[Pt(CBDCA-*O*)(NH$_3$)$_2$(L-Methionine-*S*)]: Ring-Opened Adduct of the Anticancer Drug Carboplatin ("Paraplatin"). Detection of a Similar Complex in Urine by NMR Spectroscopy

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Received January 31, 1994

Carboplatin ("Paraplatin"), [Pt(CBDCA-*O*,*O*')(NH$_3$)$_2$] (**1**),[1] is a second-generation square-planar Pt(II) complex[2] widely used for the treatment of cancer,[3] and there is much current interest in comparisons of the properties of **1** with those of the first-generation drug cisplatin, *cis*-[PtCl$_2$(NH$_3$)$_2$]. The amino acid L-methionine (L-HMet) appears to play a role in the metabolism of cisplatin, the adduct [Pt(L-Met-*S*,*N*)$_2$] having been detected both in chemical systems[4,5] and in the urine of patients treated with cisplatin.[6] However, no studies of carboplatin−methionine interactions have been reported. We report here the unexpected finding that the ring-opened complex [Pt(CBDCA-*O*)(NH$_3$)$_2$(L-HMet-*S*)] (**2**) is a remarkably stable intermediate in reactions of carboplatin with L-HMet, and moreover that a similar (perhaps the same) species is present as a major metabolite in the urine of animals treated with carboplatin.

First, we studied the reaction of [^{15}N]**1** with 1 mol equiv of L-HMet (10 mM) in 50 mM phosphate buffer (90% H$_2$O, 10% D$_2$O) at pH* 6.9, 298 K. During the first few hours of the reaction, two major new cross-peaks of similar intensity appeared in the [^{1}H,^{15}N] NMR spectrum[7−9] at −78.8/4.34, 4.32 ppm (**2a**) and −43.0/4.26 ppm (**2b**), Figure 1A. These cross-peaks clearly belong to NH$_3$ ligands in the same complex (**2**) since they have similar intensities which rise and fall together as the reaction proceeds. They also arise from a relatively stable complex which is present in solution for at least 24 h during the course of the reaction. The ^{15}N shifts of NH$_3$ ligands in Pt(II) complexes are diagnostic of the *trans* ligand[10] and allow

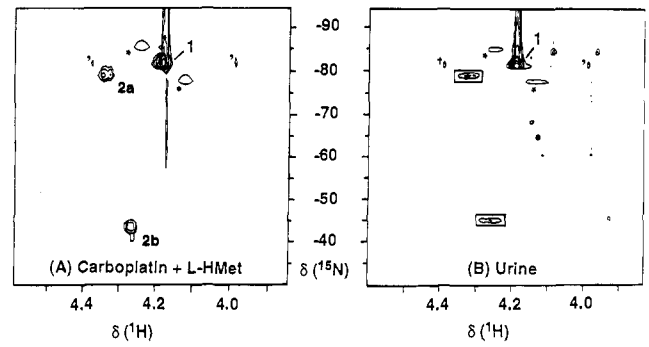

Figure 1. 500 MHz 2D [^{1}H,^{15}N] HMQC NMR spectra at 298 K of (A) 10 mM [^{15}N]**1** and 10 mM L-HMet in 50 mM phosphate buffer pH* 7 (10% D$_2$O, 90% H$_2$O) 3.5 h after mixing, and (B) urine (pH 5.96, 10% D$_2$O added) collected from 0 to 24 h after treatment of mice with [^{15}N]**1**. Peaks labeled with * are ^{195}Pt satellites, and those labeled with † are artifacts of decoupling. The streaking of the peak for **1** in the ^{15}N dimension is due to its very high intensity relative to other signals. The boxed cross-peaks in spectrum B have shifts close to those of **2** in spectrum A. Spectral accumulation times: 40 min for spectrum A and 14 h for spectrum B.

assignment of peaks **2a** and **2b** to NH$_3$ ligands *trans* to O and S ligands, respectively. Their temperature-dependent behavior (Figure D1, supplementary material) is consistent with the presence of a chiral center in **2**, the sulfur of coordinated L-HMet. At high temperature, there is rapid inversion of S, and only exchange-averaged peaks are seen, whereas at low temperature, peaks **2a** and **2b** each resolve into two separate peaks assignable to the diastereomers in slow exchange on the NMR time scale (k = 22.2 s^{-1}, $\Delta G^{\ddagger}$ = 66.5 kJ mol^{-1} for peak **2a** at 298 K). The CH$_2$ region of the 1D ^{1}H NMR spectrum is complicated by overlap of CBDCA and L-HMet peaks, but in the 2D DQF-COSY spectrum, two sets of CBDCA cross-peaks are clearly seen and can be assigned to uncomplexed CBDCA and monodentate CBDCA. The shifts for the latter (2.36 and 1.86 ppm) are close to those observed previously for Pt-bound monodentate CBDCA.[11] A ^{195}Pt{^{1}H} NMR spectrum of a similar 1:1 reaction mixture at high concentration (60 mM) gave rise to a broadened peak at −2641 ppm (relative to external PtCl$_6^{2-}$), a shift which is within the range expected for PtN$_2$SO coordination (−2500 to −2700 ppm).[12]

The coordinated S can only arise from L-HMet, and the DQF-COSY spectrum strongly suggests that the O ligand is monodentate CBDCA. Other possible O ligands can be ruled out: phosphate because the ^{1}H/^{15}N spectra are the same in H$_2$O alone, S,O-chelated L-HMet because this is stable only at very low pH,[13] and H$_2$O/OH since no pH dependence of ^{1}H/^{15}N shifts was observed (pH 3−7). These data provide strong evidence for the intermediate **2** being the ring-opened adduct [Pt(CBDCA-*O*)(NH$_3$)$_2$(L-HMet-*S*)] (**2**), a complex which may be stabilized by intramolecular H-bonding, e.g., between the carboxylate O and *cis* ammine. At later reaction times (24 h), ^{15}N/^{1}H peaks assignable to [Pt(L-Met-*S*,*N*)(NH$_3$)$_2$]$^+$ (**3**) (−61.8/4.17, 4.21 ppm and −41.8/4.33, 4.38 ppm) become more prominent in the [^{1}H,^{15}N] spectrum, and peaks for unbound CBDCA continue

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(1) Abbreviations: CBDCA, 1,1-cyclobutanedicarboxylate; L-HMet, L-methionine; pH*, pH meter reading; TSP, sodium trimethylsilyl-d_4 propionate.

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(10) Approx Pt−^{15}NH$_3$ shift ranges for ^{15}N *trans* to O, −70 to −90; N, Cl, −50 to −70; S, −30 to −50 ppm, e.g.: Ismail, I. M.; Sadler, P. J. *ACS Symp. Ser.* **1983**, *209*, 171−190. Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 4685−4693.

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(12) The ^{195}Pt shift range for PtN$_2$SO coordination is −2500 to −2700 ppm: (a) Appleton, T. G.; Connor, J. W.; Hall, J. R. *Inorg. Chem.* **1988**, *27*, 130−137. (b) Appleton, T. G.; Connor, J. W.; Hall, J. R.; Prenzler, P. D. *Inorg. Chem.* **1989**, *28*, 2030−2037. This is readily distinguished from, e.g., PtN$_2$S$_2$ and PtN$_4$, as discussed: (c) Bancroft, D. P.; Lepre, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 6860−6871.

(13) [Pt(L-Met-*S*,*O*)(NH$_3$)$_2$]$^+$ has different ^{15}N shifts and is unstable above pH 2: ref 12a.

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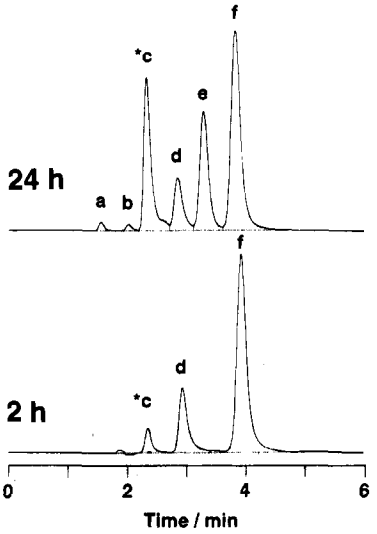

Figure 2. HPLC chromatograms of a solution containing L-HMet and **1** (5 mM each), pH 5.9, after reaction at 298 K for 2 and 24 h, with monitoring at 210 nm. Peak assignments: a, CBDCA; b, *trans*-[Pt(L-Met-*S*,*N*)$_2$]; c, complex 2; d, L-HMet; e, *cis*-[Pt(L-Met-*S*,*N*)$_2$]; f, complex **1** (plus **3**?). The ϵ_{210} values of the bis complexes[5] are >2× those of the other species.

to increase in intensity in the ^{1}H NMR spectrum (2.34 and 1.82 ppm). Also, peaks for complexes [Pt(L-Met-*S*,*N*)(NH$_3$)(X)] which have lost an NH$_3$ ligand (high *trans* effect of S; X may be S-bound L-HMet) and for the bis adduct [Pt(L-Met-*S*,*N*)$_2$] are present.

In view of the apparent stability of the ring-opened complex **2**, attempts were made to isolate it by HPLC.[14] Figure 2 shows chromatograms from an equimolar mixture (5 mM) of L-HMet and **1**, pH 5.9, after reaction at 298 K for 2 and 24 h. The first new peak to appear was peak c (elution time 2.3 min). This fraction had a ^{1}H NMR spectrum consistent with the proposed formulation of **2** and on standing gave rise to peaks for the chelated complex **3** with displacement of CBDCA. Moreover, the [^{1}H,^{15}N] NMR spectrum of fraction c isolated from a similar reaction of ^{15}N-L-HMet and [^{15}N]**1** contained only peaks **2a** and **2b**.

Second, we studied samples of urine collected from mice treated with [^{15}N]**1**.[15] These gave rise to a pair of ^{15}N/^{1}H cross-peaks of similar intensity (Figure 1B) and with shifts very close to those of **2**, along with several other sets of minor cross-peaks. The urine from a different set of animals gave rise to similar spectra. The pair of peaks represents the major NH$_3$-containing metabolite in urine, ca. 8.6% of the observable Pt if we assume that all the detected metabolites are diammine species (Table 1). The 2D COSY ^{1}H NMR spectrum of this urine contained α,β/γ cross-peaks for the CBDCA protons of **1** and a second set which may belong to monodentate CBDCA or to free CBDCA, or to both since they have similar shifts and the peaks are broader for urine than model systems (*vide supra*). These data suggest that the major metabolite of **1** may be closely related to **2**, although other *cis*-Pt(II) diammine complexes with S and O as *trans* ligands cannot be ruled out at this stage. For example, the S ligand may be an N-blocked derivative of L-HMet (e.g., a peptide), explaining why **3** is apparently not detected in urine. The metabolite must be formed directly in the body since it was not detected in control urine incubated with [^{15}N]**1** for 24 h (Figure D2, supplementary material), unlike several of the more minor metabolites, Table 1. Another notable metabolite peak is that at −45.5/3.89 ppm which has shifts similar to those of the bridged glutathione (GSH) complex[12b] [Pt(NH$_3$)$_2$(μ-GS)]$_2^{2+}$. GSH complexation of platinum is thought to form part of a cellular resistance mechanism, and GSH has been administered to patients as a chemoprotector.[16]

Table 1. ^{1}H and ^{15}N NMR Chemical Shifts for Urine of Mice Treated with [^{15}N]**1**

δ(^{15}N)	NH$_3$ trans to	δ(^{1}H)	intensity[a] (% NH$_3$)	assignment
−81.3[b]	O	4.17[b]	85.8	[Pt(CBDCA-*O*,*O*′)(NH$_3$)$_2$][c] (**1**)
−84.4[b]	O	4.07[b]	1.1	[Pt(HPO$_4$)$_x$(NH$_3$)$_2$][c]
−84.1[b]	O	3.94[b]	0.9	[PtCl(CBDCA-*O*)(NH$_3$)$_2$]$^-$ [c]
−68.3[b]	Cl	4.13[b]	1.1	[PtCl(CBDCA-*O*)(NH$_3$)$_2$]$^-$ [c]
−65.0[b]	Cl or N	4.11[b]	1.1	?
−64.9[b]	Cl or N	4.03[b]	0.4	?
−65.8[b]	Cl or N	3.83[b]	<0.2[d]	?
−78.8	O	4.32	4.2	[Pt(CBDCA-*O*)(NH$_3$)$_2$-(L-HMet-*S*)][e] (**2**)
−44.9	S	4.24	4.4	[Pt(CBDCA-*O*)(NH$_3$)$_2$-(L-HMet-*S*)][e] (**2**)
−45.5	S	3.89	1.0	?[f]
−45.0	S	3.97	<0.2[d]	?

[a] Estimated from 2D slices. [b] Peaks also seen in spectra of control urine to which [^{15}N]**1** had been added. [c] Shifts in ref 11. [d] Just detectable in 2D spectra. [e] Tentative. [f] Shifts similar to [Pt(NH$_3$)$_2$(μ-GS)]$_2^{2+}$.

This work illustrates the power of modern NMR methods for speciating Pt drugs in intact biofluids and demonstrates the advantages of [^{1}H,^{15}N] NMR over ^{1}H NMR alone[17] for such studies: the detection limits are low (ca. 20 μM) since ^{15}N-editing removes background interference from the large number of other substances in urine, and the ^{15}N shift range is large and diagnostic of the *trans* ligand. Adducts of carboplatin with monodentate S ligands may play a role in the mechanism of action of this drug and its toxic side effects. For example, recent work has shown that intermolecular displacement reactions of monodentate S-bound L-HMet (in contrast to chelated S,N-bound L-HMet) by N7 of guanine can occur,[18] and related intramolecular displacements have also been reported.[19]

Acknowledgment. We thank the MRC, Royal Society, SERC, NECRC, ULIRS, and EC COST (D1-92-002) and HCM (ERBCHRXCT 920016) programs for their support for this work, Dr. Tom Frenkiel (MRC Biomedical NMR Centre, Mill Hill) for advice on inverse probe experiments, Drs. Peng Bin and Jo Calvete (Cancer Research Unit, University of Newcastle upon Tyne) for their assistance, and Johnson Matthey for the gift of some of the carboplatin.

Supplementary Material Available: Temperature dependence of the ^{1}H{^{15}N} NMR spectrum of a similar reaction mixture to that in Figure 1 (Figure D1), and 2D [^{1}H,^{15}N] HMQC NMR spectrum of control mouse urine which had been incubated with 5 mM [^{15}N]**1** for 24 h at 310 K (Figure D2) (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(14) HPLC was carried out on a PLRP-S column (250 × 4.6 mm, 100 Å, 5 μm, Polymer Labs) with H_2O as the eluant.

(15) Solutions of 5 mg mL^{-1} of **1** and [^{15}N]**1** in 5% (w/v) glucose were each administered intravenously (tail) to two groups of five female Balb C mice (19−23 g, Comparative Biology Centre, University of Newcastle upon Tyne) in metabolism cages (food and water available *ad libitum*) as a bolus dose of 50 mg/kg. Urine was then collected onto ice/salt for 24 h and was stored frozen until it was analyzed. Total Pt concentrations in the urine, determined by atomic absorption spectroscopy (Harland, S. R.; Newell, D. R.; Siddik, Z. H.; Chadwick, R.; Calvert, A. H.; Harrap, K. R. *Cancer Res.* **1984**, *44*, 1693−1697), were 2.4 and 3.6 mM after administration of **1**, and 2.6 and 3.3 mM for [^{15}N]**1**, corresponding to 74%, and 43 and 83% of the administered dose, respectively. These data are consistent with those reported previously: Siddik, Z. H.; Newell, D. R.; Boxall, F. E.; Harrap, K. R. *Biochem. Pharmacol.* **1987**, *36*, 1925−1932. These data confirm that ^{15}N does not alter the renal handling of the drug.

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