Metal Ion–Biomolecule Interactions. Part IX. Metal Ion–C_5 Binding in a Pyrimidine Nucleoside. Ready Formation of C_5–Hg–N and C_5–Hg–S Bonds

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Received March 22, 1984

Abstract

The reaction of mercuric acetate with N_3-methylmercurated uridine, [CH_3Hg(Uri)], yielded a polymeric, dimethyl sulfoxide-soluble compound possessing a regular C–Hg–N bond system. In the presence of 6-thioguanosine, the C–Hg–N bond system was cleaved quantitatively to generate a monomeric species containing an exclusively C–Hg–S bond system. The ^1H, ^{13}C and ^{199}Hg nmr characteristics of the two systems are reported.

Introduction

Of the known nucleoside-derived organometallic compounds, the Hg(II)-containing nucleosides are among the most versatile [1]. They function both as tools for biochemical research as well as valuable synthetic intermediates [2]. The syntheses and properties of 5-mercuripyrimidine nucleosides and nucleotides, as well as mercury(II)-containing polynucleotides, by chemical modification and by enzymatic polymerization of mercurated substrates, have been described [3, 4]. Unlike mercurinucleotides which tend to be water-soluble, the known mercurinucleosides are water-insoluble species. In the case of the complexes of the uracil-derived nucleosides, the suggested explanation of this observation is that the complexes are polymeric in nature with a C–Hg–N bonding sequence [5]. There exists, however, no detailed information regarding the regularity of this linkage or the degree of polymerization of the species. This structural uncertainty is not of particular consequence when the derivatives are used as synthetic intermediates but is a distinct limitation when the derivatives are used as biochemical probes.

In the course of our studies of the interactions of CH_3Hg(II) and Hg(II) species with purine/pyrimidine bases, nucleosides and nucleotides [6], we have had occasion to investigate the reactions of uridine with a number of metal ions. We have found that in aqueous solution, the conventional mercurating agent, mercuric acetate, rapidly mercurates the N_3-methylmercurated uridine complex, [CH_3Hg(Uri)] (**2**), at the C_5 position to yield a dimethyl sulfoxide-soluble, polymeric substance which is formulated as $[-Uri-Hg-\cdot 2H_2O]_n$ (**3**). Reaction of **3** with 6-thioguanosine in aqueous solution leads to the rapid cleavage of the Hg–N bonds in **3** by 6-thioguanosine and the formation of a new complex, **4**, containing mercury(II) bridging uridine *via* the C_5 atom, and 6-thioguanosine *via* the S_6 atom. Complexes **3** and **4** have been analysed by ^1H, ^{13}C and ^{199}Hg nmr as well as by elemental analysis.

Experimental

^1H nmr spectra of the complexes dissolved in $(CD_3)_2SO$ were recorded on a Bruker HX-60 instrument operating at 60 MHz in the Fourier transform mode. ^{13}C nmr spectra were recorded on a Bruker CXP-200 instrument operating at 50.307 MHz. Chemical shifts are referenced relative to internal tetramethylsilane (TMS). ^{199}Hg nmr spectra were recorded on a Bruker CXP-200 instrument operating at 35.830524 MHz. ^{199}Hg chemical shifts are referenced relative to external neat $(CH_3)_2Hg$. All spectra were recorded at room temperature (25 ± 2 °C). Microanalyses were performed by Guelph Chemical Laboratories Limited.

Uridine, **1** (Sigma), 6-thioguanosine (Sigma), $[(CH_3Hg)_3O]OH$ (Alfa) and $Hg(OAc)_2$ (Alfa) were used as received. The N_3-methylmercurated uridine complex, [CH_3Hg(Uri)], **2**, was prepared as described elsewhere [6h].

Uridine Complexes

$[-Uri-Hg-\cdot 2H_2O]_n$, 3

To a hot solution of [CH_3Hg(Uri)], (**2**) (0.208 g, 0.543 mmol) in 50 ml of distilled water was added dropwise a solution of mercuric(II) acetate (0.0729, 0.227 mmol) in 5 ml of distilled water. After stir-

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0020-1693/84/$3.00

ring the reaction mixture for 5 h at 100 °C, the white solid present in the reaction mixture was collected by filtration, washed with 100 ml of hot water and dried to constant weight in a vacuum desiccator (0.101 g, 97%). *Anal.* Calcd. for **3** ($C_9H_{14}N_2O_8$-Hg): C = 22.56; H = 2.92; N = 5.85. Found: C = 23.17; H = 2.64; N = 5.99. ^{1}H nmr chemical shift data: N_3–H = – (11.33); C_5–H = – (5.90d); C_6–H = 7.74s (7.90d). (Values in parentheses are those for **1**). ^{13}C nmr chemical shift data: C_2 = 154.28 (150.86); C_4 = 169.28 (163.25); C_5 = 123.12 (101.88). C_6 = 146.13 (140.49). (Values in parentheses are those for **1**). ^{199}Hg nmr chemical shift data: –1191 ppm.

(Uri–Hg–6-ThioGuoH)·3H$_2$O, 4

To a stirred solution of 6-thioguanosine (0.058 g, 0.194 mmol) in 15 ml of water was added a suspension of **3** (0.093 g, 0.194 mmol) in 5 ml of water. The thick white precipitate, which formed in the course of stirring the mixture overnight, was collected by filtration, washed with water and dried in a desiccator (0.149 g, 98%). *Anal.*: Calcd. for 4 ($C_{19}H_{29}N_7O_{12}$S Hg): C = 29.23; H = 3.71; N = 12.56. Found: C = 29.08; H = 3.17; N = 12.30. ^{1}H nmr chemical shift data: (a) for the uridine moiety of **4**: N_3–H = 11.32 (11.33); C_5–H = – (5.90d); C_6–H = 7.74s (7.90d); (b) for the 6-thioguanosine moiety of **4**: N_1–H = – (11.97); NH_2 = 6.49 (6.82); C_8–H = 8.26 (8.15). Values in parentheses are those for uridine, **1**, and 6-thioguanosine, respectively. ^{13}C nmr chemical shift data: (a) for the uridine moiety of **4**: C_2 = 150.50 (150.86); C_4 = 167.29 (163.25); C_5 = 125.70 (101.88); C_6 = 145.79 (140.49); (b) for the 6-thioguanosine moiety of **4**: C_2 = 159.76 (153.1); C_4 = 150.79 (147.9); C_8 = 139.04 (138.5); C_5 = 131.86 (128.5).

Values in brackets are those for uridine, **1**, and 6-thioguanosine, respectively. ^{199}Hg nmr chemical shift data: –883 ppm.

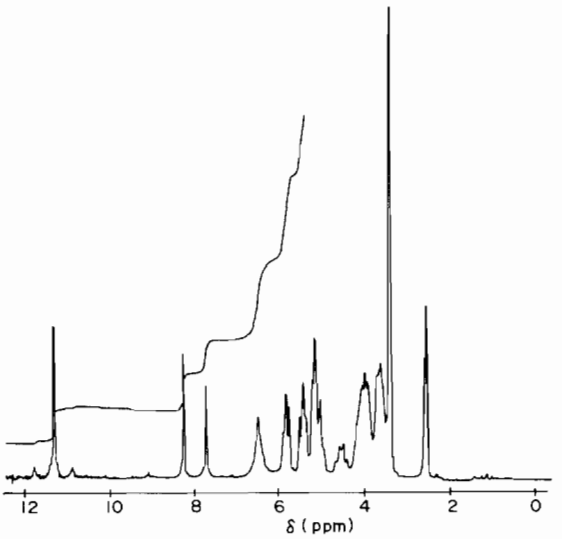
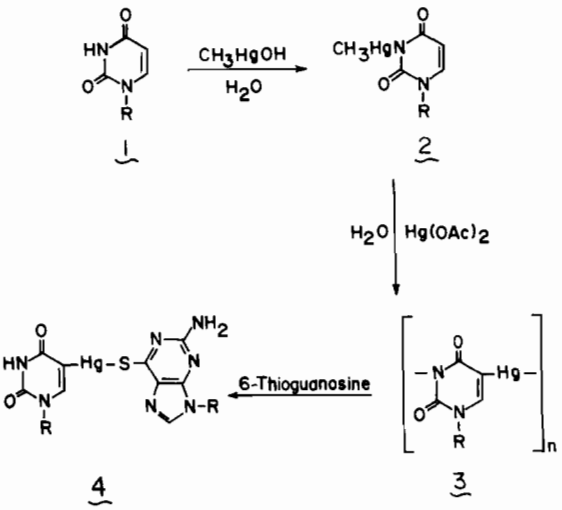


Fig. 1. Proton magnetic resonance spectrum of Uri–Hg–6-ThioGuoH·3H$_2$O, 4, in $(CD_3)_2SO$ at 25.0 °C.



Scheme I

Results and Discussion

Complex **3** could be isolated from the reaction of equimolar mixtures of the N_3-methylmercurated uridine complex, [CH_3Hg(Uri)], (**2**), and mercuric acetate in aqueous solution (Scheme 1). The complex obtained in this manner, (**3**), is soluble in dimethyl sulfoxide, in marked contrast to the dimethyl sulfoxide- and dimethylformamide-insoluble products obtained by the direct reaction of mercuric acetate with uridine itself under comparable conditions in aqueous solution [1].

The ^{1}H nmr of the mercurated complex **3** shows the absence of a resonance typical of a C_5–H proton (uridine gives rise to a doublet (J = 6.75 Hz) at 5.90 ppm). The presence in **3** of a sharp singlet at 7.74 ppm, assignable to C_6–H, is in contrast to the doublet at δ = 7.90 ppm (J = 7.92 Hz) observed for uridine. The ^{13}C nmr spectrum of **3** showed downfield shifts of all the base carbon resonances, relative to the corresponding resonance in **1**, with a dramatic downfield shift of 21.24 ppm in the mercurated C_5 resonance. The resonances of the ribose carbon atoms in **3** were not shifted relative to the resonances of the corresponding ribose carbon atoms in uridine. Comparable downfield shifts in the ^{13}C resonances of the C_8 carbon have been observed in the C_8-methylmercurated complexes of inosine, guanosine, their thio-analogs, 6-thioinosine and 6-thioguanosine, and xanthosine [6c,e,g]. While the ^{1}H and ^{13}C nmr data clearly indicate that mercury(II) is bound at C_5 and N_3, they offer no evidence as to whether or not there is a regular C–Hg–N bond

sequence. The ^{199}Hg nmr spectrum of **3** shows a single ^{199}Hg resonance at −1191 ppm, relative to neat $(CH_3)_2Hg$, thus confirming the presence of inorganic mercury(II) in a C–Hg–N system. Had complex **3** contained appreciable amounts of C–Hg–C and N–Hg–N bonding, additional ^{199}Hg resonances near −700 ± 50 ppm (*cf.* −719.18 ppm for the C-bound CH_3Hg(II) in $[(CH_3Hg)_3Ino]NO_3$) or −1800 ± 100 ppm (*cf.* −1784 for the N–Hg–N sequence in [(thymidine)$_2$Hg]), would have been observed [6h].

The reaction of **3** with the known antineoplastic agent 6-thioguanosine [8] in aqueous solution gave rise to a quantitative amount of stable product **4** (Scheme 1). The ^{1}H nmr spectrum of **4** (Fig. 1) shows the resonances associated with the uridine moiety at δ = 11.32 ppm (N_3–H), δ = 7.72 ppm (singlet, C_6–H) and the resonances associated with the 6-thioguanosine moiety at δ = 8.26 and 6.49 ppm for C_8–H and NH_2 respectively. The trends in the chemical shifts of the C_8–H and NH_2 resonances of the 6-thioguanosine portion of **4** as a result of complex formation are comparable in magnitude to the trends in the ^{1}H chemical shift values in forming 6-methylmercurated thioguanosine from 6-thioguanosine [6, 9]. The ^{13}C nmr spectrum of **4** showed a dramatic downfield shift (23.8 ppm) of the C_5 resonance of the uridine part of **4**, as compared to the C_5 resonance in uridine, and an upfield shift (9.3 ppm) of the C_6 resonance in the 6-thioguanosine portion of **4**, as compared to the C_6 resonance in 6-thioguanosine. An upfield shift of this magnitude is characteristic of the formation of S-methylmercurated and S-methylated 6-thionucleosides [6g, 9]. There is a single ^{199}Hg resonance for mercury(II) in **4** at −883 ppm, relative to neat $(CH_3)_2Hg$. This value is intermediate between the values of δ for mercury(II) in C–Hg–C and S–Hg–S bond systems [6h]. The ^{199}Hg chemical shift value is thus a useful indicator of the nature of the nucleophiles bound to the mercury atom [10].

Concluding Remarks

We have found in the present study that mercuric acetate rapidly mercurates the N_3-methylmercurated uridine [CH_3Hg(Uri)], (**2**), at the C_5 position to yield a dimethyl sulfoxide-soluble, polymeric substance, $[-Uri-Hg-\cdot 2H_2O]_n$ (**3**). Reaction of **3** with 6-thioguanosine in aqueous solution leads to the rapid cleavage of the Hg–N bonds in **3** by 6-thioguanosine and the formation of a bridged complex **4** (Hg–S bridging).

The results have significance in biological systems, since thiomercuri derivatives of the type Uri–Hg–SR are acceptable as substrates for RNA polymerase [7]. The instability of 5-alkylthiomercuripyrimidine nucleoside derivatives, where alkylthio = MeS-, EtS-, CH_3COS-, CH_3CSNH and other organic thio derivatives, severely limits their use in studies of DNA replication or transcription. They are apparently demercurated as a result of thiol-catalyzed symmetrization reactions.

While the use of 6-thionucleosides as antineoplastic agents is well known, the demonstration of their ability to form stable complexes of the type represented in **4** suggests they could also have potential as substrates for RNA polymerase and thus be of use in studies of DNA replication and/or transcription.

Acknowledgements

We thank the Natural Sciences and Engineering Research Council for support of this work *via* a Strategic Grant in Environmental Toxicology.

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