

Ab initio geometry-optimization and NMR studies of chemical constituents of *Piper sarmentosum*

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ABSTRACT The structures of two compounds, γ -asarone (1-allyl-2,4,5-trimethoxybenzene) **1** and asarylaldehyde (2,4,5-trimethoxybenzaldehyde) **2** from *Piper sarmentosum*, locally known as 'kaduk', have been studied by ¹HNMR, ¹³CNMR spectroscopy and quantum chemical calculations. The NMR shifts are calculated using Hartree Fock (HF) and Density Functional Theory (DFT) methods. We find that the *ab initio* calculations are very useful in assigning the NMR peaks and provide insights on the electronic factors affecting the NMR shifts.

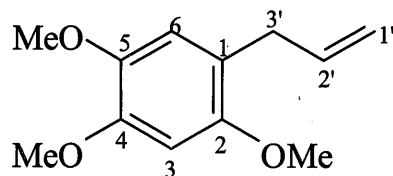
ABSTRAK Struktur sebatian, dua iaitu γ -asarone (1-allyl-2,4,5-trimethoxybenzene) **1** dan asarylaldehyde (2,4,5-trimethoxybenzaldehyde) **2** daripada pokok kaduk atau nama saintifiknya *Piper sarmentosum*, telah dikaji dengan menggunakan spektroskopi (¹HNMR, ¹³CNMR) dan pengiraan kimia kuantum. Anjakan kimia NMR dikira dengan menggunakan kaedah 'Hartree Fock' (HF) dan 'Density Functional Theory' (DFT). Kami mendapati bahawa pengiraan *ab initio* adalah sangat berguna dalam menentukan kedudukan puncak NMR. Faktor elektronik juga memberi kesan kepada anjakan kimia.

(*piper sarmentosum*, *ab initio*, HF, DFT, NMR)

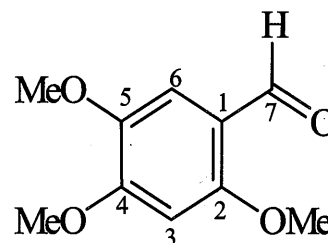
INTRODUCTION

The genus *Piper* belongs to the Piperaceae family and is widely distributed in both hemispheres [1,2,3]. The *Piper* species grow as erect or scandent herbs, shrubs or infrequently as trees. They have high commercial, economical and medicinal importance [4,5]. Several species of *Piper* are grown domestically as houseplants for their foliage. The *Piper* species are known to possess biodegradable insecticidal activity like anti bacteria and anti tumor compounds [6,7] such as β -sitosterol, pellitorine, and isoasarone.

Recently we have initiated a study to investigate the various chemical constituents of *piper sarmentosum*. During the course of investigations, we have isolated two compounds (among others) namely, γ -asarone (1-allyl-2,4,5-trimethoxybenzene) **1** and asarylaldehyde (2,4,5-trimethoxybenzaldehyde) **2** from the roots of *Piper sarmentosum* which have been collected from tasik perdana Kuala Lumpur. In this paper we report the structural and *ab initio* studies of these compounds. The bioactivity studies of these and the other compounds isolated from the *Piper sarmentosum* will be presented elsewhere.



C₁₂H₁₆O₄ **1**



C₁₀H₁₂O₄ **2**

Several trimethoxybenzene compounds are known to occur in nature. The compound **1** (γ -asarone) was first isolated from *caesulia axillaries* oil by Devgan and Bokadia [8]. Masuda et al⁷. have isolated γ -asarone from the dry leaves of *Piper sarmentosum* and have reported the ¹H NMR of this compound. They have observed three phenolic methyl signals [δ 3.83, 3.87, 3.90], the signals of para located protons on the benzene ring [δ 6.53 (1H, s), 6.70 (1H,s)] and allyl signals [δ 5.97 (1H,m), δ 4.77-5.27 (2H, m), 3.33 (2H, d, J=6.0Hz)]. Even though there is no uncertainty about the structure of compound **1**, it is not possible to assign the individual NMR peaks to the corresponding protons, without further experimental or theoretical studies. For example, among the three phenolic methyl signals, which one corresponds to phenolic methyl at C-2, C-4 or C-5 position? Similarly, between the two signals of para located protons, which signal corresponds to protons at C-3 or C-6 position?

In the absence of any other data, the assignments of NMR peaks of similar protons are made on the basis on the electronic factors. It is well known that the presence of substituents in a highly delocalized system like phenyl rings affects the electron distributions and these effects are explained on the basis of inductive and mesomeric effects. In general the π -donor groups (OMe) deshield the α nuclei and shield the β nuclei causing a downfield shift of the α nuclei and an up field shift of the β nuclei. Similarly, the π -acceptor groups ($>C=O$) causes downfield shifts to both α and β nuclei, with the effect at the β position being larger.

In compound **1**, H-3 is flanked by two OMe groups. The strong electron donating OMe groups are expected to shield the H-3 protons causing an up field shift. However, H-6 is flanked by a methoxy and a propenyl group. C-1 is directly attached to a propenyl carbon, which is a weak electron-donating group. The presence of the olefinic C-2' and C-1' might reduce its electron donating capacity. Even though the net effect of OMe and propenyl group in H-6 is not clear, one may anticipate that the net effect might be less than that exerted by two OMe group on H-3. Hence H-3 should be more shielded (up field) compared to H-6.

In the case of compound **2**, H-3 is flanked by two OMe groups where as H-6 is flanked by an

electron donating OMe group and an electron withdrawing aldehyde group. Hence H-3 should be shielded more compared to H-6 causing an up field shift of H-3 relative to that of H-6. In the present work we wish to calculate the chemical shift values of these protons using *ab initio* quantum mechanical calculations so as to confirm these arguments.

METHODOLOGY

The ¹HNMR and ¹³CNMR spectra of the compounds were recorded in CDCl₃ on JEOL JNM-LA 400 NMR spectrometer. All electronic structure and NMR chemical shift calculations were done using Gaussian 98 quantum mechanical packages [9]. The structures of the compounds **1** and **2** are first optimized at the Hartree-Fock (HF) level theory using the 6-31G** basis set. Density functional theory (DFT) calculations using Becke's three-parameter gradient corrected exchange functional [10] with the Lee-Yang-Parr gradient corrected correlation (B3LYP) functional [11] were also performed using the 6-31G** basis set. The nuclear magnetic shielding values of the proton and the carbon atoms were calculated using the gauge-including atomic orbital (GIAO) method [12-14] at the B3LYP/6-31G** level, using the B3LYP/6-31G** optimized structure. The calculation of the nuclear shielding of the reference compound, tetramethyl silane (TMS) was also carried out at the same level of theory. The NMR shifts were obtained as the difference between the magnetic shielding values of the compounds and the TMS.

RESULT AND DISCUSSION

The ¹HNMR spectrum of compound **1** showed the characteristics of an olefinic system. A multiplet at δ 5.8- δ 5.9, representing one proton, can be attributed to the olefinic proton H-2'. A multiplet at δ 4.93- δ 5.00 (two protons) was assigned to the protons attached to the sp^2 C-1'. The signal of the aromatic proton situated *para* to each other (H-6 and H-3) on the benzene ring was revealed at δ 6.6 and δ 6.4 respectively. Three methoxy singlets were observed at δ 3.72, δ 3.74 and δ 3.80. A *dt* at δ 3.25 can be attributed to the C-3' methylene. For compound **2**, the spectrum showed three singlet at δ 3.80, δ 3.85 and δ 3.90 corresponding to three methoxyl groups directly attached to the aromatic ring. A singlet at δ 10.25 could be assigned to the aldehyde proton. Two

singlets at $\delta 7.25$ and $\delta 6.43$ may be attributed to the para located protons in the phenyl ring. These results are in excellent agreement with that of Masuda et al [7]. However, in their experimental studies Masuda et al [7] have not done the $^1\text{H NMR}$ proton assignments of H-3 and H-6. The assignments may be established through the 2D NMR experiment, HMBC. However, we could not carry out this study, since the amount of sample available is very limited. Hence we have resorted to *ab initio* NMR studies on compound **1** and **2** to predict the chemical shift of H-3 and H-6.

The structures of compounds **1** and **2**, optimized using HF and DFT methods show that, even though the benzene ring is planar as expected, the methoxyl groups deviate from the plane of the benzene ring. The oxygen atoms of the OMe groups lie in the plane of benzene where as the O-Me bond deviate from the benzene ring. The dihedral angles of the O-Me groups with respect to the benzene rings are in the range of 105° - 119° . For the OMe attached to C-2, C-4, and C-5, the value of $^1\text{H NMR}$ shifts are different from each other.

The calculated $^1\text{H NMR}$ and $^{13}\text{C NMR}$ shielding tensor values are given in Tables 1 and 2. We found that the *ab initio* NMR shielding values are uniformly higher than the experimental values. This may be due to the smaller basis set that we have used in the calculations. It has been already reported that the *ab initio* NMR shielding values depend on the method of calculation and the basis set involved. An improved basis set with added diffuse functions might give better agreement with the experimental results. In the present study we are interested only in the relative positions of the chemical shifts and we observe that the *ab initio* values are in excellent qualitative agreement with the experimental values. When a molecule is placed in a magnetic field, currents are set up in the molecule. The currents flow around atoms, within bond and from atom to atom around benzene ring. The general regions of chemical shifts of aromatic and hetero aromatic protons lie between $\delta 6$ to $\delta 9$, i.e. towards lower field. The position taken by an entering group in substituted benzene appears to be intimately connected with the value of the electron density at the *ortho*, *meta* and *para* position. Highet and Highet [15] reported that the chemical shifts of protons on aromatic rings reflect the charge density on the carbon atom to

which the protons are attached and the observations on substitute benzenes suggest that electron-withdrawing substituents produce downfield shifts in the *ortho* and *para* protons, whereas electron-donating substituents produce an up field shift: the *ortho* protons are generally more affected than the *para* protons.

For compound **1**, the experimental and *ab initio* values predict that H-3 is slightly more shielded compared to H-6 (Table 1). The H-3 is surrounded by two methoxyl groups, where as H-6 is surrounded by one methoxyl group and a propenyl group at the *ortho* positions. The propenyl is a weaker electron-donating group compared to the methoxyl group. This results in more increase in electron density around H-3 and cause it resonate at high field compared to H-6. Anisotropic effect of the field on the chemical shift of nearby nuclei is dependent upon the orientation of the nucleus in question with respect to the bond. The π -bonds are especially effective in influencing the chemical shift of nearby atoms, so that the olefinic carbon atoms and the hydrogens bonded to them are shifted downfield. From our *ab initio* calculation it is now clear that the $^1\text{H NMR}$ signal at $\delta 6.46$ is due to H-3 and the $^1\text{H NMR}$ signal at $\delta 6.62$ is due to H-6. In addition, our calculation also demonstrate that the ^{14}C chemical shift for C-3 is slightly higher than that of C-6 thus reaffirming the interpretation of the $^1\text{H NMR}$ assignment for H-3 and H-6.

As for the carbons, from the calculations, C-5 ($\delta 144$) is the most shielded compared to carbons 2 and 4. This is due to perhaps it being *ortho* and *para* to two carbons attached to the methoxyls. C-2 is most deshielded as it is *ortho* to the weakly electron donating propenyl group.

For compound **2**, we found that H-6 is significantly more deshielded compared to H-3, which is in good agreement with our experimental values. This implies that the aldehyde group has more deshielding effect on H-6 compared to that of the propenyl group. From our *ab initio* calculation it is now clear that the $^1\text{H NMR}$ peak at $\delta 6.43$ is due to H-3 and the $^1\text{H NMR}$ peak at $\delta 7.25$ is due to H-6. The values of $^{13}\text{C NMR}$ signals for quaternary carbons are $\delta 158$, $\delta 155$, $\delta 143$ and $\delta 117$ due to C-4, C-2, C-5 and C-1 respectively. C-2 and C-4 are more deshielded than C-5 since they are *para* and *ortho* to the aldehyde bearing C-1.

Table 1. Experimental ^1H NMR and ^{13}C NMR chemical shifts (in δ ppm) and the results from *ab initio* calculations (GIAO) for compound 1

No. of Carbon	^1H NMR		^{13}C NMR	
	Exp. #	B3LYP/6-31G**	Exp. #	B3LYP/6-31G**
1			120.4	122
2			151.7	151
3	6.46	7.19	98.5	103
4			148.3	150
5			143.4	144
6	6.62	7.59	114.4	123
1'	4.95	5.46	115.5	112
2'	5.90	6.15	137.7	134
3'	3.25	3.46	34	41
C2-OMe	3.72	4.22, 3.82, 3.53 (3.86)	57	54
C4-OMe	3.74	3.98, 3.56, 3.88 (3.81)	57	59
C5-OMe	3.80	3.53, 4.59, 3.98 (4.03)	57	59

#Assignments of experimental NMR signals are done on the basis of *ab initio* calculations. The values given within the parenthesis are the average of the NMR shift of the three hydrogens of the OMe group.

Table 2. Experimental ^1H NMR and ^{13}C NMR chemical shifts (in δ ppm) and the results from *ab initio* calculations (GIAO) for compound 2

No of Carbon	^1H NMR		^{13}C NMR	
	Exp. #	B3LYP/6-31G**	Exp. #	B3LYP/6-31G**
1			109	119.9
2			155	155.4
3	6.43	6.68	96	103.6
4			158	156.8
5			143	144.5
6	7.25	8.18	117	122.9
7	10.25	10.75	188	179.1
C2-OMe	3.80	4.32, 3.93, 3.91 (4.05)	56	54.5
C4-OMe	3.85	4.07, 3.59, 5.16 (4.27)	56	58.5
C5-OMe	3.90	3.60, 4.37, 4.07 (4.01)	56	59.8

#Assignments of experimental NMR signals were done on the basis of *ab initio* calculations. The values given within the parenthesis are the average of the NMR shift of the three hydrogens of the OMe group.

CONCLUSION

The *ab initio* calculations showed that even though the oxygen atom of the methoxyl groups lie in the same plane as that of benzene, the CH_3

groups of methoxyl are bent away from the benzene ring. For compound 1, H-3 and H-6 protons are deshielded with H-3 being slightly more deshielded compared to H-6 because of the adjacent propenyl group. The *ab initio* ^1H NMR

values also show that H-6 of compound 2 is more deshielded compared to H-6 of compound 1 because of the better electron withdrawing character of the aldehyde group. Furthermore ^{13}C NMR peak for C-1 is up field. By using *ab initio* calculation we are thus able to unambiguously assign the NMR peaks and identify the electronic factors influencing the NMR signal.

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