Reactions of *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ with Glutathione

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Abstract

A study of the reaction between *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ and glutathione (GSH) was undertaken to confirm the identity of the products formed. In alkaline solution, the platinum products observed were mononuclear species, while in acidic solution, the oligomeric products were products obtained. The mass spectrometry of the reaction in alkaline solution showed a sulfur-bridged dinuclear platinum(II) species, *trans*-[{Pt(SG)$_2$(^{15}NH$_3$)$_2$}$_2$(SG)]$^+$ giving m/z 1380 and the lost of two ammines was observed.

Keywords: Thiolate, Oligomers, 2D NMR, Glutathione

1. Introduction

The chemistry of cisplatin interacting with thiol groups has been extensively studied (Appleton et al., 1989; Dedon and Borch, 1987; Odenheimer and. Wolf, 1982; El-Khateeb et al., 1999; Appleton et al., 2003; Hadi and Appleton, 2006) the corresponding reactions of the trans analogue on the other hand are relatively unexplored due to the lack of antitumour activity of *trans*-diammineplatinum(II) complexes (Berners-Price and Kuchel, 1990; Van Beusichem and Farrell, 1990; Hadi and Appleton, 2005, Oehlsen et al., 2003). The reaction of platinum(II) complexes with sulfur-containing ligands is of interest as it is believed that before the platinum(II) complexes reaching the DNA, they will bind first to the constituents of cells containing sulfur donor (Borch, and Pleasants, 1979; Borch et al., 1980; Bodener et al., 1986a,b; El-Khateeb et al., 1999; Gale et al., 1982; Hadi and Appleton, 2006; Johnson et al., 1985).

Lempers *et al.* (1988) and Lempers and Reedijk (1990) have shown that in the reaction of [PtCl(dien)]$^+$ with glutathione, a sulfur donor, the products obtained depended on the pH. At pH < 7, the favoured coordinating mode of the thiol group was to bridge two platinum centers, whereas at pH > 7 mononuclear species were preferred. At pH < 7, the thiol group is still protonated, so it is not readily available for coordination. A thiol already coordinated to the metal is still a good nucleophile, and therefore at lower pH a second metal will preferentially bind with this coordinated thiol, forming a bridged species. At high pH a greater percentage of the free thiol is deprotonated and the preferred binding mode is non-bridging.

It has been shown that when *cis*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ reacts with thiols at low pH, the preferred products involved sulfur bridges at the concentration of platinum complex ~0.1 M, and the thiolate tends to bridge more in acidic condition than in basic condition(Appleton et al., 1990). This observation is also useful in characterizing the products obtained from the reactions carried out in this study. In this paper, we reported the study of the reaction between *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ and glutathione (GSH).

2. Experiment

2.1 Starting Materials

trans-[Pt(^{15}NH$_3$)$_2$(NO$_3$)$_2$] was prepared based on the procedure as previously described (Appleton et al., 1992). Glutathione (GSH) was used as supplied by Sigma – Aldrich Chemical Company without further purification.

^{15}NH$_4$)$_2$SO$_4$ (99% ^{15}N, Cambridge Isotopes) was supplied by Novachem, Melbourne, Australia.

2.2 Preparation of trans-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ (1)

trans-[Pt(^{15}NH$_3$)$_2$(NO$_3$)$_2$] was converted to *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ in aqueous solution with the following procedure: A certain amount of *trans*-[Pt(^{15}NH$_3$)$_2$(NO$_3$)$_2$] (based on the concentration desired, normally either 1 mM or 5 mM) was weighed out, then it is dissolved by warming in 2 mL of water for about 30 minutes. 0.1 M nitric acid was added to adjust the pH. Any solid remaining was removed by gravity filtration to give a solution containing *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ (**1**) which was then checked with ^{15}N NMR.

2.3 Reaction of trans-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ (1) with GSH

The reaction was followed and monitored routinely with 2D [^{1}H, ^{15}N] HSQC NMR. The procedure used in this reaction is as follows: To a small bottle containing solid GSH (0.307 mg, 1 mmol) was added 0.5 mL 1 mM *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ (**1**) (0.5 mmol). The pH was adjusted to ~2.0 under argon gas. The solution was immediately transferred to a 5-mm NMR tube, then placed in the AV400 NMR spectrometer which has been tuned for ^{15}N NMR and accumulation 2D [^{1}H, ^{15}N] HSQC NMR spectrum was commenced. The spectrum was monitored periodically. 30 hours after the initial mixing, the pH was adjusted to > 7 and the spectrum was again monitored. The reaction was also carried out at the same way with the initial pH of the solution was > 7.

2.4 NMR Spectra

The 1D 40.54 MHz ^{15}N NMR spectra were recorded using DEPT pulse sequence (Berners-Price and Kuchel, 1990) to increase the sensitivity in a Bruker Avance 400 MHz spectrometer with a 5 mm broadband multinuclear probe. The number of scans used to obtain spectra was normally 250 - 500. A recycle time of 3.54 s was used with pulse width of 12.55 μs (tilt angle of 45 degrees). The number of data points used was 32 K. Chemical shifts are reported relative to 2.5 M (^{15}NH$_4$)$_2$SO$_4$ in 1 M H$_2$SO$_4$ (δ_N = 0.00) in coaxial capillary.

The 2D [^{1}H, ^{15}N] heteronuclear single-quantum coherence (HSQC) NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (^{1}H, 400.1 MHz; ^{15}N, 40.54 MHz) using the sequence of Stonehouse *et al.* (1994).

2.5 Electrospray Mass spectrometry

The following method was used to prepare sample for ES-MS: To a small bottle containing solid GSH (1.535 mg, 5 mmol) was added 0.5 mL 5 mM solution (**1**) (2.5 mmol) and pH was adjusted to ~2.0 under argon gas. The bottle was then sealed with parafilm to minimise the oxidation of GSH. The mole ratio of GSH and Platinum complex was 2 :1. The reaction mixture was left for 45 minutes to 1 hour then electrospray ionization mass spectrometry (ES-MS) was undertaken.

3. Results and Discussion

The reaction of (**1**) with GSH was followed by 2D [^{1}H, ^{15}N] NMR with the initial concentration of (**1**) was 1 mM. The mole ratio used in this reaction any condition was 1 : 2 platinum complex to GSH. As indicated by the 2D NMR spectra, the reaction between *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ and glutathione tends to mimic those of *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ with H$_3$accys (Hadi and Appleton, 2005). Based on the series of these NMR spectra (Figure 1 - 3), the reactions occurred both in acidic and alkaline solution are shown in Scheme 1

The reaction was carried out initially at higher pH (pH > 7). In this reaction, apart from a peak due to *trans*-[Pt(OH)$_2$(^{15}NH$_3$)$_2$] (**2**) (δ_N/δ_H –62.59/3.67 ppm), two new peaks were present in the 2D NMR spectrum (Figure 1), labelled as **A** (δ_N/δ_H –59.26/3.77 ppm) and **B** (δ_N/δ_H –63.44/3.47 ppm). Peak **B** was slightly more intense than **A**. No change occurred with time.

As thiolate bridging is not enhanced at higher pH (Appleton et al., 1989; Lempers et al., 1988; Lempers and Reedijk, (1990; Hadi and Appleton, 2006) these two peaks were assigned to *trans*-[Pt(OH)(SG)(^{15}NH$_3$)$_2$] (**3**) and *trans*-[Pt(SG)$_2$(^{15}NH$_3$)$_2$] (**4**) respectively. This can be seen from the two NMR spectra in Figure 1 and 3. In Figure 1, the reaction was undertaken at higher pH, as a result (**3**) was dominant in the mixture reaction while in Figure 3, the NMR spectrum was run from a sample at a moderate pH, so (**3**) is expected to be less in the mixture reaction. From the spectrum, it can also be seen that the ^{15}N chemical shift for (**3**) and (**4**) is similar, but in the ^{1}H NMR, there is a greater separation on their chemical shifts. These results are consistent with those reported by Oehlsen *et al.* (2003) who detected *trans*-[Pt(SG)$_2$(NH$_3$)$_2$] and [{*trans*-(GS)Pt(NH$_3$)$_2$}$_2$(SG)] in reactions between *trans*-[PtCl$_2$(^{15}NH$_3$)$_2$)] with glutathione. When the pH of the solution was lowered to ~2, the two peaks above disappeared, and new peaks (the strongest of which are labelled **C**, **D**, **E** and **F**) were present in the 2D NMR spectrum, a similar observation as in acidic condition as discussed in the next discussion.

Then the reaction between *trans*-[Pt(^{15}NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ (**1**) and GSH was carried out in acidic solution (pH ~2). 1 hour after mixing, apart from the peak from starting material (**1**) (δ_N/δ_H –62.39/4.08 ppm) four major peaks were present in the 2D NMR spectrum. They are labelled as **C** (δ_N/δ_H –55.55/4.18 ppm), **D** (δ_N/δ_H –55.93/4.13 ppm), **E** (δ_N/δ_H

–60.23/3.79 ppm) and **F** (δ_N/δ_H –59.54/3.74 ppm). This spectrum did not change significantly for a further 30 hours (Figure 2) and was similar to the spectrum described above, from the solution which was initially alkaline, then acidified. When the pH of this solution mixture was increased to about 7, all these peaks disappeared and two peaks appeared in the 2D NMR spectrum corresponding to those labeled **A** and **B** from reaction at higher pH (Figure 3).

The peaks observed from the reaction of (**1**) at lower pH are assigned to sulfur-bridged complexes. The formation of dinuclear, trinuclear, tetranuclear and pentanuclear platinum complexes would be possible in this reaction.

If the dinuclear platinum complex (**5**) is formed, in the 2D NMR, it would give rise to a single peak at the region near δ_N/δ_H –60/3.7 ppm, the region for terminal $Pt(NH_3)_2$. When the trinuclear complex (**6**) is formed, it would give rise to two peaks in the NMR spectrum, one for terminal $Pt(NH_3)_2$ and one for "internal" $Pt(NH_3)_2$ at the region δ_N/δ_H –55/4.1 ppm, with the intensity ratio of 2 : 1. If the tetranuclear species (**7**) is present in the mixture reaction, it would also give rise to two peaks, but in this complex the intensity of terminal $Pt(NH_3)_2$: "internal" $Pt(NH_3)_2$ would be in 1 : 1 ratio. For a pentanuclear (**8**) species, there would be one peak from terminal $Pt(NH_3)_2$, and two different peaks for internal $Pt(NH_3)_2$ with intensity ratios 2 : 2 : 1. The spectrum shown in Figure 2 showed poorly resolved peaks in the "terminal" region (labelled **E** and **F**) and four clearly resolved peaks in the "internal" region (most intense labelled **C** and **D**). The presence of four distinct "internal" peaks indicates that oligomers up to pentanuclear must be formed in the solution.

From the mass spectrometry data obtained at low pH, the strongest peaks were from complex (**5**) with the m/z 1380 (Figure 4). The separation between isotope lines is showed that charge was +1. The isotope pattern obtained corresponded to the pattern expected for 2 platinum atoms.

There were also two other peaks present in the spectrum with the difference of 36 amu between one peak to another. From this difference, these peaks are due to the sequential loss of two ammine ligands at a time ($^{15}NH_3$ = 18 amu).

However, when the reaction was carried out at higher pH (pH > 7), the mass spectrometry data did not show any peaks. This is probably due to the formation of platinum complexes with neutral or negative charge.

4. Conclusion

From this study, the results obtained clearly support the previous studies that in the reaction of both *cis-* and *trans*-diaquaplatinum(II) complexes with thiols, the tendency to bridge is more in acidic condition than in basic condition. The preferred products in such reaction always involve sulfur bridges when the reaction is carried out at low pH.
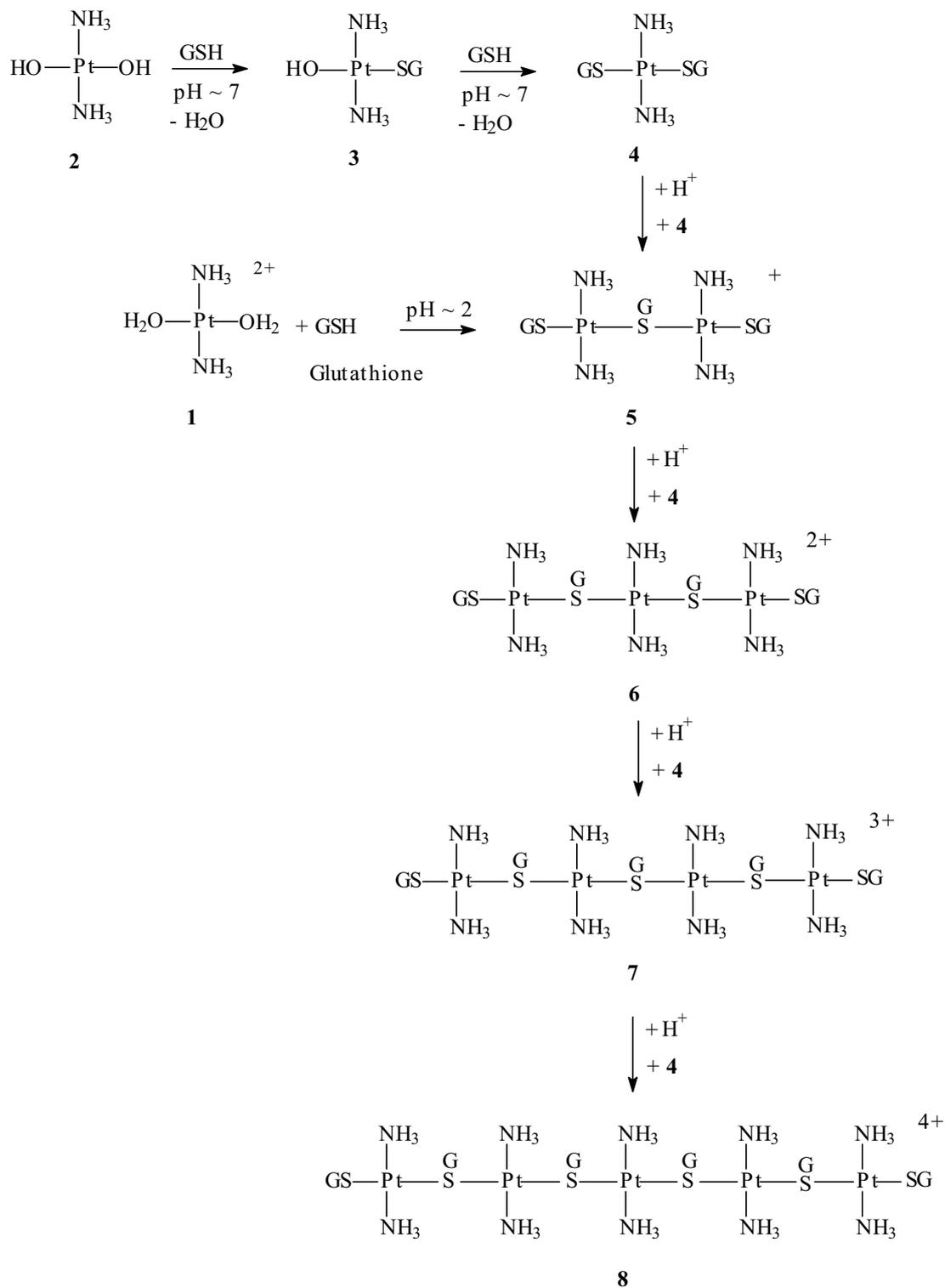
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$$
\underset{\textbf{2}}{\overset{\displaystyle \text{NH}_3}{\underset{\displaystyle \text{NH}_3}{\text{HO}-\text{Pt}-\text{OH}}}}
\xrightarrow[\substack{\text{pH} \sim 7 \\ -\text{H}_2\text{O}}]{\text{GSH}}
\underset{\textbf{3}}{\overset{\displaystyle \text{NH}_3}{\underset{\displaystyle \text{NH}_3}{\text{HO}-\text{Pt}-\text{SG}}}}
\xrightarrow[\substack{\text{pH} \sim 7 \\ -\text{H}_2\text{O}}]{\text{GSH}}
\underset{\textbf{4}}{\overset{\displaystyle \text{NH}_3}{\underset{\displaystyle \text{NH}_3}{\text{GS}-\text{Pt}-\text{SG}}}}
$$

4 $\xrightarrow[+\ \textbf{4}]{+\ \text{H}^+}$

$$
\underset{\textbf{1}}{\overset{\displaystyle \text{NH}_3}{\underset{\displaystyle \text{NH}_3}{\text{H}_2\text{O}-\text{Pt}-\text{OH}_2}}}^{2+} + \text{GSH (Glutathione)}
\xrightarrow{\text{pH} \sim 2}
\underset{\textbf{5}}{\left[\text{GS}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{SG}\right]^{+}}
$$

5 $\xrightarrow[+\ \textbf{4}]{+\ \text{H}^+}$

$$
\underset{\textbf{6}}{\left[\text{GS}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{SG}\right]^{2+}}
$$

6 $\xrightarrow[+\ \textbf{4}]{+\ \text{H}^+}$

$$
\underset{\textbf{7}}{\left[\text{GS}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{SG}\right]^{3+}}
$$

7 $\xrightarrow[+\ \textbf{4}]{+\ \text{H}^+}$

$$
\underset{\textbf{8}}{\left[\text{GS}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{S(G)}-\text{Pt(NH}_3)_2-\text{SG}\right]^{4+}}
$$

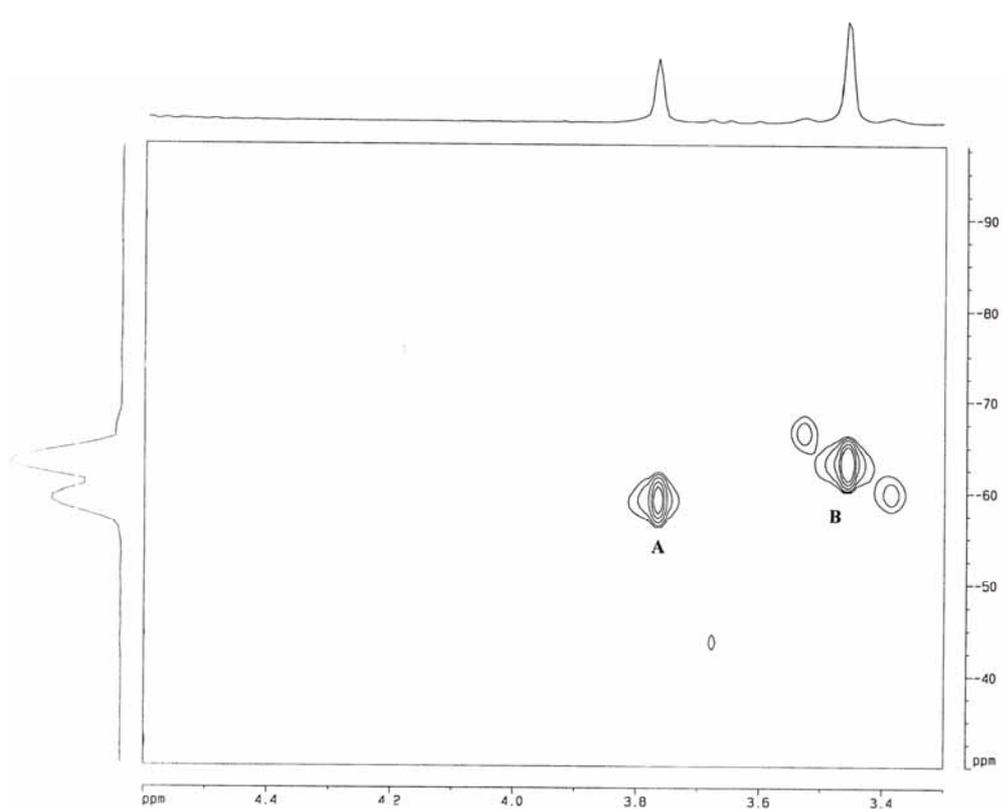Scheme 1. Reaction of (**1**) with GSH in alkaline and acidic conditions

Figure 1. 2D [^{1}H, ^{15}N] HSQC NMR spectrum of obtained from the reaction of 1 mM (**1**) and GSH in 1:2 mole ratio at the pH ~9, 1 hour after the reaction commenced
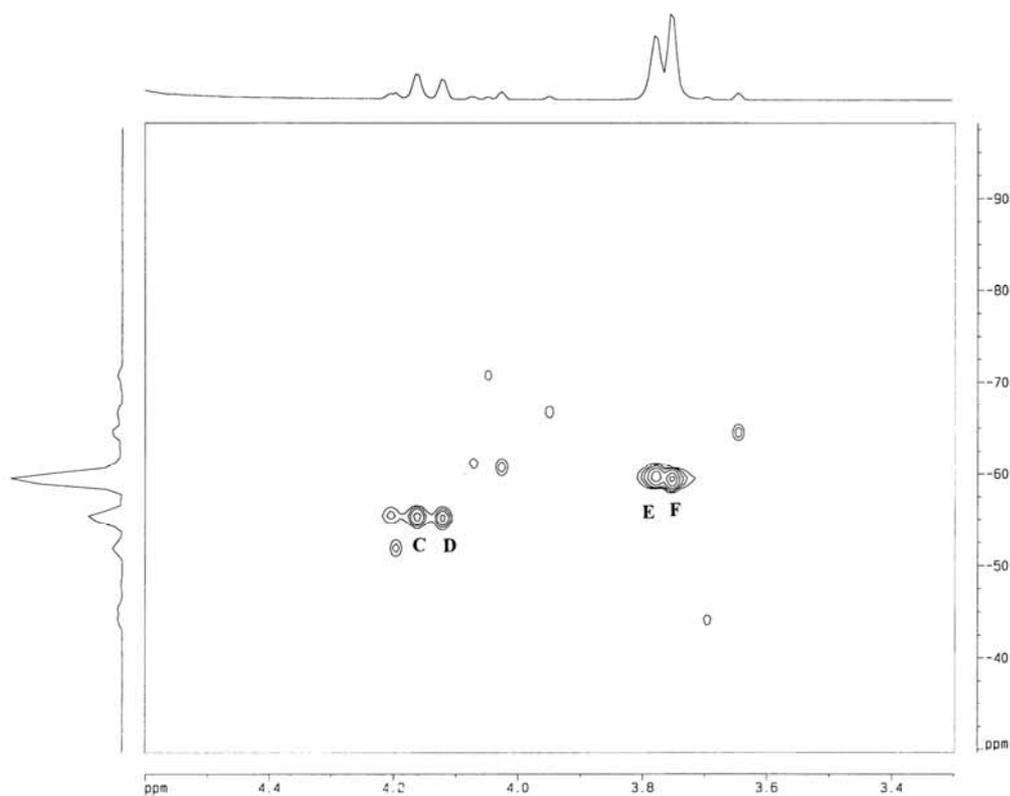

Figure 2. 2D [^{1}H, ^{15}N] HSQC NMR spectrum of obtained from the reaction of 1 mM (**1**) and GSH in 1:2 mole ratio at pH ~2, 30 hours after the reaction commenced
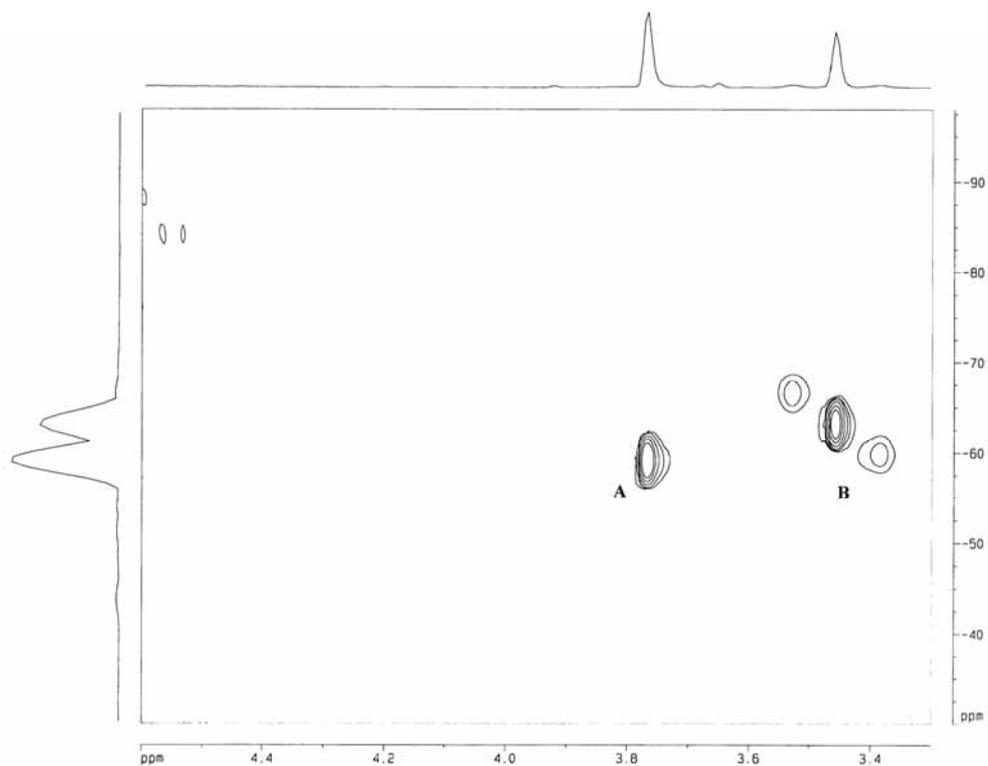
Figure 3. 2D [^{1}H, ^{15}N] HSQC NMR spectrum of obtained from the reaction of 1 mM (**1**) and GSH in 1:2 mole ratio at initially pH ~2, then the pH was adjusted to ~7, 32 hours after the reaction commenced
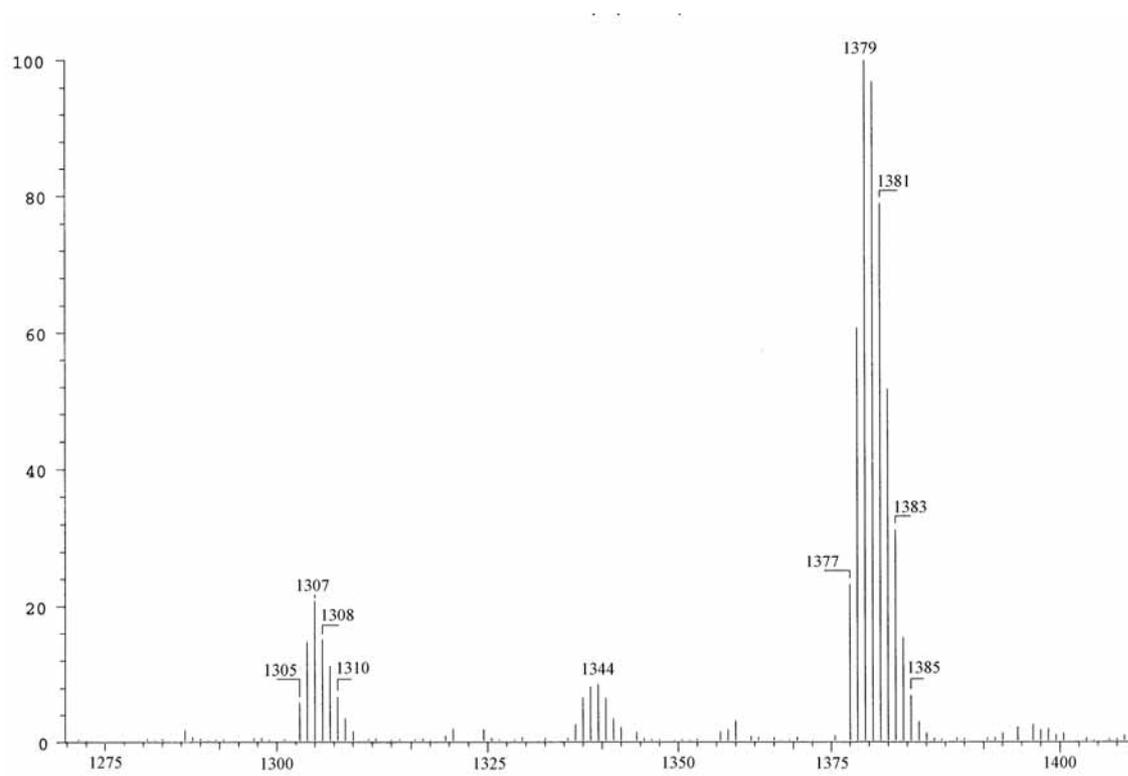

Figure 4. Electrospray mass spectrometry obtained from the reaction of 5 mM (**1**) and GSH in 1 : 2 mole ratio, taken 1 hour after mixing.