Thermodynamic Considerations in Co-ordination. Part XXIV.[1] Gibbs Free-energy Changes, Enthalpies, and Entropies of Formation of Complexes of Glycinate, Glycylglycinate, Glycylglycylglycinate, Cysteinate, and Glutathionate with Hydrogen and Lead(II) Ions and Suggested Aqueous Structures

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Values of $\Delta G^{\ominus}$, $\Delta H^{\ominus}$, and $\Delta S^{\ominus}$ for the title systems are reported for aqueous solutions at 25 °C and I = 3.00 mol dm^{-3} $Na[ClO_4]$. The results are used to suggest structures for the various complexes present in solution.

CURRENTLY the medical profession is intensely interested in lead pollution (for example, the 1974 volume of *The Lancet* contained eight articles concerning lead). The design of improved drugs for removing lead from plasma is germane to this interest. A previous publication [2] suggested that glutathionate [γ-L-glutamyl-L-cysteinylglycinate, Glu(Cys-GlyO)] is more selective for Cd^{II} and Pb^{II} than drugs currently in the pharmacopoeia. This suggestion has now been manifested in pharmacological trials using animals loaded with heavy metal ions. However, before a new pharmaceutical is marketed it is prudent to know as much as possible about the molecular chemistry of its mode of action. This involves knowledge of the products of ligand metabolism, the selectivity of the ligand for the pollutant cation with respect to the essential metal ions *in vivo*, the major species present at physiological pH values, the extent to which the ligand and its complexes partition into a cell membrane, and the structures of the complexes formed. In principle, this information ought to be determined *in vivo*. In practice, the complicated chemical matrix of plasma renders this impracticable and so there is recourse to *in vitro* studies in aqueous solution (as contrasted with structures determined in the solid state where crystal lattice forces may dictate an isomer not normally present *in vivo*).

The metabolic products of glutathione, Glu(Cys-Gly), are already known to be simple, easily assimilated, endogenous amino-acids. The selectivity of Pb^{II} compared to Zn^{II}, and the major species present in plasma, are shown in Table 5 and Figure 2 of Part XXII [2] of this series. Currently, we are investigating (*i*) the partition coefficients of complexes of Glu(Cys-GlyO) into an organic solvent, and (*ii*) the influence of the chloride ligand present in plasma. This paper reports our opinions concerning the structures of lead complexes, as derived from enthalpies and entropies of formation. Since Glu(Cys-Gly) is a peptide of L-glutamic acid, L-cysteine, and glycine, certain parent ligands [glycinate (GlyO), glycylglycinate (Gly-GlyO), glycylglycylglycinate (Gly-Gly-GlyO), and cysteinate (CysO)] were also studied with protons and Pb^{II} as precursor data to our discussions of lead(II) complexes of Glu(Cys-GlyO).

EXPERIMENTAL

Materials.—The following compounds were used: glycine, Gly (Fisons, AnalaR) (Found: C, 32.1; H, 6.50; N, 18.5. Calc. for $C_2H_5NO_2$: C, 32.0; H, 6.70; N, 18.6%); glycylglycine, Gly-Gly (Koch–Light) (Found: C, 36.3; H, 6.40; N, 21.1. Calc. for $C_4H_8N_2O_3$: C, 36.4; H, 6.10; N, 21.2%); glycylglycylglycine, Gly-Gly-Gly (Koch–Light) (Found: C, 38.0; H, 6.10; N, 22.1. Calc. for $C_6H_{11}N_3O_4$: C, 38.1; H, 5.85; N, 22.3%); cysteine, Cys (E. Merck, A.G.)

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[2] A. M. Corrie, M. D. Walker, and D. R. Williams, *J.C.S. Dalton*, 1976, 1012.

(Found: C, 29.75; H, 5.90; N, 11.4. Calc. for $C_3H_7NO_2S$: C, 29.75; H, 5.80; N, 11.55%); glutathione, Glu(Cys-Gly) (Sigma) (Found: C, 38.8; H, 5.75; N, 13.5. Calc. for $C_{10}H_{17}N_3O_6S$: C, 39.1; H, 5.60; N, 13.7%).

The preparation and standardisation of perchloric acid and sodium perchlorate were as described in ref. 3. Lead(II) perchlorate solution was prepared and analysed as in ref. 4 and water purified as in ref. 5.

Methods.—The potentiometric approach was as described in ref. 6, studies being carried out at 10, 25, and 40 °C, $I = 3.00$ mol dm^{-3} Na[ClO_4]. Concentration formation constants were refined from the titration data using the SCOGS [7] and MINIQUAD [8] computer programs.

The calorimetry was as described in ref. 3. For all ligands studied, protonation constants at 25 °C have already been reported.[2,9,10] Heats of protonation of the ligands were obtained calorimetrically, the values for protonation of CysO having previously been measured under the same experimental conditions in this laboratory.[10] However, for the lead(II) complexing reactions, calorimetry could not be used for the following reasons. In the case of GlyO and its peptides the maximum $\bar{Z}$ attainable before precipitation was *ca.* 1.0 and, even below this, the heat contribution from lead hydroxo-species [11,12] was considerable, thus making the net heat output low and the heats of the complex-formation reactions insignificant compared to the corrections which had to be applied. For Pb^{II}–CysO and Pb^{II}–Glu(Cys-GlyO) systems, only very low concentrations of metal (*ca.* 1×10^{-3} mol dm^{-3}) could be used because of precipitation of insoluble complexes, and so once again sufficient heat output could not be realised.

Thus, for the lead(II) complex-formation reactions, potentiometric studies were made at 10, 25, and 40 °C and enthalpies of reaction calculated from the temperature variation of the formation constants. This method is much less satisfactory than direct calorimetry because of its inherent, and often unjustified, assumption that $\Delta H^\ominus$ does not vary within the temperature range studied, and also because formation constants do not change greatly over this temperature range and so have to be measured with great accuracy in order to give an accurate value for $\Delta H^\ominus$.

RESULTS AND DISCUSSION

Ligand Protonations.—The enthalpies for ligand protonation are presented in Table 1 together with the Gibbs free energies and the entropies calculated using the formation constants previously published.[2,9,10] Our work generally gave more negative enthalpies than that of other workers as one would expect in our more concentrated ionic background. These calorimetrically determined enthalpies were then used in the calculation of the thermodynamic data for the lead(II)–ligand complexing reactions (Table 2).

In the case of Pb^{II}–CysO and Pb^{II}–Glu(Cys-GlyO) interactions, the species 210 * and 21–1 are also present but they are minor species occurring only in low concentrations at high pH and so their formation constants cannot be found with sufficient accuracy to allow enthalpy determinations. For GlyO, Gly-GlyO, and Gly-Gly-GlyO, $\Delta H_1^\ominus$ is assigned to the protonation of the amino-group, which is seen to be an enthalpy-dependent process, and $\Delta H_{1,2}^\ominus$ to the protonation of the carboxylate group which is essentially an electrostatic phenomenon and is entropy dependent. However, for CysO and Glu(Cys-GlyO), only composite values of the thermodynamic functions are given since some concurrent ionisation of the mercapto- and amino-groups, and of the two carboxylate groups in Glu(Cys-GlyO), will be occurring.

Lead(II)–Ligand Interactions.—Glycine and its peptides. Evans and Monk,[13] from potentiometric studies, suggested that the binding of Pb^{II} to glycine peptides was through the amino-group and either the nitrogen or oxygen atom of the first peptide link. Rabenstein and Libich [14] from n.m.r. studies, and Nag and Banerjee [15] from polarography, showed that at low pH the metal is bound to the carboxylate end of the ligand with the amino-end still protonated, and at high pH the amino-end and the peptide oxygen are bound. Rabenstein and Libich consider that at high pH the carboxylate group is probably also bound to the metal and that polynuclear complex formation may be occurring. Their work also shows that very little ionisation of the peptide hydrogen can be present.

From our work we see that $\Delta H_{110}^\ominus$ is very similar for GlyO and its peptides, the differences in formation constants being entropy determined. This indicates that the binding is the same in all three cases and so the peptides are probably bound by their amino- and peptide oxygen groups. The large positive entropy change in the case of lead(II)–glycinate complex formation reflects the more effective charge neutralisation on bonding of the carboxylate group, the smaller values for the peptides suggesting that their carboxylate groups are not bound.

There are two bonding possibilities for the 111 species found at lower pH. (*i*) Binding of GlyO to Pb^{II} by the carboxylate group with the amino-group protonated. (For the protonation of the GlyO amino-group we found $\Delta H^\ominus = -51.2$ kJ mol^{-1} and for lead(II)–acetate complex formation $\Delta H^\ominus = -0.25$ kJ mol^{-1}.[16] Thus for the above type of binding we would expect $\Delta H_{111}^\ominus \approx -51.4$ kJ mol^{-1}.) (*ii*) Binding of GlyO to Pb^{II} by the amino-group with the carboxylate group protonated.

* Defined in the footnote to Table 2.

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TABLE 1

Gibbs free-energy changes, enthalpies, and entropies for the protonation of GlyO, Gly-GlyO, Gly-Gly-GlyO, CysO, and Glu(Cys-GlyO) at 25 °C and I = 3.00 mol dm^{-3} Na[ClO$_4$]. $\Delta G^\ominus$ and $\Delta H^\ominus$ are in units of kJ mol^{-1}, $\Delta S^\ominus$ are in J K^{-1} mol^{-1}. n Denotes the number of calorimetric measurements

		n	Literature data (θ_c/°C, I/mol dm^{-3}, values)			Ref.
(*a*) GlyO						
$-\Delta G_1^\ominus$	57.49 ± 0.05		30	0.09	ΔH_1 −42.7, ΔS_1 39.8	*a*
$-\Delta G_{1,2}^\ominus$	15.31 ± 0.11		25	0	ΔH_1 −44.22, ΔS_1 38.9	*b*
$-\Delta H_1^\ominus$	51.2 ± 1.3	40	25	0	ΔH_1 −44.4, ΔS_1 37.7; ΔH_2 −5.9, ΔS_2 25.1	*c*
$-\Delta H_{1,2}^\ominus$	9.0 ± 0.4					
$\Delta S_1^\ominus$	20.9 ± 3.1		25	0	ΔH_1 −41.8, ΔS_1 46.9; ΔH_1 −45.0, ΔS_1 36.0	*d*
$\Delta S_{1,2}^\ominus$	21.1 ± 1.7					
			25	0.1	ΔH_1 −42.7, ΔS_1 39.8; ΔH_2 −4.6, ΔS_2 30.1	*e*
			25	0	ΔH_1 −44.5, ΔS_1 37.3; ΔH_2 12.1	*f*
			25	0	ΔH_1 −44.2, ΔS_1 39.2	*g*
			25	0	ΔH_2 −4.1, ΔS_2 31.4	*h*
					ΔH_2 −4.9, ΔS_2 28.9	*i*
			25	0.2	ΔH_1 −46.5; ΔH_2 −4.4	*j*
			25	0.1	ΔH_1 −46.7, ΔS_1 28.3	*k*
			20		ΔH_1 −41.9	*l*
(*b*) Gly-GlyO						
$-\Delta G_1^\ominus$	48.88 ± 0.04		30	0.09	ΔH_1 −50.2, ΔS_1 12.6	*a*
$-\Delta G_{1,2}^\ominus$	20.04 ± 0.08		25	0	ΔH_2 −0.13, ΔS_2 59.7	*m*
$-\Delta H_1^\ominus$	48.7 ± 1.6	37	25	0.06	ΔH_1 −44.0, ΔS_1 9.2; ΔH_2 −1.09, ΔS_2 57.8	*n*
$-\Delta H_{1,2}^\ominus$	5.6 ± 0.3					
$\Delta S_1^\ominus$	0.8 ± 5.3		25	0.1	ΔH_1 −44.4, ΔS_1 6.3; ΔH_2 −0.13, ΔS_2 58.2	*o*
$\Delta S_{1,2}^\ominus$	48.3 ± 1.3					
			25	0	ΔH_1 −43.4, ΔS_1 12.6	*p*
(*c*) Gly-Gly-GlyO						
$-\Delta G_1^\ominus$	49.10 ± 0.05		30	0.09	ΔH_1 −23.0, ΔS_1 71.2	*a*
$-\Delta G_{1,2}^\ominus$	20.74 ± 0.17		25	0.1	ΔH_1 −42.3, ΔS_1 8.8; ΔH_2 −0.8 ΔS_2 58.2	*o*
$-\Delta H_1^\ominus$	49.5 ± 1.4	34				
$-\Delta H_{1,2}^\ominus$	4.0 ± 0.2					
$\Delta S_1^\ominus$	−1.3 ± 4.7					
$\Delta S_{1,2}^\ominus$	56.2 ± 0.6					
(*d*) CysO [10]						
$-\Delta G_1^\ominus$	61.13 ± 0.18					
$-\Delta G_{1,2}^\ominus$	50.12 ± 0.23					
$-\Delta G_{2,3}^\ominus$	13.7 ± 1.7					
$-\Delta H_1^\ominus$	40.4 ± 1.0					
$-\Delta H_{1,2}^\ominus$	38.8 ± 1.5	31				
$-\Delta H_{2,3}^\ominus$	−1.4 ± 1.5					
$\Delta S_1^\ominus$	69.5 ± 3.9					
$\Delta S_{1,2}^\ominus$	38.0 ± 5.8					
$\Delta S_{2,3}^\ominus$	50.6 ± 10.7					
(*e*) Glu(Cys-GlyO)						
$-\Delta G_1^\ominus$	56.41 ± 0.11		25	0	ΔH_1 −34.87, ΔS_1 77.44	*q*
$-\Delta G_{1,2}^\ominus$	52.31 ± 0.20				ΔH_2 −31.65, ΔS_2 66.56	
$-\Delta G_{2,3}^\ominus$	21.80 ± 0.33				ΔH_3 −0.71, ΔS_3 69.07	
$-\Delta G_{3,4}^\ominus$	14.82 ± 0.43				ΔH_4 −1.88, ΔS_4 34.33	
$-\Delta H_1^\ominus$	37.1 ± 1.0	36				
$-\Delta H_{1,2}^\ominus$	35.1 ± 1.4					
$-\Delta H_{2,3}^\ominus$	1.8 ± 1.9					
$-\Delta H_{3,4}^\ominus$	4.6 ± 3.4					
$\Delta S_1^\ominus$	64.6 ± 3.7					
$\Delta S_{1,2}^\ominus$	57.9 ± 5.7					
$\Delta S_{2,3}^\ominus$	67.1 ± 7.4					
$\Delta S_{3,4}^\ominus$	49.7 ± 12.7					

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(For the protonation of the GlyO carboxylate group we found $\Delta H^{\ominus} = -9.0$ kJ mol^{-1}. For the binding of Cd^{II} to ammonia or to ethylamine $\Delta H^{\ominus} \approx -14.8$ kJ mol^{-1},[17,18] and we would expect the corresponding value for Pb^{II} to be similar. Thus for the above type of bonding we would expect $\Delta H_{111}^{\ominus} \approx -23.8$ kJ mol^{-1}.) The value found experimentally for $\Delta H_{111}^{\ominus}$ is -25.1 kJ mol^{-1}, and so it would appear that the second type of binding is occurring contrary to the view of Rabenstein and Libich.[14] In the species 111 in the case of Gly-Gly-GlyO it would appear that it is the free carboxylate group that is being protonated, but for Gly-Gly-GlyO $\Delta H_{111}^{\ominus}$ is more negative than we would expect. The bonding is not merely through the oxygen and nitrogen atoms as in the GlyO case but that the sulphur atom is also involved. The large positive value of $\Delta S^{\ominus}$ indicates that effective charge neutralisation has occurred and so both the mercapto- and the carboxylato-groups are bound to the metal. The tridentate nature of CysO towards Pb^{II} is also shown by its reluctance to form a 210 species whereas Zn^{II} forms such a species in preference to the 110 species.[2] Tridentate binding of CysO to Pb^{II} but not to Zn^{II} has also been proposed by other workers.[19-23]

For the reaction 110 ⟶ 111 we find $\Delta H^{\ominus} = -14.5$ kJ mol^{-1} and there are three bonding possibilities.

TABLE 2

Log formation constants (log β_{pqr}) * at 10, 25, and 40 °C and $\Delta G^{\ominus}$, $\Delta H^{\ominus}$, and $\Delta S^{\ominus}$ for the lead complexes at 25 °C and $I = 3.00$ mol dm^{-3} Na[ClO$_4$]. $\Delta G^{\ominus}$ and $\Delta H^{\ominus}$ are in units of kJ mol^{-1}, $\Delta S^{\ominus}$ are in J K^{-1} mol^{-1}; n denotes the number of experimental observations

				log β_{pqr}								
	p	q	r	10 °C	(n)	25 °C	(n)	40 °C	(n)	$\Delta G^{\ominus}$	$\Delta H^{\ominus}$	$\Delta S^{\ominus}$
GlyO	1	1	0	5.893 ± 0.034	(165)	5.752 ± 0.045	(131)	5.675 ± 0.027	(179)	−32.84 ± 0.25	−12.4 ± 3	68.6 ± 11
	1	1	1	12.212 ± 0.079		11.880 ± 0.108		11.772 ± 0.047		−67.82 ± 0.61	−25.1 ± 4	143.2 ± 15
	1	1	−1	−2.071 ± 0.038		−1.886 ± 0.050		−1.781 ± 0.026		10.76 ± 0.29	16.5 ± 3	19.2 ± 11
Gly-	1	1	0	4.005 ± 0.034	(139)	3.824 ± 0.036	(99)	3.782 ± 0.043	(156)	−21.83 ± 0.20	−12.7 ± 5	30.5 ± 17
GlyO	1	1	1	10.405 ± 0.059		10.007 ± 0.063		9.739 ± 0.077		−57.13 ± 0.35	−37.8 ± 2	34.3 ± 8
Gly-	1	1	0	4.143 ± 0.038	(96)	3.967 ± 0.022	(103)	3.879 ± 0.020	(97)	−22.65 ± 0.12	−15.0 ± 1	25.5 ± 4
Gly-	1	1	1	10.840 ± 0.046		10.618 ± 0.021		10.352 ± 0.020		−60.62 ± 0.12	−27.5 ± 2	111.0 ± 7
GlyO	1	1	−1	−3.524 ± 0.061		−3.401 ± 0.024		−3.132 ± 0.017		19.43 ± 0.13	22.0 ± 4	8.7 ± 14
CysO	1	1	0	13.579 ± 0.027	(96)	13.207 ± 0.041	(147)	12.828 ± 0.019	(179)	−75.40 ± 0.23	−42.4 ± 2	110.6 ± 7
	1	1	1	17.974 ± 0.069		17.434 ± 0.112		16.968 ± 0.047		−99.53 ± 0.64	−56.9 ± 3	142.9 ± 12
	2	1	1	28.417 ± 0.065		27.301 ± 0.122		26.445 ± 0.058		−155.86 ± 0.69	−111.8 ± 4	147.8 ± 16
Glu	1	1	0	10.769 ± 0.032	(147)	9.913 ± 0.035	(128)	9.583 ± 0.028	(113)	−56.60 ± 0.20	−67.6 ± 5	−36.8 ± 17
(Cys-	1	1	1	17.618 ± 0.015		16.821 ± 0.017		16.167 ± 0.013		−96.03 ± 0.10	−82.2 ± 2	46.4 ± 7
GlyO)	2	1	1	24.453 ± 0.023		23.400 ± 0.025		22.665 ± 0.026		−133.59 ± 0.14	−101.4 ± 3	107.9 ± 10
	2	1	2	33.765 ± 0.020		32.313 ± 0.022		31.234 ± 0.019		−184.48 ± 0.13	−143.5 ± 2	137.6 ± 7

* The formation constant for the general complex $A_pB_qH_r$ is β_{pqr} (A = ligand, B = metal, and H = proton).

Pb^{II}–Gly-GlyO system is also unusual in that a 11–1 species is not detected and a similar situation occurs in the case of Zn^{II}–Gly-GlyO.[9] The reason for these differences is not obvious.

In the Pb^{II}–GlyO 110 complex there are no ionisable protons other than those of co-ordinated water molecules which may ionise to give a Pb–OH bond. For the formation of this bond Carell and Olin [12] found $\Delta H^{\ominus}$ between 20.9 and 41.8 kJ mol^{-1} and $\Delta S^{\ominus}$ between −81.1 and −1.1 J K^{-1} mol^{-1}. These values can be compared with our values for the reaction 110 ⟶ 11–1 of $\Delta H^{\ominus} = 28.9$ kJ mol^{-1} and $\Delta S^{\ominus} = -49.4$ J K^{-1} mol^{-1} which certainly fall within this range. In the Pb^{II}–Gly-Gly-GlyO system there is the possibility that ionisation of the peptide proton is occurring with binding of the peptide nitrogen to the metal. However, our values of $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ for 110 ⟶ 11–1 still fall within the range for lead(II)–hydroxide bond formation.

Cysteine.—For the Pb^{II}–CysO 110 complex we find a high negative enthalpy (−42.4 kJ mol^{-1}) showing that (*i*) N and O bound to Pb^{II} with S protonated. For the binding of the GlyO N and O to Pb^{II} we have $\Delta H^{\ominus} = -12.4$ kJ mol^{-1} and for the protonation of the CysO mercapto-group we have $\Delta H^{\ominus} = -40.4$ kJ mol^{-1}. So for this type of bonding we expect $\Delta H_{111}^{\ominus} \approx -52.8$ kJ mol^{-1} and $\Delta H^{\ominus}$ for 110 ⟶ 111 to be *ca.* −10.4 kJ mol^{-1}. (*ii*) N and S bound to Pb^{II} with the carboxylate group protonated. For the reaction 110 ⟶ 111 for the GlyO case, where the carboxylate group is being protonated, we have $\Delta H^{\ominus} = -12.7$ kJ mol^{-1} and we would expect a similar value here. (*iii*) S and O bound to Pb^{II} with the amino-group protonated. We would expect $\Delta H^{\ominus}$ for the reaction 110 ⟶ 111 to be approximately equal to the enthalpy of protonation of the amino-group (−38.8 kJ mol^{-1}) minus the value for the binding of the amino-group to Pb^{II} (−14.8 kJ mol^{-1}) which gives *ca.* −24.0 kJ mol^{-1}. Thus, our experimental value is closest to that for carboxylate protonation but no definite assignment can be made.

For the 211 species, if we assume that one ligand is

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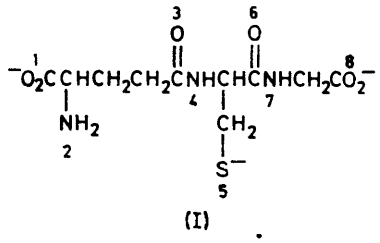
tridentate, this leaves $\Delta H^{\ominus} = -69.4$ kJ mol^{-1} to be accounted for. This amount is closest to the expected value for the second ligand being bound to the metal through S and O with the amine group protonated.

Glutathione.—In the ligand Glu(Cys-GlyO) there are eight sites through which bonding to the metal may occur [structure (I)]. Fuhr and Rabenstein [24] found no detectable binding of Pb^{II} to group 2, but only to 5 and



(I)

to 1 and 8 in some regions of pH. For example, they found 8 bound up to high pH where it is replaced by a hydroxo-group. Also they found no evidence for the lead(II)-promoted ionisation of the peptide proton.

Our results show that $\Delta H_{110}^{\ominus}$ is more negative than for Pb^{II}–CysO complex formation showing that more bonding is occurring. The negative value of $\Delta S_{110}^{\ominus}$ may be due to strain in the rings formed due to this multiple bonding. To obtain such a highly negative enthalpy of formation we believe, contrary to Fuhr and Rabenstein, that the amino-group 2 must be bound together with **1** and 5. To make up the remaining enthalpy, group 8 may be involved as they suggest or, alternatively, the peptide links. We have shown from our GlyO peptides that the oxygen of the peptide links rather than the nitrogen tends to bind to Pb^{II} and so groups 3 or 6 are more likely to be bound than 4 or 7. For the reaction 110 ⟶ 111 for CysO we have $\Delta H^{\ominus} = -14.5$ kJ mol^{-1} and for Glu(Cys-GlyO) we have $\Delta H^{\ominus} = -14.6$ kJ mol^{-1}, suggesting that the same process is occurring in each case, probably carboxylate protonation.

For the complex 211, $\Delta H^{\ominus} = -101.4$ kJ mol^{-1} whereas $\Delta H_{110}^{\ominus} + \Delta H_{111}^{\ominus} = -149.8$ kJ mol^{-1}. Thus, the bonding to the metal must be different for 211 with fewer groups being bound, presumably for steric reasons. If we assume that the first ligand is complexed as in the 111 species, then the binding of the second ligand has to account only for $\Delta H^{\ominus} = -19.2$ kJ mol^{-1} which is too low for the involvement of both the nitrogen and the sulphur atoms. For the reaction 211 ⟶ 212, $\Delta H^{\ominus} = -42.1$ kJ mol^{-1} which would correspond approximately to the protonation of a free nitrogen or sulphur atom on the second ligand.

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