NMR solution structure study of one saturated sulphur-containing amides from *Glycosmis lucida*

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**ABSTRACT**

One sulphur-containing amide (N-[2-(4-Hydroxyphenyl)-ethyl]-3-methanesulfonyl-N-methyl-propionamide) which was isolated from *Glycosmis lucida* Wall ex Huang had a different NMR profile with this kind of compounds' normal case. Based on the information obtained by nuclear magnetic resonance pectroscopy (NMR) and mass spectrometry (MS), its configurations in solution were investigated. The results indicated that the compound would have two stable configurations in solution as the double bond switched between C–N and C–O in an appropriate rate. This phenomenon was clearly exposed by the one dimension selective NOE (1D-NOE) experiments. This conclusion would play an active role in the structure analysis work of this kind of compounds.

**1. Introduction**

*Glycosmis lucida* Wall ex Huang of the Rutaceae family is a shrub plant which is widely distributed in South Asia, south-east Asia and north-east of Australia. In China, these plants mainly located in the southern part of Yunnan province and the south-east part of Tibet (The flora of China 1997). Previous phytochemical studies indicated that *Glycosmis* species contain alkaloids, flavonoids, terpenes, sulfer-containing amide and so on (Govindachari et al. 1966; Greger et al. 1993; Hofer et al. 1995, 2000; Cuong et al. 2004; Ito et al. 2004; Rahmaniz et al. 2004;

**KEYWORDS**

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Wang et al. 2006; Lukaseder et al. 2009; Yang et al. 2015). Among them, sulphur-containing amides were only isolated from Rutaceae plants and have been recognised as the characteristic compounds of these plants (The flora of China 1997). All these compounds contained a substituent of methyl sulphonylpropenoic acid are assigned to two patterns (unsaturated one and saturated one) depending on whether there was a double bond in their skeleton structure. This kind of compounds had good biological activity, such as antifeedant activity, antileishmanial activity and antifungal activity. These attracted the attention of many researchers (Greger et al. 1992; Astelbauer et al. 2011; Yang et al. 2015; Guo et al. 2016).

Structural analysis was the first step for compound research. NMR technique was one of the important tools. However, NMR structural analysis of sulphur-containing amides was challengeable, since S, N and O co-existed in these molecules (Ouyang et al. 2006). Moreover, some of unusual appearances were observed in ¹H and ¹³C NMR spectra, and this made the assignment of NMR signals even more difficult. Based on NMR technology, such as several 1D and 2D experiments, structure and conformation of the compound was determined. For example, ¹H NMR experiments at different temperatures were useful to examine whether chemical exchange took place in solution. Because the exchange rate would change at various temperature. Also, 1D-NOE was a useful tool to solve the problems of compounds’ spatial configuration. It was based on transient NOE, the same as NOESY. In NOESY spectrum, cross-relaxation gives negative cross-peaks, whereas chemical exchange gives positive cross-peaks (Claridge 2010). So, 1D-NOE spectrum could provide clear information about Nuclear Overhauser Effect and chemical exchange.

My partner had separated a new saturated sulphur-containing amides from G. lucida and it was confirmed as N-[2-(4-Hydroxyphenyl)-ethyl]-3-methanesulfonyl-N-methyl-propionamide (see its structure in Figure S1) (Yang et al. 2015). But this sample’s NMR spectra showed some unusual appearances and it has not been studied. In ¹H NMR spectrum, although most of the signals could well agree to the reported data of suphur-containing amide (Greger et al. 1994; Hinterberger et al. 1998; Rahmani et al. 2010), some unusual appearances could be observed in NMR spectra. The feature and shapes of some resonant peaks were more or less different from those in normal case. In ¹³C NMR spectrum, most of the signals appeared in pair as shown in Figure S2. These indicated that the testing sample might contain two compounds with similar molecular structures or have two chemical conformers in solution. This phenomenon was found by Rahmani M and his partners when they investigated the structure of an unsaturated sulphur-containing amide by NMR (Rahmani et al. 2010). However, the explanation of cis–trans isomers coexistence could not face the saturated cases.

In this work, structure of this saturated sulphur-containing amides (labeled as Compound 1) in the solution was thoroughly studied through regular 1D and 2D NMR experiments, variable temperature experiments and 1D-NOE experiments. A reasonable interpretation would be given to these unusual appearances and its NMR signals assignment would be given correctly.

2. Results and discussion

2.1. Conformation of compound 1 in the solution

First, ¹H NMR experiments at different temperatures were used to figure out whether the sample had two compounds or it had two configurations in solution through chemical
exchange. Figure S3 was the $^1$H NMR spectra of variable temperature experiments. It is seen from the diagram that, as the temperature increases, the peaks had the trend of coming together. Take the peak at $\delta_H 9.0$ as an example. At 298 K, we could see two single peaks. With the increase in temperature, the peaks become broad and turn into a peak at 358 K. It indicated that chemical exchange took place in solution. The higher the temperature, the faster is the exchange rate.

On this basis, 1D-NOE experiment was chosen to further confirm how the chemical exchange took place in solution. It was found that four s-signals related to the protons of S–CH$_3$ and N–CH$_3$ could be clearly observed at $\delta_H 2.83$, $\delta_H 2.93$, $\delta_H 2.94$ and $\delta_H 2.99$ in $^1$H NMR spectrum (Figure S2). Among them, the two signals at $\delta_H 2.83$ and $\delta_H 2.99$ were separated with others and another two signals were overlapped. In 1D-NOE experiments, three resonant peaks at $\delta_H 2.83$, $\delta_H 2.93$ and $\delta_H 2.99$ were excited separately and a serial of 1D-NOE spectra were obtained (Figure S4). In these spectra, there was a batch of NOE signals with relatively lower intensities and contrary phase respective to the exciting signal, meanwhile, the signals with relatively higher intensities and same phase respective to the exciting signal could be observed as well. Based on the mechanism of this experiment, the later signals were exchange signals. When the resonant peak at $\delta_H 2.83$ was excited, the same phase signal at $\delta_H 2.93$ could be observed. This meant that both signals were generated by the protons of identical groups appeared in different solution conformers which could exchange in a certain rate. As same, when the resonant peak at $\delta_H 2.99$ was excited, there was only an exchange signal appearing at $\delta_H 2.94$. It was predicted that this pair of signals belonged to the protons of other identical groups. According to the influence of heteroatom effect and HMBC spectrum (Figure S5), it was confirmed that resonant peaks at $\delta_H 2.83$ and $\delta_H 2.93$ were signals of –NCH$_3$ and resonant peaks at $\delta_H 2.94$ and $\delta_H 2.99$ were signals of –SCH$_3$.

According to the structure of sulphur-containing amide, and considering the enol-form could be exchanged with the keto-form of the compound, it could be inferred that the chemical exchange might be caused by the rearrangement of a double bond between C–O and C–N (Figure 1).

Besides exchange signals given in Figure S4, the enlarged NOE signals were shown in this figure. It was found that the profiles of the NOE signals were quite different when two resonant peaks at $\delta_H 2.93$ and $\delta_H 2.83$ were excited separately. It had been predicted that the above two resonant peaks belonged to the protons of identical groups (–NCH$_3$) in different solution conformers. When the resonant peak at $\delta_H 2.93$ was excited, the spectrum provided five triplets at $\delta_H 2.62$, $\delta_H 2.74$, $\delta_H 3.43$, $\delta_H 2.56$ and $\delta_H 3.18$. The first three peaks were the

![Diagram of chemical bond rearrangement](image-url)
NOE signals of \( \text{H2}' \), H2 and H1’ respectively in one conformer (A). The last two (\( \delta_H 2.56 \) and \( \delta_H 3.18 \)) should be NOE signal of H2 and H3 from other conformer (B), since the resonant peak at \( \delta_H 2.94 \) (–SCH3) was simultaneously excited. When the resonant peak at \( \delta_H 2.83 \) was excited, the spectrum only presented two triplets at \( \delta_H 2.71 \) and \( \delta_H 3.48 \) which were NOE signals of H2’ and H1’ correspondingly in conformer B. All the above information exposed that the two proton signals of –NCH3 should be related to two different conformers. Conformer A had a structural form as shown in the left of Figure 1. Since the double bond of carbonyl would not limit the rotation of other functional groups, –NCH3 would be close to H2 frequently. So the NOE signals of H2 were observed along with the NOE signals of H1’ and H2’ as the resonant peak (\( \delta_H 2.93 \)) of –NCH3 was excited. Conformer B had a structural form as shown in the right of Figure 1. In this case, the double bond appeared at C-N. –NCH3 and ethane group connected to methanesulfonyl formed a E-configuration, and the distance between –NCH3 and H2 was too long to produce nucleus overhauser enhancement. Therefore, when the resonant peak (\( \delta_H 2.83 \)) of –NCH3 was excited, only the NOE signals of H1’ and H2’ were observed. From all above analysis, it could be confirmed that in the testing solution, compound 1 had two conformers which could be exchanged at a rather lower rate than NMR time scale Spin Dynamics (Basics of Nuclear Magnetic Resonance 2007). So the signals of them were presented in \(^1\text{H} \) and \(^{13}\text{C} \) NMR spectrum at the same time.

2.2. NMR signal assignment of compound 1

Compound 1 had the molecular formula C\(_{13}\)H\(_{19}\)NO\(_4\)S as revealed by the high-resolution HR-FAB-MS quasi molecular ion peak at \( m/\varepsilon 286.1109 \) [M + H]\(^+\), 308.0928 [M + Na]\(^+\). For the structural elucidation of Compound 1, the regular 1D and 2D NMR experiments, including \(^1\text{H} \) NMR, \(^{13}\text{C} \) NMR, DEPT, COSY, HSQC and HMBC were recorded. Also, NMR experiments using different deuterated solvents and different concentrations were made to confirm the structure of this compound. Their \(^1\text{H} \) NMR spectra were shown in Figure S6. From this figure, we could know that concentration had no influence on the appearance of \(^1\text{H} \) NMR spectrum. In MeOD-\( d_4 \), reactive hydrogen was exchanged with deuterium and it cannot be seen other variation except that. In pyridine-\( d_5 \), four methyl signals could be seen obviously. All these indicated that Compound 1 had two stable configurations in solution and the NMR data in DMSO-\( d_6 \) were representative.

Based on the previous discussions, the spectra in DMSO-\( d_6 \) were analysed and the NMR signals assignments were fulfilled for this compound and the NMR signal assignment is listed in Table S1.

3. Conclusion

NMR was a very useful tool which could supply a plenty of information with different kinds of experiments. In the process of NMR analysis, it was found that the compound usually existed in a form of a pair of conformers in solution. With \(^1\text{H} \) NMR experiments at different temperatures, this phenomenon was investigated. It was confirmed that the chemical exchange took place as a double bond was rearranged between the conformers. Depending on 1D-NOE, N-CH\(_3\), S-CH\(_3\), H2, 3, 1’ and 2’ in different conformers were precisely assigned. This view would provide the evidence for NMR elucidation of sulphur-containing amides.
Supplementary material

Supplementary material relating to this article is available online, alongside experimental part, Figures S1–S7 and Table S1.

Disclosure statement

No potential conflict of interest was reported by the authors.

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