temperature. Diethyl ether was added, and the mixture was cooled to −20 °C. A pale-yellow solid precipitated after standing overnight. This product was filtered off, washed with diethyl ether, and dried in vacuo (yield: 68 mg; 82%). Anal. Calcd for $C_{39}H_{48}Cl_3IrN_3O_8P_2Tl$: C, 37.42; H, 3.87; N, 3.36; Cl, 8.50. Found: C, 36.64; H, 3.81; N, 3.04; Cl, 7.60.

$[Ir(PPh_3)_2(AcO)(CO)(\mu AcO)Tl(AcO)_2]$. The complex has been prepared following modification of the original procedure.[15] A 30-mg sample of $Ag(CH_3COO)$ (0.179 mmol) was added to a suspension of $Ir(CO)Cl(PPh_3)_2$ (140 mg, 0.179 mmol) in dry CH_2Cl_2 (8 mL) and the reaction mixture was rapidly stirred for 5 min in the dark. Solid $Tl(CH_3COO)_3 \cdot 1.5H_2O$ (73 mg, 0.179 mmol) was then added to the yellow suspension. Immediately the suspension turned deep orange (not red as previously reported). A clear orange solution was obtained after approximately 30 min. After being stirred for 1.5 h, the solution turned yellow, and after 3 h (overall reaction time) a white solid (AgCl) was removed by filtration. Hexane (15 mL) was then added to the yellow filtrate, and the mixture was cooled to −20 °C. A cream microcrystalline solid precipitated overnight (yield: 71 mg; 33%). Further workup on the mother liquor gave another crop of the same product (yield: 43 mg; 20%).

X-ray Data Collection. [Tl(crown-P_2)Ir(CO)Cl](NO_3) (5). Yellow-orange crystals were obtained by slow diffusion of diethyl ether into a dichloromethane solution of the complex. They were coated with a light hydrocarbon oil to prevent cracking upon exposure to air. The crystal was mounted on a glass fiber with silicon grease and placed into the 130 K nitrogen stream of a Syntex $P2_1$ diffractometer with a modified LT-1 low-temperature apparatus. The space group was determined to be $P\bar{1}$. The two-check reflection showed only random fluctuations (<2%) in intensity throughout the data collection. The data were corrected for Lorentz and polarization effects. Crystal data are given in Table IV.

$(CH_3CO_2)_2Tl(\mu\text{-}O_2CCH_3)Ir(CO)(PPh_3)_2(O_2CCH_3)\cdot CHCl_3$. Colorless plates were obtained by slow diffusion of ethyl ether into a chloroform solution of the complexes. These were handled as described above for **5**. There was no decay in the intensity of two-check reflections during data collection.

Solution and Refinement of Structures. [Tl(crown-P_2)Ir(CO)Cl]$(NO_3)\cdot CH_2Cl_2$. The structure was solved by Patterson and difference Fourier methods. Computer programs are from SHELTL, Version 5, installed on a Data General Eclipse computer. Neutral atom scattering factors and corrections for anomalous dispersion are from a standard source.[30] The disorder in the carbonyl group and chloride bonded to Ir was modeled by assigning 60% weight to one arrangement (A) and 40% weight to the other (B). The positional parameters for the carbonyl groups were taken from a final difference map and fixed. Thermal parameters for all the atoms in question were allowed to refine and are reasonable for the model. Hydrogen atoms were included at calculated positions with use of a riding model and C–H distances of 0.96 Å. The thermal parameters for the hydrogen atoms were fixed at 1.2 times the thermal parameter of the bonded carbon. An absorption correction was applied.[3] Final refinement was carried out with anisotropic thermal parameters for Ir and Tl atoms. The largest peak in a final difference map had a value of 4.6 e Å^{-3}, located 0.81 Å from Tl.

$(CH_3CO_2)_2Tl(\mu\text{-}O_2CCH_3)Ir(CO)(PPh_3)_2(O_2CCH_3)\cdot CHCl_3$. The structure was solved by Patterson and difference Fourier methods. Hydrogen atoms were included at calculated positions with use of a riding model and C–H distances of 0.96 Å. The thermal parameters for the hydrogen atoms were fixed at 1.2 times the equivalent isotropic thermal parameter of the bonded carbon. An absorption correction was applied.[31] Final refinement was carried out with anisotropic thermal parameters for Ir, Tl, P, and Cl atoms. The largest peak in the final difference map had a value of 1.60 e Å^{-3} and was located 0.85 Å from Ir.

Physical Measurements. The $^{31}P\{^1H\}$ NMR spectra were recorded on a General Electric QE-300 NMR spectrometer that operates at 121.4 MHz with an external 85% phosphoric acid standard and the high field positive convention for reporting chemical shifts. Infrared spectra were recorded on an IBM IR32 spectrometer. Electronic spectra were recorded with a Hewlett-Packard 8450A spectrometer. Uncorrected emission spectra were obtained through the use of a Perkin-Elmer MPF-44B fluorescence spectrometer.

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Supplementary Material Available: Tables of atomic coordinates, bond distances, bond angles, anisotropic thermal parameters, hydrogen atom positions, and crystal refinement data for **6** and **9** (10 pages); listings of observed and calculated structure factors (62 pages). Ordering information is given on any current masthead page.

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Chromium(VI) Forms a Thiolate Complex with Glutathione

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Abstract: Reaction of potassium dichromate with the tripeptide glutathione resulted in the formation of a 1:1 complex of Cr^{VI} with glutathione. The red Cr^{VI}–glutathione adduct was stable for ~60 min at 4 °C and I = 1.5 M. ^1H, ^{13}C, and ^{17}O NMR studies showed that glutathione acts as a monodentate ligand and binds to Cr^{VI} through the cysteinyl thiolate group, forming a $GSCrO_3^-$ complex. No evidence was obtained for involvement of the other possible ligating groups, e.g., glutamyl amino and carboxylate, glycinyl carboxylate, or peptide backbone, in binding to Cr^{VI}; however, Cr^{VI}-induced conformational changes in the glutamyl and cysteinyl side chains of the tripeptide. EPR studies showed that two chromium(V) species (g = 1.987 and g = 1.973) are formed upon reaction of potassium dichromate with glutathione. Chromium–glutathione complexes may be involved in producing the high levels of chromium(VI)-induced DNA damage in cells having high concentrations of glutathione and may play an important role in the carcinogenicity of chromium(VI) compounds.

Introduction

Chromium(VI) compounds have been shown to be human carcinogens in several epidemiological studies.[1-3] Chromium(VI) toxicity and mutagenicity has been the subject of a recent review.[4] In contrast, chromium(III) compounds are relatively nontoxic and noncarcinogenic. However, chromium(VI) does not react with

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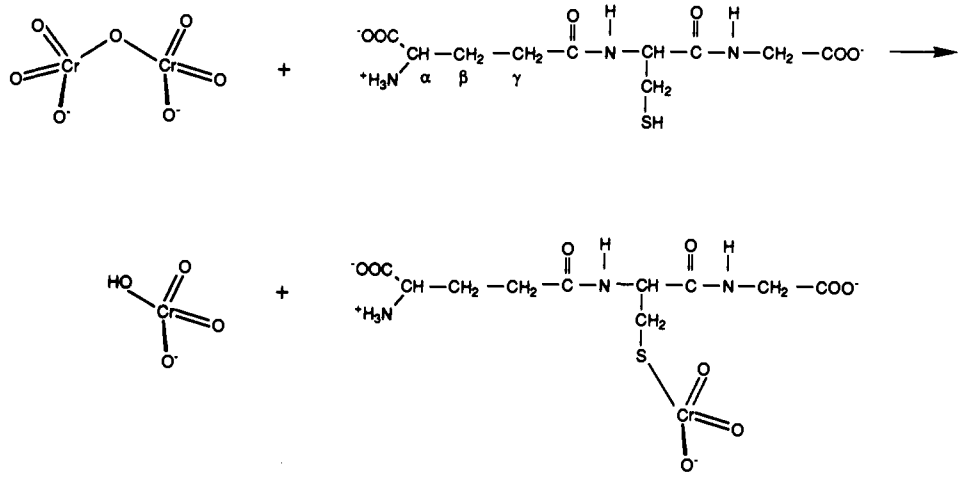
Scheme I



DNA in vitro, but chromium(III) reacts slowly with DNA to form DNA–Cr(III) complexes.[5] To explain these results, Connett and Wetterhahn[6] proposed the uptake-reduction model for chromium(VI) carcinogenicity. Chromium(III) compounds, which form octahedral complexes that are kinetically inert to substitution, are not readily carried across the cell membrane. In contrast, tetrahedral chromium(VI) oxoanions are readily carried across the cell membrane by the anion transport system and are reduced by intracellular components.[7] Chromium(VI) is ultimately reduced to chromium(III), which forms stable complexes with DNA.[4] An important part of the carcinogenesis mechanism is thought to be the intracellular reduction of chromium(VI) which generates putative DNA-damaging species, such as Cr(V) and other radical species.

Connett and Wetterhahn[8] compared rates of chromium(VI) reduction by small redox-active cellular components under physiological conditions and found that the highest rates of reduction occurred with compounds containing a thiol moiety. Glutathione (γ-glutamylcysteinylglycine, GSH) is a biologically important reductant which exists in mammalian cells at concentrations up to 8 mM, making it the most prevalent intracellular thiol.[9] Glutathione has been shown to react with chromium(VI) in vitro under physiological conditions.[6,10] The ability of carcinogenic chromium(VI) compounds to damage DNA has been shown to increase as glutathione concentrations increase both in vitro and in cultured cells.[11,12] Glutathione has been postulated to reduce the chromium(VI), via a Cr^{VI}–glutathione thioester intermediate.[6,8,13] (eq 1). McAuley and Olatunji[13] performed

$$GSH + Cr^{VI} \rightarrow GSCr^{VI} \quad (1)$$

stopped flow kinetic studies on the reaction of GSH with chromium(VI) under acidic conditions and hypothesized the existence of a GS–Cr^{VI} thioester intermediate. Connett and Wetterhahn[8] also observed the formation of a putative GS–Cr^{VI} thioester spectroscopically under physiological pH. The high concentrations of thiol, relative to chromium^{VI}, used in these studies to provide conditions for pseudo-first-order kinetics, also led to facile reduction of chromium(VI). Since both of these studies used only UV–vis spectroscopy to detect the GS–Cr^{VI} thioester, no detailed structural information could be obtained on the proposed intermediate complex. In addition, no information as to the relative reactivity of GSH with chromate/hydrogen chromate, in comparison to dichromate, was obtained.

The formation of chromium(VI)–oxy esters is expected to be favored over the formation of chromium(VI)–thioesters, since chromium(VI) is considered a *hard* acid and sulfur is a *soft* base.[14] Although no characterized chromium(VI)–thiolate complex has been reported, molybdenum(VI) and tungsten(VI) have been shown to form chelate complexes with cysteine having thiolate ligation to the metal.[15] NMR studies have shown that the ^1H and ^{13}C resonances of glutathione are extremely sensitive to the environment and, therefore, changes in chemical shift can be used to determine binding sites of metal complexes.[9,16-22] In order to further elucidate the mechanism by which chromium(VI) reacts with GSH, we have studied the reaction of dichromate with glutathione by using ^1H, ^{13}C, and ^{17}O NMR, EPR, and UV–vis spectroscopy under conditions which favor the formation of the putative GS–Cr^{VI} thioester. This study provides the first structural evidence for a chromium(VI)–glutathione thioester, which has the cysteinyl thiolate of glutathione bound to chromium(VI) (Scheme I).

Experimental Section

Materials. Reduced glutathione (GSH) and oxidized glutathione (GSSG) (Sigma Chemical Co., St. Louis, MO) and potassium dichromate (Fisher Scientific, Fair Lawn, NJ) were used as received. All solutions were made up in 99.8% D_2O (Aldrich Chemical Co., Milwaukee, WI), with the exception of the solutions used for ^{17}O NMR measurements which were prepared with 10.8 atom % ^{17}O H_2O and 2–3% D_2O (Icon Services, Summit, NJ) added to provide a lock signal. pH

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Table I. ^1H and ^{13}C Chemical Shifts for Reduced Glutathione (GSH), Chromium(VI)–Glutathione Complex ($GSCrO_3^-$), and Oxidized Glutathione (GSSG)[c]

	γ-glutamyl			cysteinyl		glycinal
	βCH_2	γCH_2	αCH	CH_2	CH	CH_2
			GSH			
^1H	1.86	2.24	3.46	2.63	4.27	3.45
^{13}C	25.8	31.0	53.7	25.3	55.4	43.1
			$GSCrO_3^-$			
^1H	1.90[a]	2.25[a]	3.51	3.22, 3.06[b]	4.39	3.48
^{13}C	25.9	31.0	53.8	36.3	53.8	43.1
			GSSG			
^1H	1.86	2.23	3.47	3.01, 2.66[b]	4.45	3.46
^{13}C	25.9	31.0	53.7	38.2	52.2	43.2

[a] There is little or no fine structure observed with these protons. [b] The diastereotopic protons of the cysteinyl methylene are resolved into two separate signals in the $GSCr^{VI}$ thioester and GSSG. [c] These shifts occurred under the following conditions: pH* = 5.75 ± 0.05 for GSH, pH* = 5.70 ± 0.05 for GSSG, and $GSCrO_3^-$ at 277 K. All shifts are reported relative to TMS; ^1H shifts are accurate to within ±0.02 ppm, and ^{13}C shifts are within ±0.1 ppm.

measurements were made by using a Corning Model 140 pH meter with a microcombination electrode. The pH values were uncorrected for deuterium isotope effect and are denoted by pH*. The initial pH* of all solutions was adjusted to 4.9 with either DNO_3 or NaOD (Aldrich Chemical Co., Milwaukee, WI). Ionic strength was adjusted to I = 1.5 M with KCl (Fisher Scientific, Fair Lawn, NJ). All solutions were bubbled with argon for 10 min to prevent possible air oxidation of GSH to GSSG. All measurements were made on solutions that were prepared under identical conditions, preequilibrated, and then maintained at 4 ± 1 °C. No buffers were employed in order to prevent overlap in the NMR spectra and possible competing reactions. Conditions of ionic strength, temperature, and stoichiometry were chosen to stabilize the chromium(VI) thioester and to slow the reduction of chromium(VI) to chromium(III). The final Cr(VI) concentration after mixing was 0.080 M, and the GSH concentration after mixing was 0.040 M for all experiments. Upon mixing Cr(VI) and GSH, a dark red solution was observed. Since no buffers were employed, pH* increased from 5.3 to 5.8 over a 65-min time period. All spectroscopic measurements were made in less than 70 min after mixing, at which time increasing amounts of oxidized glutathione were observed in the reaction mixture by NMR.

NMR Spectroscopy. NMR spectra were obtained on a Varian XL-300. The temperature for all runs was maintained at 4 ± 1 °C. Proton and ^{13}C chemical shifts are reported relative to an external tetramethylsilane (TMS) reference. Reference spectra were obtained with a 10-mm coaxial tube which contained 10% TMS in CCl_4 in the outer tube. 3-(Trimethylsilyl)propionic acid (TSP) was not employed as a reference[23] due to the possibility of the production of paramagnetic species in the reaction of chromium(VI) with glutathione which could alter the chemical shift of an internal reference. Coupling constants were estimated with the aid of the spin simulation program, LAOCOON. Standard Varian software was used for data acquisition and processing.

COSY3[24] spectra of the glutathione and dichromate reaction mixture were acquired with 12 transients per 128 increments in a 1024 × 1024 matrix with 960 points and a sweep width of 1200 Hz. A Gaussian apodization function was used for resolution enhancement.

^{17}O NMR spectra were acquired at a frequency of 40.662 MHz. Samples were not spun and for Cr(VI)–SG measurements were run unlocked. Samples that contained chromium(VI) only were locked on D_2O and used for shimming purposes. The reference for all spectra was the solvent line which was ~95% H_2O/5% D_2O, approximately 10% enriched in ^{17}O. Sweep widths were 100 000 Hz, acquisition times were 0.040 s, and 90° pulses were employed. Typically 10–12 000 transients were collected per spectrum. Baseline corrections were applied to aid presentation. Total collection time per spectrum was approximately 15 min.

^{13}C NMR spectra of the carbon skeleton of the Cr^{VI}–glutathione complex were acquired at 75.429 MHz with broad band proton decoupling, by using a 15–20° flip angle and a sweep width of 3200 Hz.

(23) Under these conditions the signal of the methyl protons of internal TSP is ~0.3 ppm upfield of external TMS, in addition, the ^{13}C signal of internal dioxane is 66.1 ppm relative to external TMS.

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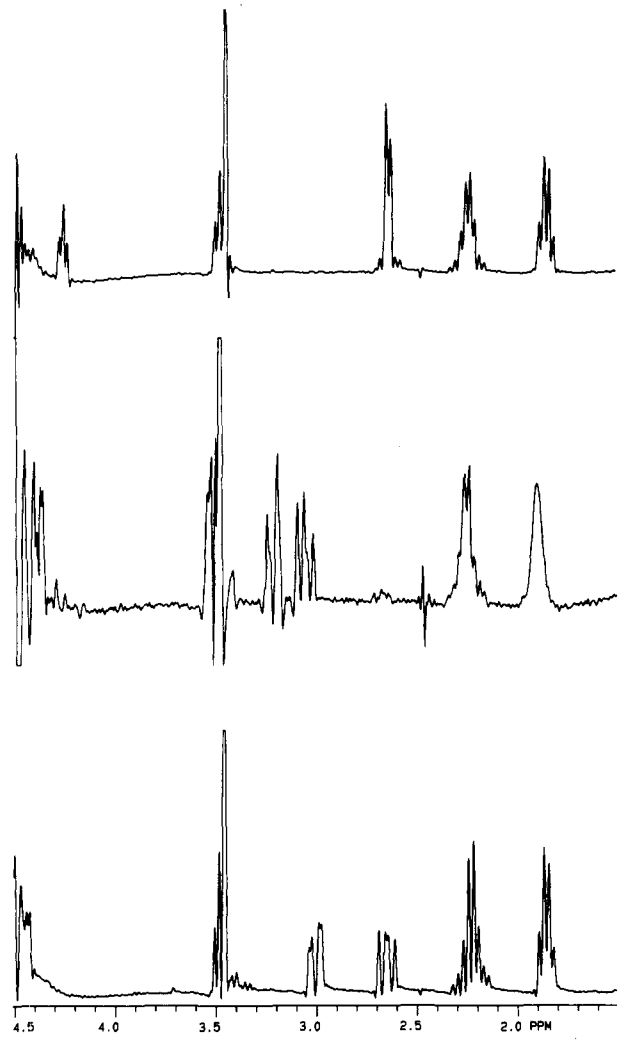

Figure 1. Proton NMR spectra of glutathione, the glutathione–chromium(VI)–thioester, and oxidized glutathione. (top) The 300-MHz ^1H NMR spectrum of 0.040 M GSH in D_2O. The following conditions were used: pH* = 5.7, I = 1.5 M. The 256 transients were accumulated at 277 K. (middle) ^1H NMR spectrum of the reaction mixture of 0.040 M potassium dichromate and 0.040 M GSH in D_2O. The following conditions were used: I = 1.5 M, pH* varied from 5.6 to 5.7. The 256 transients were accumulated with an acquisition time of 0.4 s and a delay between pulses of 1.0 s at 277 K. The spectrum was acquired from 11–17 min after mixing. (bottom) ^1H NMR spectrum of 0.020 M GSSG acquired under the same conditions as the top spectrum.

Nearly 3000 transients were accumulated which took approximately 50 min.

UV–Visible Absorption Spectroscopy. UV–visible absorption spectra were taken on a Perkin-Elmer Lambda 9 spectrophotometer equipped with a Lauda K-4R circulating bath set at 4 ± 1 °C. Due to the concentrated solutions and high extinction coefficients of chromium(VI) and the chromium(VI)–GSH complex, a quartz cell with a 0.010-mm path length (Hellma Corp.) was used. This cell led to path length errors of ~10%, so all runs were performed in quadruplicate and averaged.

EPR Spectroscopy. EPR spectra were acquired on a Bruker ESP 300 equipped with a homemade frequency detector and an Er 4111T VT temperature controller set at 4 °C. Spectra were acquired at a frequency of 9.418 GHz with a center field of 3380–3400 G, a sweep width of 75–100 G, a modulation amplitude of 1.0 G, and microwave power of 20 mW.

Results and Discussion

^1H and ^{13}C NMR Spectroscopy. The reaction of glutathione with potassium dichromate was studied by NMR spectroscopy. Glutathione has several ionizable groups, which affect the chemical shifts of the ^1H and ^{13}C resonances as a function of pH.[17,18,20] It was therefore important to distinguish between chemical shifts varying as a function of changing pH or the binding to chromium(VI). However, over the pH range of interest, the changes

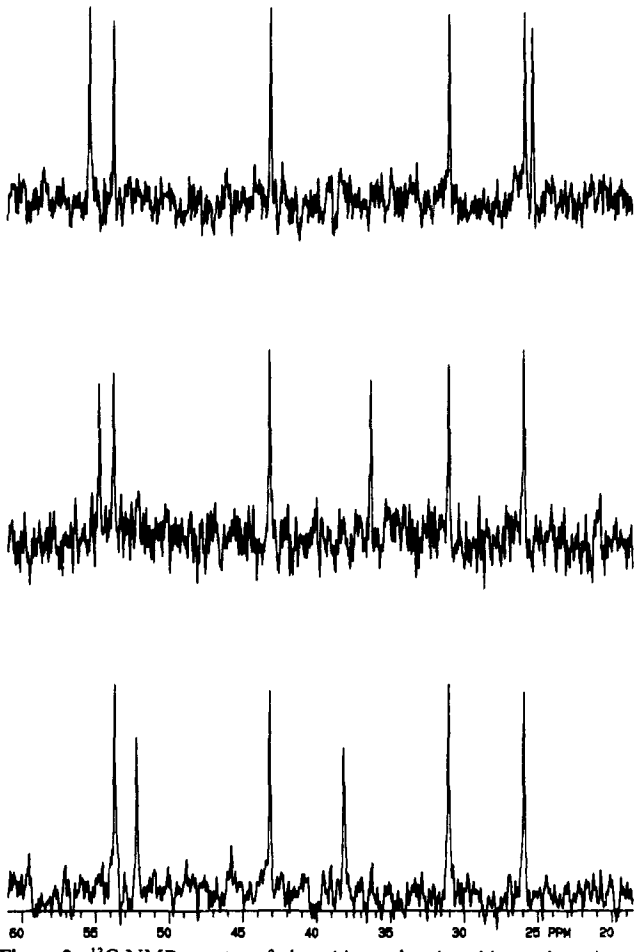

Figure 2. ^{13}C NMR spectra of glutathione, the glutathione–chromium(VI)–thioester, and oxidizied glutathione. (top) The 75.429-MHz ^{13}C NMR spectrum of 0.040 M GSH in D_2O. The following conditions were used: pH* = 5.7, I = 1.5 M. Approximately 2500 transients were accumulated at 277 K. (middle) ^{13}C NMR spectrum of the reaction mixture of 0.040 M potassium dichromate and 0.040 M GSH in D_2O. The following conditions were used: I = 1.5 M, pH* varied from 5.6 to 5.7. Approximately 3000 transients were accumulated with an acquisition time of 0.7 s at 277 K. The spectrum was acquired from 6 to 54 min after mixing. (bottom) ^{13}C NMR spectrum of 0.020 M GSSG acquired under the same conditions as the top spectrum. Approximately 6700 transients were accumulated at 277 K.

in chemical shifts attributed to differences in protonation of the thiol, carboxylate and amine groups are rather small and can be easily distinguished from changes in chemical shifts due to the complexation of chromium(VI). The cysteinyl methylene and methyne protons of glutathione showed the largest change in chemical shift upon addition of dichromate (Table I). The diastereotopic cysteinyl methylene protons, which appear as a multiplet at 2.63 ppm in uncomplexed glutathione, were split into two distinct signals at 3.22 and 3.06 ppm upon reaction with dichromate (Figure 1). The cysteinyl methyne proton underwent a smaller shift to 4.39 ppm from its uncomplexed position at 4.27 ppm (Figure 1, Table I). Conformation of this coupling network was accomplished with a COSY spectrum, which showed cross peaks between the two cysteinyl methylene protons and the methyne proton, in an analogous fashion to GSSG. Coupling constants for the cysteinyl protons of the chromium(VI)–glutathione complex were estimated to be $J_{gem} = -14$ Hz, $J_{AX} = 9$ Hz, and $J_{AM} = 4$ Hz. The coupling constants of the cysteinyl protons of GSSG were estimated at $J_{gem} = -14$ Hz, $J_{Ax} = 10$ Hz, and $J_{AM} = 4$ Hz, which is in good agreement with the literature.[25] The ^1H NMR results are consistent with binding of the cysteinyl thiolate to chromium(VI). The cysteinyl thiolate coordination

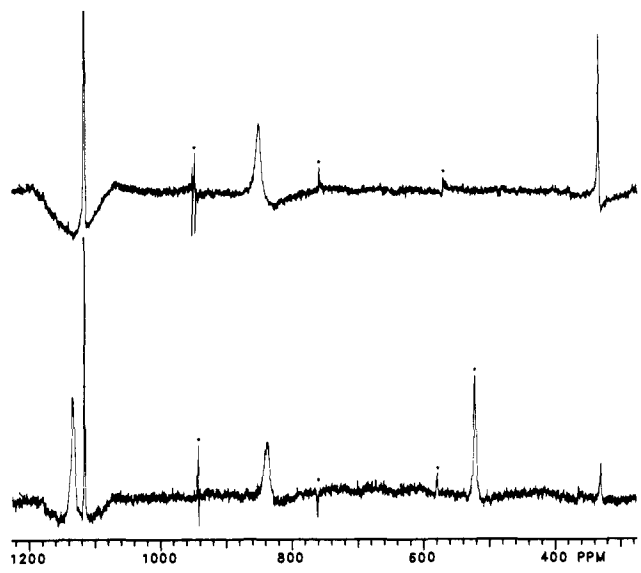

Figure 3. A comparison between the ^{17}O NMR spectra of chromium(VI) and the reaction mixture containing the $GSCrO_3^-$ complex. (top) A 40.662-MHz ^{17}O NMR spectrum of 0.075 M potassium dichromate in 95% H_2O/5% D_2O enriched ~10% in ^{17}O. The following conditions were used: I = 1.5 M, pH = 5.2. Approximately 15000 transients were accumulated with an acquisition time of 0.04 s at 277 K. 90° pulses were employed, and 10 Hz of line broadening was used. The sweep width was 100000 Hz. (bottom) An ^{17}O NMR spectrum of the reaction mixture of 0.039 M potassium dichromate and 0.039 M GSH in 95% H_2O/5% D_2O enriched ~10% in ^{17}O. The following conditions were used: I = 1.5 M, and initial pH of both reactants was 4.9. Approximately 11000 transients were accumulated with an acquisition time of 0.04 s at 277 K. 90° pulses were employed, and 10 Hz of line broadening was used. The sweep width was 100000 Hz. The spectrum was acquired 13–26 min after mixing, and the pH varied from 5.6 to 5.7 over this time period. The peaks marked with "*" are artifacts.

was confirmed by ^{13}C NMR studies which showed that the cysteinyl methylene carbon signal shifted from 25.3 to 36.3 ppm and the methyne shifted from 55.4 to 53.8 ppm upon reaction of glutathione with dichromate (Figure 2, Table I).

Reaction of dichromate with glutathione also resulted in changes of the chemical shifts of the glutamyl protons compared with uncomplexed glutathione. However, the magnitude of the changes in chemical shift were small, ~0.04 ppm downfield for the β-methylene protons (Table I). In addition some line broadening was observed, especially on the β-methylene protons at 1.90 ppm (Figure 1). Due to the overlap of the glutamyl methyne and the glycinal protons at 3.46 ppm and the loss of multiplet structure of the β-methylene protons, no coupling constants could be determined for the glutamyl moiety. In addition, the COSY spectrum showed that the cross peak between the glutamyl methyne and the β-methylene protons had decreased to the noise level. It is possible that the reaction produced paramagnetic species such as chromium(V) or chromium(III) which shifted and broadened the glutamyl resonances. EPR experiments clearly showed the presence of a small amount of chromium(V) (vide infra), suggesting a possible fast exchange of a Cr(V) species with the glutamyl residue. It is also possible that the chromium(VI) affected the conformation of the glutamyl moiety and restricted its rotation. Englander and Wand[26] have observed the loss of the methyne cross peak in glutamyl spin patterns in some proteins and suggested that changes in transverse relaxation times and J coupling constants were responsible. However, the ^{13}C spectrum of the glutamyl region did not show any significant changes in chemical shift (Figure 2, Table I). There were no changes in the ^1H and ^{13}C spectra of the glycinyl moiety upon reaction of glutathione with dichromate (Figure 2, Table I).

^{17}O NMR Spectroscopy. ^{17}O NMR studies of the reaction of GSH with dichromate revealed a new signal with a chemical shift

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of 1133 ppm, which was ~20 ppm downfield of the terminal oxygens of dichromate (Figure 3). In addition, signals from both the terminal (1115 ppm) and bridging oxygens (329 ppm) of dichromate and the oxygens of chromate/hydrogen chromate (835–845 ppm) were still observed. Filowitz et al.[27] observed that ^{17}O chemical shifts were largely a function of electronic environment. Downfield shifts correlate with increased π-bonding of oxygens to the metal center. The fact that the new signal is within 20 ppm of the terminal oxygens of dichromate indicates that the oxygens on chromium(VI) in the glutathione complex are in a similar electronic environment and are consistent with a $GSCrO_3^-$ complex. Similar trends in chemical shift are observed for molybdenum complexes, MoO_4^{2-} and $Mo_2O_7^{2-}$.[28,29] Sulfur substitution of oxygen on molybdenum(VI) leads to deshielding and therefore downfield shifts of the remaining oxygens bound to the metal. In addition, the ^{95}Mo spectra showed a similar downfield shift of molybdenum(VI) resonances upon substitution of sulfide for oxide ligands.[28,29]

The line width of the new ^{17}O signal (180–200 Hz) at 1133 ppm assigned to the $GSCrO_3^-$ complex was broader than the terminal oxygens of dichromate (~30 Hz) or the line width of chromate oxygens at pH* = 13 (13 Hz). It has been shown that the electric field gradient around a tetrahedral metal oxoanion is isoelectronic; however, increased asymmetry of the electric field leads to shortened T_1's of the ^{17}O nucleus. Filowitz et al.[27] noted the increased line width of the bridging oxygen of the $Mo_2O_7^{2-}$ anion, in comparison to the terminal oxygens, and attributed the increase in line width to the larger electric field around the bridging oxygen. Similar results can be seen for dichromate (vide infra). Therefore, the greater line width of the chromium(VI)–glutathione oxygen signal can be attributed to the decreased symmetry of the electric field gradient for the $GSCrO_3^-$ complex when compared to dichromate or chromate due to distortion of the tetrahedral environment upon formation of the Cr–S bond.[30,31]

Under our conditions the ^{17}O line width of chromate does vary with pH*, but neither the terminal nor the bridging oxygens of dichromate show a similar dependence. Therefore, it is unlikely that any exchange processes between dichromate and chromate occur. There is also no evidence of exchange for the chromium(VI)–glutathione complex with any other species. In addition, the line width of the chromium(VI)–glutathione thioester oxygen signal does not show a dependence on pH* within the narrow range observed. Early ^{17}O NMR experiments[32,33] suggested the broad line width of the signal from chromate/hydrogen chromate was indicative of chemical exchange with dichromate. However, the previous work was performed at much higher chromium(VI) concentration compared to our study. Under our conditions, chromate preferentially undergoes exchange with hydrogen chromate.

The oxygen signals of chromate/hydrogen chromate in the reaction mixture had a line width which decreased from ~600 to ~400 Hz with the increase in pH* (4.9–5.8) over the course of the reaction. However, the line width of chromate/hydrogen chromate, measured from a pure 0.20 M Cr(VI) solution with I = ~1.0 M, decreased from 300 to 150 Hz over the pH* range 5.1–5.8. The line width of the chromate/hydrogen chromate signal in this pH* range is much greater than chromate at pH* = 13, suggesting that there is a fast chemical exchange between chromate and hydrogen chromate at pH* 5–6. The chemical shift of the chromate/hydrogen chromate oxygens also showed a dependence on pH*. The chromate/hydrogen chromate oxygen signal shifted from ~860 ppm at pH* = 5.0 to ~840 ppm at pH* = 6.0 and was 815 ppm at pH* = 13. Therefore, the line width of chromate/hydrogen chromate in the reaction mixture can be explained by the changing pH* during the acquisition period.

The dichromate bridging oxygen line width increased from ~65 Hz at the start of the reaction to ~90 Hz after 40 min; however, the line width was difficult to measure given the small size of this signal. Neither the dichromate oxygens nor the $GSCrO_3^-$ oxygens showed significant changes in line width or chemical shift with time or increasing pH*. The line width of the bridging oxygens of dichromate, measured from solutions of pure chromium(VI), range between 45 and 65 Hz over the pH* range 2–6. Previous workers had attributed the larger line width of the bridging oxygen of dichromate, in comparison with the terminal oxygens of dichromate, to chemical exchange with chromate;[33] however, it is likely that the change in the electric field gradient is responsible for the larger line widths of the bridging oxygens of dichromate.

Dichromate may form a chromium(VI) thioester more readily than chromate because of the lability of chromate as a leaving group. ^{17}O NMR results showed that at the 1:1 ratio of glutathione and dichromate some chromate/hydrogen chromate and dichromate is still present after mixing, yet no free glutathione is observed in the proton and ^{13}C NMR spectra. Formation of $GSCrO_3^-$ from the reaction of glutathione and dichromate would result in the liberation of hydrogen chromate (eq 2), which would then reequilibrate to form dichromate and chromate (eq 3 and 4).

$$Cr_2O_7^{2-} + GSH \rightarrow GSCrO_3^- + HCrO_4^- \quad (2)$$

$$2HCrO_4^- \rightleftharpoons Cr_2O_7^{2-} + H_2O \quad (3)$$

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} + H^+ \quad (4)$$

Our evidence for the proposed 1:1 glutathione/chromium(VI) stoichiometry is based on NMR titration experiments. At substoichiometric amounts of glutathione to dichromate, no changes in the NMR spectrum of the glutathione ligand were observed when compared with the 1:1 ratio. When greater than stoichiometric ratios of glutathione to dichromate were reacted, much larger amounts of chromium(V) were formed, and a new glutathione–chromium complex was formed (data not shown).

Glutathione complexes with various metal ions have been reviewed by Rabenstein et al.[9] Glutathione binds primarily through the cysteinyl thiolate group to Zn^{II}, Cd^{II}, Pb^{II}, and Hg^{II}, which are considered *soft* acids.[14] In contrast, glutathione primarily binds through the glycinal carboxylate and the glutamyl carboxylate and amine to Ni^{II} at neutral pH.[34] VO^{2+} has been shown to bind through both the glutamyl and the glycinal moieties of glutathione.[22] Cu^{II}, Ag^{I}, and Au^{I} form complexes with glutathione of variable stoichiometry, with the cysteinyl thiolate generally being the preferred coordination site.[35–37] Molybdenum thiol complexes have been the subject of much discussion in the literature.[15,38–40] Recent evidence suggests that cysteine (Cys) binds through the thiolate, amine, and carboxylate groups to form a 1:1 tridentate cysteine–Mo^{VI} complex, followed by reduction to a Cys–Mo^{V} complex, $[Mo_2O_4(Cys)_2]^{2-}$.[15,37] Reactions of molybdenum(VI) with glutathione are thought to follow a similar pathway.[39] Our NMR data indicate that chromium(VI) binds to glutathione solely through the cysteinyl thiolate.

EPR Spectroscopy. The NMR studies clearly show that dichromate readily reacts with glutathione to form a chromium(VI)–thiolate complex. Surprisingly, chromate does not appear to react in a similar fashion with glutathione. EPR experiments by this group and others have shown the formation of several chromium(V) complexes from the reaction of chromate and

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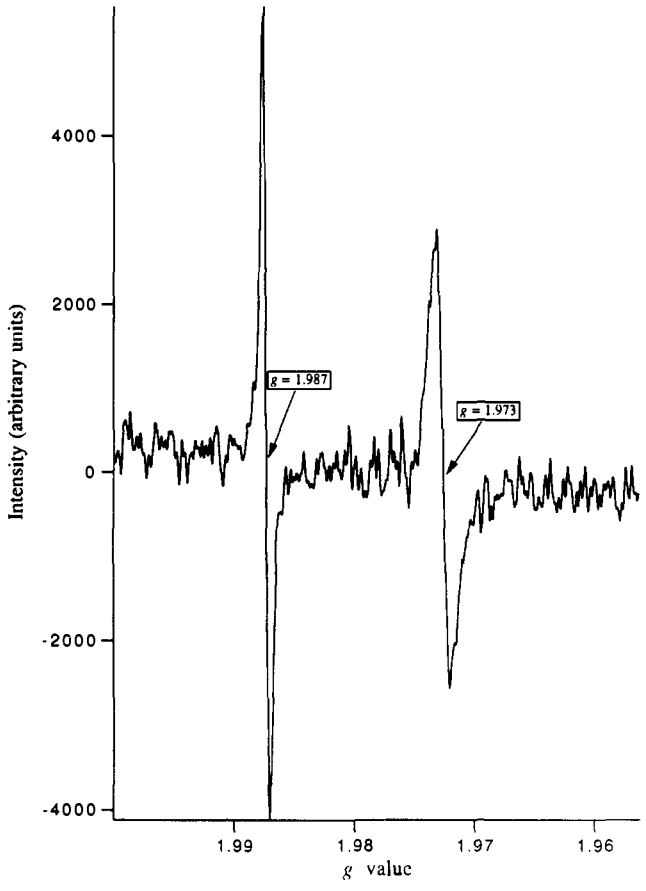

Figure 4. The EPR spectrum of chromium(V) species formed upon the reaction of dichromate with glutathione. Conditions for the reaction of potassium dichromate and glutathione are described in Figure 1. The spectrum was acquired with the following parameters: frequency = 9.418 GHz, with a center field of 3400 G and a sweep width of 75 G. Microwave power was 20 mW, with a modulation amplitude of 1.0 G.

glutathione; however, the structures of these complexes are still unknown.[41-43] Under our conditions, EPR studies of the reaction of dichromate with glutathione showed the formation of two chromium(V) complexes (Figure 4). Both chromium(V) signals showed minimal changes in intensity over a 60-min time period. One of the EPR signals, with a g value of 1.973 and a ΔH of 1.9 G, has not been reported previously in studies of the reaction of chromium(VI) with glutathione. A second EPR signal, with a g value of 1.987 and a ΔH of 1.1 G, has been previously reported by Goodgame and Joy[41] and Aiyar et al.[43] The basic conditions used by both these groups favored the chromate form of chromium(VI), as opposed to dichromate. Under our conditions, ^{17}O NMR showed that ~15% of the initial chromium(VI) exists as chromate/hydrogen chromate. Therefore, we can tentatively assign the signal at g = 1.987 to be from a chromium(V)-glutathione complex, formed from the reaction of chromate and glutathione. This result suggests that chromium(VI) may undergo a one-electron reduction in the absence of excess thiol. It has been suggested that the structure of the g = 1.987 complex is most likely a square-pyramidal $[Cr{=}O]^{3+}$ species with two molecules of thiol bound in a bidentate fashion, through the cysteinyl sulfhydryl and amide groups of glutathione.[42,43]

Chromium(V) complexes formed from ethanethiol and propanethiol have g values of 1.974, which are quite close to the EPR signal observed at g = 1.973.[44] These complexes were proposed

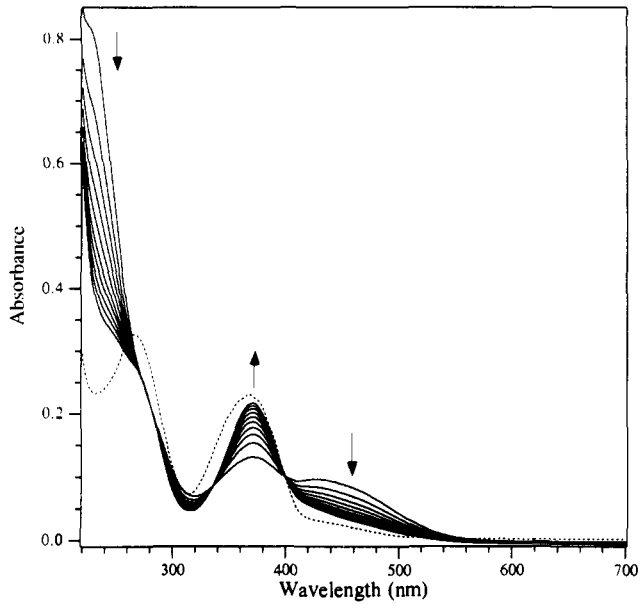

Figure 5. A time course of the reaction of GSH and chromium(VI) as monitored by UV-visible absorption spectroscopy. Conditions for the reaction mixture of potassium dichromate and glutathione are described in Figure 1. Path length of the cells used was 0.010 mm, so the average of four runs is presented. The time course follows the reaction for 1-67 min after mixing, each cycle took 6 min, and the pH* varied from 5.2 to 5.9 over this time period. The dashed line is 0.080 M chromium(VI) at pH* = 5.8, I = 1.5 M.

to possess a distorted tetrahedral structure with monodentate binding through the thiol. However, both species were only detectable at liquid nitrogen temperatures; at room temperature the signal was too broad to be observed. Therefore, we propose a different structure for the g = 1.973 species observed in the reaction of chromium(VI) with glutathione. The proposed paramagnetic complex is a square-pyramidal chromium(V), with a Cr=O unit, three coordinated oxygens from oxo, aquo, carboxylate, or carbonyl ligands, and a thiolate group from a bound glutathione molecule. This species would have a g value similar to what has been proposed for the ethanethiol and propanethiol complexes yet should also possess a narrow line width at 4 °C.

UV-Visible Absorption Spectroscopy. UV-visible spectral studies showed that the reaction of glutathione with dichromate resulted in an absorption band at 373 nm which was assigned to the $GSCrO_3^-$ thioester (Figure 5). Chromium(VI) at pH* = 5.8 has an absorption band at 370 nm. This shift to higher wavelength upon ligation of glutathione is consistent with sulfur displacing an oxygen bound to the chromium(VI) metal center. In addition a shoulder from 400-500 nm and a band in the UV region were observed. Plots of wavelength vs time at 425 and 240 nm showed a steady decrease of absorbance with time. After 65 min most of the species absorbing in these regions have disappeared. However, the amount of chromium(VI)-glutathione thioester over this time period of the reaction showed only ~20% decay as monitored by ^1H NMR integration. Also, the integration of the EPR signals at g = 1.987 and g = 1.973 from the chromium(V)-glutathione complex showed minimal change for over 90 min. Integration of the ^{17}O spectra showed little change in total Cr(VI) concentration and minimal changes in the concentration of the $GSCrO_3^-$ thioester over a 60-min time period. Therefore, the shoulder from 400-500 nm and the peak in the UV region at 240 nm do not correlate with either the chromium(VI)- or the chromium(V)-glutathione complexes which have been observed by NMR and EPR spectroscopy.

The UV-visible spectrum of chromate/hydrogen chromate does not show a significant absorbance in the 400-500-nm region (Figure 5). Therefore, release of chromate/hydrogen chromate upon thioester formation, cannot account for the size of the observed absorbance at 400-500 nm. It is possible that the 400-500-nm band and the band at 240 nm correspond to a transient

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chromium(IV) or EPR silent chromium(V) species, with a significantly larger extinction coefficient than the chromium(VI) glutathione thioester; however, we have no other spectroscopic evidence for such an intermediate. McAuley and Olatunji have tentatively assigned a peak at ~420–430 nm with an extinction coefficient of $\sim 1.25 \times 10^3$ M^{-1} cm^{-1} to the chromium(VI) thioester formed from glutathione and hydrogen chromate.[45] They determined the following equilibrium:

$$GSH + [(HO)CrO_3]^- \overset{K_1}{\rightleftharpoons} [(GS)CrO_3]^- + H_2O \quad (5)$$

$$K_1 = 1440\ M^{-1} \text{ at } T = 25\ ^\circ C$$

Under their conditions of large excess ligand-to-metal ratios, the absorbance from hydrogen chromate at 350 nm was no longer observable, once the putative thioester had formed. However, the ^{17}O NMR results clearly indicate that under our conditions unreacted chromium(VI) remains in the reaction mixture. Connett and Wetterhahn[8] have also reported a chromium(VI) thioester formed from the reaction of chromate and glutathione at pH = 7.4. The UV-visible absorption spectrum observed is quite similar to that obtained under the conditions reported herein. However, the NMR data obtained at pH* = 8.0 show no $GSCrO_3^-$ complex but rather different glutathione–chromium complexes (data not shown).

Summary

On the basis of extensive NMR studies, we conclude that the most likely structure for the product of the reaction of glutathione and dichromate is a monodentate thiolate chromium(VI)–glutathione complex, $GSCrO_3^-$, which has been proposed previously.[6,8,10,13] The formation of this thiolate complex is unexpected, as glutathione possesses a number of other possible chromium(VI) binding sites. Chromium(VI) is considered a "hard" acid and sulfur a "soft" base, and therefore the "expected" preferred site of chromium(VI) binding to glutathione would be a carboxylate or possibly the amine group.[14] However, the reactivity of the thiolate of glutathione with chromium(VI) seems to outweigh these potential thermodynamic considerations of metal–ligand stability. Most of the previous metal–thiolate complexes of glutathione have been low-valent metal species.[9] We have presented evidence for a high-valent metal–thiolate complex formed from the reaction of dichromate with glutathione, i.e., $GSCrO_3^-$. Several of the low-valent metal–thiolate complexes are not sensitive to the state of protonation of the thiol ($pK_a = 8.93$).[9] In contrast, we have seen little evidence of reaction between chromium(VI) and glutathione over pH 9.[46] This is in agreement with Connett and Wetterhahn's hypothesis that the proton on the thiol plays an important role in the formation of the $GSCrO_3^-$ complex.[8]

The initial species of chromium(VI) appears to be important in thioester formation. Chromate/hydrogen chromate will form chromium(V) complexes with glutathione quite readily, as shown by the wealth of EPR data; however, we have no evidence for the formation of $GSCrO_3^-$ thioester with chromate as the initial species of chromium(VI). We hypothesize that the lability of chromate/hydrogen chromate as a leaving group from dichromate assists in the formation of the $GSCrO_3^-$ thioester. In comparison, the leaving group from chromate or hydrogen chromate would be oxide/hydroxide or hydroxide/water, and therefore redox processes appear to be favored over ligand substitution reactions of chromate/hydrogen chromate with glutathione. Further studies are being undertaken to identify the species formed at higher ratios of glutathione to chromium(VI).

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Preparations, Structures, and Properties of M_3X_{13} Type Molybdenum and Tungsten Trimers with Eight Cluster Electrons

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Abstract: The reactions of $MoCl_3 \cdot 3H_2O$ and $(Et_4N)_3W_2Cl_9$ with excess amounts of a mixture of acetic anhydride and acetic acid or 1 M hydrochloric acid solution of acetic acid, followed by suitable workup procedures, result in the isolation of complexes whose common feature is the presence of a $[M_3OCl_6(OAc)_3]^-$ unit. This unit consists of an equilateral triangle of tungsten and molybdenum atoms with one capping oxygen atom above the triangle plane. Each M–M edge is bridged by an acetate group (above the plane) and a chloride ligand (below the plane). Each metal atom is further linked with a terminal chloride atom so that a distorted octahedral coordination environment is completed. The average oxidation state of the metal atoms for these two complexes is 3.33, and eight d electrons are delocalized over the M_3 cluster. The average metal–metal bond distances for these two complexes are 2.570 (2) and 2.567 (1) Å, respectively. Additional cations are present as necessary for electroneutrality. Two such complexes have been structurally characterized by X-ray diffraction with the following crystallographic data: $(Me_4N)[Mo_3(\mu_3\text{-}O)(\mu\text{-}Cl)_3(\mu\text{-}OAc)_3Cl_3] \cdot 2HOAc$ (**1**), orthorhombic *Pnma*, $a = 7.684$ (2) Å, $b = 13.856$ (3) Å, $c = 28.495$ (4) Å, $V = 3034$ (1) Å^3, $Z = 4$, $R = 0.034$, $R_w = 0.041$; $(Et_4N)[W_3(\mu_3\text{-}O)(\mu\text{-}Cl)_3(\mu\text{-}OAc)_3Cl_3] \cdot Me_2CO$ (**2**), monoclinic, $P2_1/c$, $a = 13.996$ (2) Å, $b = 15.999$ (4) Å, $c = 14.031$ (3) Å, $\beta = 98.01$ (1)°, $V = 3110$ (1) Å^3, $Z = 4$, $R = 0.034$, $R_w = 0.047$.

Introduction

The chemistry of triangular M–M bonded cluster compounds of molybdenum and tungsten has been extensively studied in recent years.[1] One of the structural types (though there are others[2-5]) is often designated the M_3X_{13} type. It consists of three MX_6 octahedra fused together so that each octahedron shares one X

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