}O_2NS)_3Hg_3(HCl)_3HgCl_4$)(M.W. 1488.3) requires C 12.10; H 2.23; O 6.45; N 2.82; Hg 53.91; Cl 16.68; S 6.46].

Following refrigeration, the mother liquor yielded second and third fractions (0.206 and 0.169 g, respectively) which were shown by X-ray diffraction to be methionine.

Mercury(II) acetate complex with methionine. A solution of D,L-methionine (0.745 g, 0.005 mol, Sigma 99.5 %) was obtained in 95 % ethanol (20 ml) with heating and magnetic stirring by addition of a minimum of water (4 – 5 ml). To this solution, a solution of mercury(II) acetate (0.798 g, 0.0025 mol, Merck 99 %) in methanol (10 ml) was slowly added. A gelatinous precipitate formed when nearly half the mercurial reagent had been added. After standing for 2 h, the mixture was filtered, and the product was washed with 95 % ethanol and dried over NaOH *in vacuo*. The yield was 1.065 g or 48 %, and the solid was found to decompose above 215°. X-Ray diffraction showed the solid to be crystalline. [Found: C 24.01; H 3.91; O 13.33 and 13.38; N 5.95; Hg 41.96; S 12.72 and 12.67. $(C_5H_{10}O_2NS)_2Hg$ (M.W. 497.01) requires C 24.16; H 4.06; O 12.88; N 5.64; Hg 40.36; S 12.90].

Lithium methioninate. The conditions reported by Halbert and Rogerson [11] for the preparation of a lithium salt of methionine (composition not reported) were used. A mixture of D,L-methionine (3.00 g, Sigma) and freshly opened lithium hydroxide monohydrate (0.800 g, BDH) in 95 % ethanol (40 ml) was heated (50 – 60°) with magnetic stirring. A solution was soon obtained. On cooling to room temperature, the solution deposited a small quantity of solid which did not increase with refrigeration. This solid (0.138 g) was shown by X-ray diffraction to be methionine.

The mother liquor was reduced to approximately 10 ml by rotary evaporation, then the concentrate was treated with diethyl ether (~ 15 ml) in small portions until turbidity was obtained. The mixture was refrigerated and treated periodically with more ether to obtain glistening, white flakes. The solid was collected, washed with ether-ethanol (1:1) and dried over NaOH *in vacuo*. The product (2.16 g) was found to be crystalline by X-ray diffraction and infrared spectra showed a marked change from the parent compound. [Found: C 38.40; H 7.08; N 9.00. $C_5H_{10}O_2NSLi$ ((M.W. 155.14) requires C 38.71; H 6.50; N. 9.03].

Bis(methioninato)mercury(II) from lithium methioninate and mercury(II) acetate or mercury(II) chloride. To a magnetically stirred solution of lithium methioninate (0.500 g, 0.00323 mol), prepared at room temperature from water (2 ml) and 95 % ethanol (10 ml), was added dropwise a warm (40°C) solution of mercury(II) acetate

(1.015 g, 0.00323 mol, Merck, *p.a.*) in methanol (10 ml). Precipitation was immediate. The solid was too finely divided for suction filtration, thus the mixture was centrifuged (10 000 rpm) and washed three times with 95 % ethanol. The dry product (0.498 g, 62 % yield) was shown by X-ray diffraction to be crystalline and identical to the product obtained directly from methionine with mercury(II) acetate.

To an identical aqueous ethanolic solution of lithium methioninate (as above) was added dropwise a solution of mercury(II) chloride (0.875 g, 0.00323 mol, BDH Analar) in 95 % ethanol (10 ml). Precipitation was immediate and copious. After collection and washing with 95 % ethanol the dry solid (0.814 g, 100 % yield) was shown by X-ray diffraction to be crystalline and identical to the product obtained directly from methionine with mercury(II) acetate.

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