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DFT-GIAO ^{1}H and ^{13}C-NMR Chemical Shifts Calculation of *Uncaria longiflora* Alkaloids

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Recently, the structure elucidation of three new chiral alkaloids, namely isoformosaninol (**1**), formosaninol (**2**), and longiflorine (**3**), isolated from the leaves of *U. longiflora* var. *pteropoda* (Miq.) Ridsdale has been reported. Their molecular structures were initially determined by using experimental NMR data, following a systematic method for establishing the absolute configuration of pentacyclic oxindole alkaloids (POAs), and the chemical correlation method based on the known chirality of their precursor, secologanin. Indeed, the integration of the information from experimental and theoretical data can be of fundamental importance for the successful elucidation of the configurational and stereostructural assignments of organic compounds, including natural products. Thus, the present work was conducted to further support the success of this integration by focusing on the NMR data, which is the most critical spectroscopic analysis in the structural elucidation of natural products. The ^{1}H and ^{13}C-NMR chemical shift values for the three alkaloids were calculated using density functional theory-gauge including atomic orbitals (DFT-GIAO) approximation at the B3LYP/6-311+G(d,p) level of theory in integral equation formalism polarizable continuum model (IEF-PCM) concerning to tetramethylsilane (TMS). Statistical error analysis between the experimental and calculated supported an excellent correlation with linearity of higher than 93% and 99% for ^{1}H and ^{13}C-NMR chemical shifts, respectively, for the three alkaloids. The correct correspondences between experimental and calculated data sets were further supported by the mean absolute error (MAE) parameter. The present findings provide insights on the usefulness of integrating experimental and calculated NMR data to ascertain structural elucidation in easing ambiguity.

Key words: *Uncaria longiflora*; Chiral Alkaloids; NMR; DFT-GIAO

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There are various techniques that have been applied in determining molecular structure and one of them is through a combination of experimental spectroscopy and simulated theoretical studies [1]. Quantum mechanical/nuclear magnetic resonance (QM / NMR) approach is widely used for organic compounds, generally comparing one cluster of experimentally determined data, such as ^{1}H and ^{13}C-NMR chemical shifts, with those predicted theoretically. This method is particularly very useful to ascertain structural elucidation of a chiral molecule in easing ambiguity of stereoassignments [2]. To facilitate the confidence on the stereoassignments, a statistical parameter measuring the deviation of the theoretical from the experimental chemical shifts, known as mean absolute error (MAEΔΔδ), is very useful for indicating the best fit between sets of experimental and calculated data to groups of stereoisomers. Comparison of statistical differences of calculated/experimental chemical shifts leads to the advantage of avoiding calibration errors, reducing systematic errors, and highlighting the most diagnostic differences between the data [3].

Many important chemical and physical properties of biological and chemical systems can be predicted from the first principles by various computational techniques [1,4]. In recent years, density functional theory (DFT) has been popularly used mainly to predict ground state energies of molecular systems. The development of better exchange-correlation functionals made it possible to calculate many molecular properties with comparable

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accuracies to traditional correlated ab initio methods, with more favorable computational costs [5]. One of the recommended hybrid functionals for NMR calculation of natural products is the hybrid functional B3LYP, along with the use of a larger basis set, and inclusion of solvent effect modelling [6-8]. This is due to its high efficiency and accuracy in modelling the electronic properties of complex polyatomic organic systems with reasonable computational resources. However, it is important to note that the use of a larger basis set will significantly increase the calculation time.

The genus *Uncaria* (Rubiaceae) represents shrubby woody climbers, comprising of approximately 34 species that are distributed worldwide, including 14 species in Malaysia [9]. To date, more than 400 compounds comprising mainly of alkaloids and terpenoids, as well flavonoids, and coumarins have been identified from the *Uncaria* species [10,11]. Of these, over 100 new structures including the three new chiral alkaloids isolated from the leaves of *Uncaria longiflora* var. *pteropda* were recently reported by our group [12]. The molecular structures of the three new alkaloids (Figure 1), isoformosaninol (**1**), formosaninol (**2**), and longiflorine (**3**), were initially determined by using experimental NMR data following a systematic method for establishing the absolute configuration of pentacyclic oxindole alkaloids (POAs), and the chemical correlation method based on the known chirality of their precursor, secologanin. With a goal to further understand the configurational and stereostructural fundamentals of the molecules, the present work was conducted to integrate the information from experimental and theoretical NMR data, which is the most critical spectroscopic analysis in the structural elucidation of natural products.

MATERIALS AND METHODS

1. Experimental

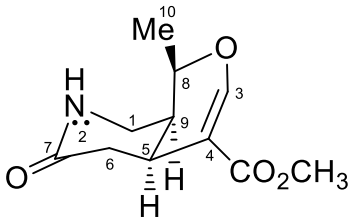
All solvents were of analytical grade purchased from Sigma-Aldrich. The alkaloids isoformosaninol (**1**), formosaninol (**2**), and longiflorine (**3**) were previously isolated from the leaves of *Uncaria longiflora* var. *pteropoda* [12]. The ^{1}H- and ^{13}C-NMR were analyzed in chloroform-*d* on Bruker 300 Ultrashield NMR spectrometer at 300 and 75 MHz, respectively. The data were analyzed using TOPSPIN 3.2 software.

2. Theoretical

Optimization and frequency calculations of the conformers for the three alkaloids were performed by using DFT method at the B3LYP/6-311+G(d,p) level of theory as implemented in Gaussian09 [13]. The minima of ground states (GSs) were confirmed by the absence of imaginary frequencies. The ^{1}H and ^{13}C-NMR chemical shift values for the three alkaloids, and isotropic chemical shielding constants, were calculated within GIAO approach at the B3LYP/6-311+G(d,p) level of theory [14]. The isotropic shielding values were used to calculate the isotropic chemical shifts δ_{cal} with respect to tetramethylsilane (TMS).

$$\delta_{iso} = \rho_{TMS} - \rho_{iso}$$

where δ_{iso} is the chemical shift, ρ_{iso} the absolute shielding, and ρ_{TMS} the absolute shielding of TMS. The solvent effects were taken into account implicitly by using the polarizable continuum model formalism IEF-PCM. In the PCM model, the substrate is embedded into a cavity surrounded by a dielectric continuum characterized by its dielectric constant (ε_{CH3CN} = 35.688) [7]. The ^{1}H and ^{13}C-NMR chemical shifts of each conformer was Boltzmann weighted (BW) for the respective alkaloid, and qualitatively analyzed through a mean absolute error (MAE) between the experimental and its corresponding calculated BW using Excel 2013 software.



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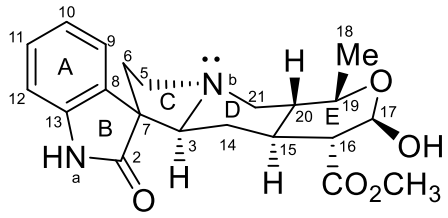


1 C7-A (Oxindole C=O below C/D plane)
2 C7-B (Oxindole C=O above C/D plane)

Figure 1. Structures of isoformosaninol (**1**), formosaninol (**2**), and longiflorine (**3**).

Note. Reprinted from "Absolute configuration of alkaloids from *Uncaria longiflora* var. *pteropoda* through experimental and theoretical approaches," by Salim, F., Yunus, Y. M., Anouar, E. H., Awang, K., Langat, M., Cordell, G. A., Ahmad, R., 2019, *Journal of Natural Products*, 82, 2933-2940.

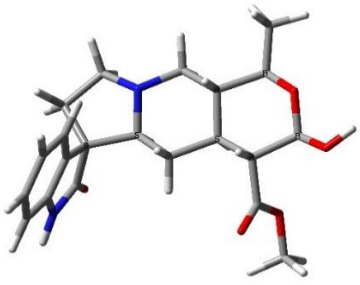
RESULTS AND DISCUSSION

Individual solutions of alkaloids **1**-**3** in deuterated chloroform were made up to ~25 mM concentration. Their ^{1}H and ^{13}C-NMR experimental spectra were acquired at 300 and 75 MHz, respectively. Theoretical calculations were attempted for alkaloids **1-3** in order to support the previous structure elucidation, as well as to obtain additional information of the electronic distribution in the alkaloids and compare them with those observed experimentally.

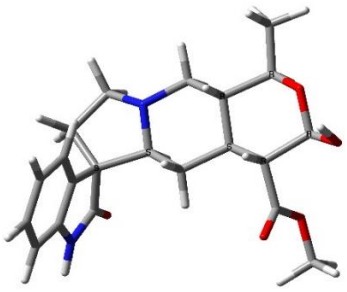
A systematic conformational search of the isomers of the proposed configurations for alkaloids **1** (3*S*, 7*S*, 15*S*, 16*R*, 17*R*, 19*R*, 20*R*), **2** (3*S*, 7*R*, 15*S*, 16*R*, 17*R*, 19*R*, 20*R*), and **3** (4*S*, 8*R*, 9*S*) was carried out using Merck molecular force field 94 (MMFF94) implemented in the Spartan 08 program. Conformers with energy below 10 kcal/mol were selected, and as shown in Figure 2 this gave 2 conformers for alkaloids **1** (**1a** 69.6 and **1b** 28.1%) and **2** (**2a** 77.5 and **2b** 21.2%), and 6 conformers for alkaloid **3** (**3a** 49.2, **3b** 42.9, **3c** 2.4, **3d** 2.1, **3e** 1.9 and **3f** 1.6%). Each selected conformer of each alkaloid was further optimized using DFT, and the minima was confirmed by the absence of imaginary frequencies built to Gaussian09 software [13]. The ^{1}H and ^{13}C-NMR chemical shift values were calculated with respect to tetramethylsilane within GIAO approach using density functional theory (DFT) at the B3LYP/6-311+G(d,p) level of theory. The calculations were accomplished in integral equation formalism polarizable continuum model (IEF-PCM) using $CDCl_3$ as solvent, as NMR data are more solvent dependent. This approach has been shown to give reasonable results [2].

Tables 1 and 2, respectively, show the ^{1}H and ^{13}C chemical shifts of the calculated conformers of each alkaloid, their weighted Boltzmann averages (BW), and the experimental data. The position of each atom in the alkaloid molecules used in the present investigation follows the biogenesis numbering system as shown in Figure 1 above [12]. As shown in Tables 1 and 2, the predicted ^{1}H and ^{13}C chemical shifts were in good agreement with the experimental data expressed by the coefficient values. Statistical error analysis between the experimental and calculated Boltzmann averages (BW) supports an excellent correlation with a linearity of higher than 93% and 99% for ^{1}H and ^{13}C-NMR chemical shifts, respectively, for the three alkaloids (Figures 3 and 4). Slightly lower correlation difference coefficients for alkaloid **3** suggest that the values are strongly influenced by the conformational effect and the prochiral carbons CH_2 that exist in its molecular structure.

The correct correspondences between experimental and theoretical data sets were further supported by the mean absolute error (MAE) parameter, shown at the bottom of Tables 1 and 2. Relatively lower MAE values obtained with ^{13}C compared to ^{1}H-NMR are related to the sensitivity of the latter and to the significantly lower isotopic abundance of the former (1.1%). Lower MAE values are important since alkaloids **1**, **2**, and **3** are chiral. As NMR is very sensitive to chirality changes, this would definitely influence the ^{1}H and ^{13}C-NMR chemical shifts of the atoms located nearby the chiral center, due to different magnetic environments [15]. Nevertheless, the amide and hydroxyl protons were not included in the MAE calculation due to the possibility of involving hydrogen bonds, whether with themselves or the solvent molecules and this would affect the observed chemical shifts [2]. Based on the consistency in the results obtained, the present findings agreed with the applicability of the B3LYP hybrid functional, along with the use of adequate Pople's basis set incorporating diffusion and polarization functions (6-311+G(d,p)), which take into account extensive solvation model (IEF-PCM) to properly calculate the ^{1}H and ^{13}C-NMR chemical shifts for the studied types of alkaloids. Thus, the present work provides insights on the usefulness of integrating experimental and theoretical NMR data to ascertain structural elucidation in easing ambiguity.

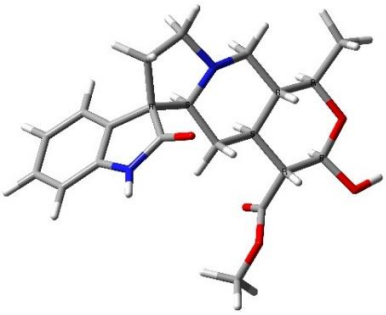


1a (0.00 kcal/mol) (69.6%)

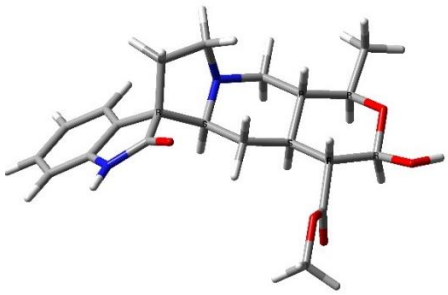


1b (0.54 kcal/mol) (28.1%)

Conformers of alkaloid **1**

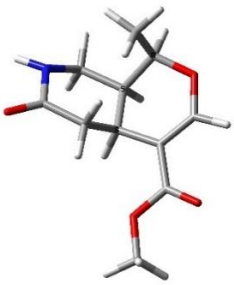


2a (0.00 kcal/mol) (77.5%)

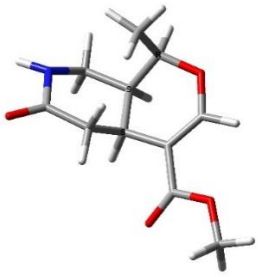


2b (0.77 kcal/mol) (21.2%)

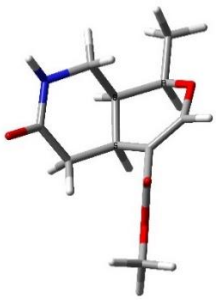
Conformers of alkaloid **2**



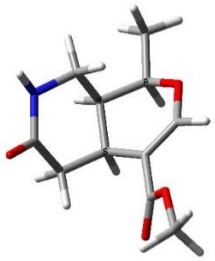
3a (-1.88 kcal/mol) (49.2%)



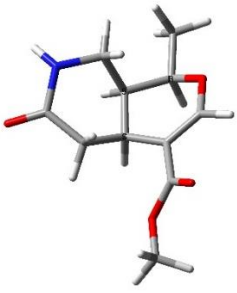
3b (-1.80 kcal/mol) (42.9%)



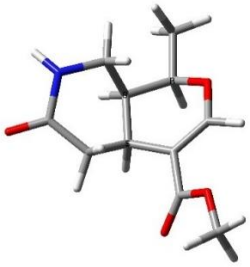
3c (-0.09 kcal/mol) (2.4%)



3d (0.00 kcal/mol) (2.1%)



3e (0.06 kcal/mol) (1.9%)



3f (0.15 kcal/mol) (1.6%)

Conformers of alkaloid **3**

Figure 2. Selected stable conformers of alkaloids **1**-**3**.

Table 1. Experimental and Calculated ^{1}H NMR Data (300 MHz, $CDCl_3$) for Alkaloids **1**-**3**.

Position	Isoformosaninol (**1**)				Formosaninol (**2**)				Position	Longiflorine (**3**)							
	δ_{Cal}			δ_{Exp}[a]	δ_{Cal}			δ_{Exp}[a]		δ_{Cal}							δ_{Exp}[a]
	1a	**1b**	**BW**		**2a**	**2b**	**BW**			**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**BW**	
H3	2.50	2.52	2.45	2.45	2.34	2.89	2.43	2.41	-	-	-	-	-	-	-	-	-
H5α	2.56	2.57	2.50	2.43	2.65	3.22	2.74	2.54	-	-	-	-	-	-	-	-	-
H5β	3.31	3.32	3.24	3.25	3.24	3.38	3.23	3.23	-	-	-	-	-	-	-	-	-
H6α	1.99	1.97	1.94	2.03	1.91	1.97	1.90	2.12	-	-	-	-	-	-	-	-	-
H6β	2.33	2.33	2.28	2.42	2.47	2.58	2.46	2.45	-	-	-	-	-	-	-	-	-
H9	7.74	7.72	7.56	7.34	7.44	7.89	7.44	7.20	-	-	-	-	-	-	-	-	-
H10	7.23	7.22	7.06	7.05	7.23	7.20	7.13	7.06	-	-	-	-	-	-	-	-	-
H11	7.45	7.44	7.28	7.21	7.41	7.42	7.32	7.21	-	-	-	-	-	-	-	-	-
H12	6.99	6.99	6.83	6.88	6.93	6.96	6.85	6.89	-	-	-	-	-	-	-	-	-
H14α	0.74	0.68	0.71	0.99	0.95	1.02	0.95	0.95	-	-	-	-	-	-	-	-	-
H14β	0.93	0.90	0.90	1.29	1.30	1.64	1.36	1.82	-	-	-	-	-	-	-	-	-
H15	1.48	1.58	1.47	2.19	1.40	1.60	1.42	2.18	H3α	2.41	2.47	2.01	1.78	1.84	2.08	2.40	2.19
H16	2.00	1.64	1.85	2.59	2.21	2.29	2.20	2.66	H3β	3.46	3.22	2.91	3.09	2.90	2.85	3.32	2.93
H17	4.97	4.71	4.78	4.92	4.92	4.97	4.87	5.21	H4	3.12	3.10	3.02	3.13	3.13	2.99	3.11	3.05
$CH_3$18	1.30	1.34	1.28	1.33	1.28	1.28	1.26	1.33	H6	7.89	7.95	7.89	8.04	8.09	7.95	7.93	7.57
H19	3.49	3.53	3.42	4.08	3.45	3.38	3.39	4.25	$CH_3$7	1.59	1.58	1.49	1.53	1.62	1.50	1.58	1.42
H20	1.17	1.10	1.12	1.55	1.29	1.40	1.30	1.57	H8	4.28	4.29	4.53	3.99	3.99	4.54	4.29	4.19
H21α	1.79	1.80	1.75	2.20	1.75	2.63	1.91	2.02	H9	2.28	2.26	2.15	2.02	1.99	2.17	2.26	1.9
H21β	3.09	3.08	3.02	3.22	3.09	3.07	3.05	3.33	H10α	3.35	3.36	3.27	3.23	3.23	3.28	3.35	3.35
OCH_3	3.63	3.63	3.55	3.62	3.58	3.75	3.57	3.58	H10β	3.38	3.36	3.91	3.24	3.25	3.88	3.39	3.65
NH	-	-	-	7.77	-	-	-	8.27	OCH_3	3.78	3.76	3.78	3.75	3.75	3.77	3.77	3.75
OH	-	-	-	3.38	-	-	-	4.60	NH	4.71	4.71	4.80	5.16	5.18	4.81	4.74	5.92
MAE	**0.24**	**0.27**	**0.24**		**0.24**	**0.31**	**0.23**		**MAE**	**0.21**	**0.19**	**0.16**	**0.21**	**0.21**	**0.17**	**0.19**	
Max. Dev	**0.71**	**0.95**	**0.74**		**0.80**	**0.87**	**0.86**		**Max. Dev**	**0.53**	**0.38**	**0.34**	**0.47**	**0.63**	**0.38**	**0.39**	

Table 2. Experimental and Calculated ^{13}C NMR Data (75 MHz, $CDCl_3$) for Alkaloids **1**-**3**.

Position	Isoformosaninol (**1**)				Formosaninol (**1**)				Position	Longiflorine (**3**)							
	δ_{Cal}			δ_{Exp}[a]	δ_{Cal}			δ_{Exp}[a]		δ_{Cal}							δ_{Exp}[a]
	1a	**1b**	**BW**		**2a**	**2b**	**BW**			**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**BW**	
N-C=O	186.22	186.08	181.90	180.68	186.84	184.65	183.95	181.05	N-C=O	176.64	176.47	177.38	178.70	178.54	177.18	176.85	170.96
C3	74.81	74.74	73.07	71.29	78.89	72.63	76.54	74.71	-	-	-	-	-	-	-	-	-
C5	55.98	55.82	54.65	53.54	57.93	50.24	55.55	54.82	-	-	-	-	-	-	-	-	-
C6	39.91	39.60	38.90	35.03	41.08	38.81	40.06	34.17	-	-	-	-	-	-	-	-	-
C7	63.05	63.06	61.60	56.90	62.34	62.85	61.64	56.19	-	-	-	-	-	-	-	-	-
C8	142.20	141.88	138.84	133.50	142.48	145.58	141.29	132.95	-	-	-	-	-	-	-	-	-
C9	130.75	130.65	127.71	124.47	128.93	129.23	127.32	123.04	-	-	-	-	-	-	-	-	-
C10	126.59	126.58	123.68	122.74	126.81	126.91	125.18	122.55	-	-	-	-	-	-	-	-	-
C11	133.00	133.08	129.96	127.95	133.14	132.78	131.33	128.04	-	-	-	-	-	-	-	-	-
C12	112.62	112.76	110.07	109.77	112.29	112.16	110.80	109.61	-	-	-	-	-	-	-	-	-
C13	147.40	147.37	144.00	139.78	147.76	146.28	145.53	140.86	-	-	-	-	-	-	-	-	-
C14	32.79	32.69	32.01	23.56	32.60	30.48	31.73	23.30	C3	34.99	36.82	39.08	41.29	41.89	39.78	36.25	35.75
C15	45.72	45.07	44.49	35.82	46.50	46.73	45.94	36.62	C4	36.88	36.83	31.91	34.06	33.88	32.09	36.58	27.94
C16	58.03	64.69	58.57	52.11	58.02	58.39	57.34	52.22	C5	112.91	113.30	114.43	118.38	118.11	114.14	113.46	109.86
C17	102.19	101.38	99.61	91.70	102.45	102.32	101.09	91.45	C6	165.37	166.19	162.23	165.05	165.90	162.95	165.78	154.82
C18	19.79	19.97	19.39	19.27	19.75	19.66	19.47	19.63	C7	18.35	18.30	18.16	18.11	18.08	18.17	18.33	18.59
C19	77.65	79.18	76.29	69.55	77.79	77.88	76.80	69.44	C8	79.79	80.05	80.89	77.89	78.20	81.21	79.96	70.68
C20	48.05	48.54	47.08	41.40	47.73	40.04	45.48	41.30	C9	38.24	38.04	36.01	46.64	46.44	36.04	38.44	35.29
C21	55.78	55.78	54.50	51.72	55.37	51.23	53.77	53.88	C10	40.88	40.93	45.84	40.35	40.36	45.81	41.12	41.78
OCH_3	53.13	53.47	52.00	51.72	53.13	53.27	52.47	51.67	OCH_3	52.51	52.64	52.50	52.53	52.60	52.66	52.62	51.38
O-C=O	183.45	180.53	178.41	171.98	183.59	183.60	181.21	172.46	O-C=O	174.22	175.63	174.48	174.56	175.58	174.95	175.05	167.15
MAE	**5.93**	**6.12**	**3.92**		**5.97**	**5.57**	**4.53**		**MAE**	**4.58**	**4.84**	**4.51**	**6.11**	**6.29**	**4.69**	**4.73**	
Max. Dev	**11.47**	**12.58**	**8.67**		**11.13**	**12.63**	**9.64**		**Max. Dev**	**10.55**	**11.37**	**10.21**	**11.35**	**11.15**	**10.53**	**10.96**	

[a]Reprinted from "Absolute configuration of alkaloids from *Uncaria longiflora* var. *pteropoda* through experimental and theoretical approaches," by Salim, F., Yunus, Y. M., Anouar, E. H., Awang, K., Langat, M., Cordell, G. A., Ahmad, R., 2019, *Journal of Natural Products*, 82, 2933-2940.

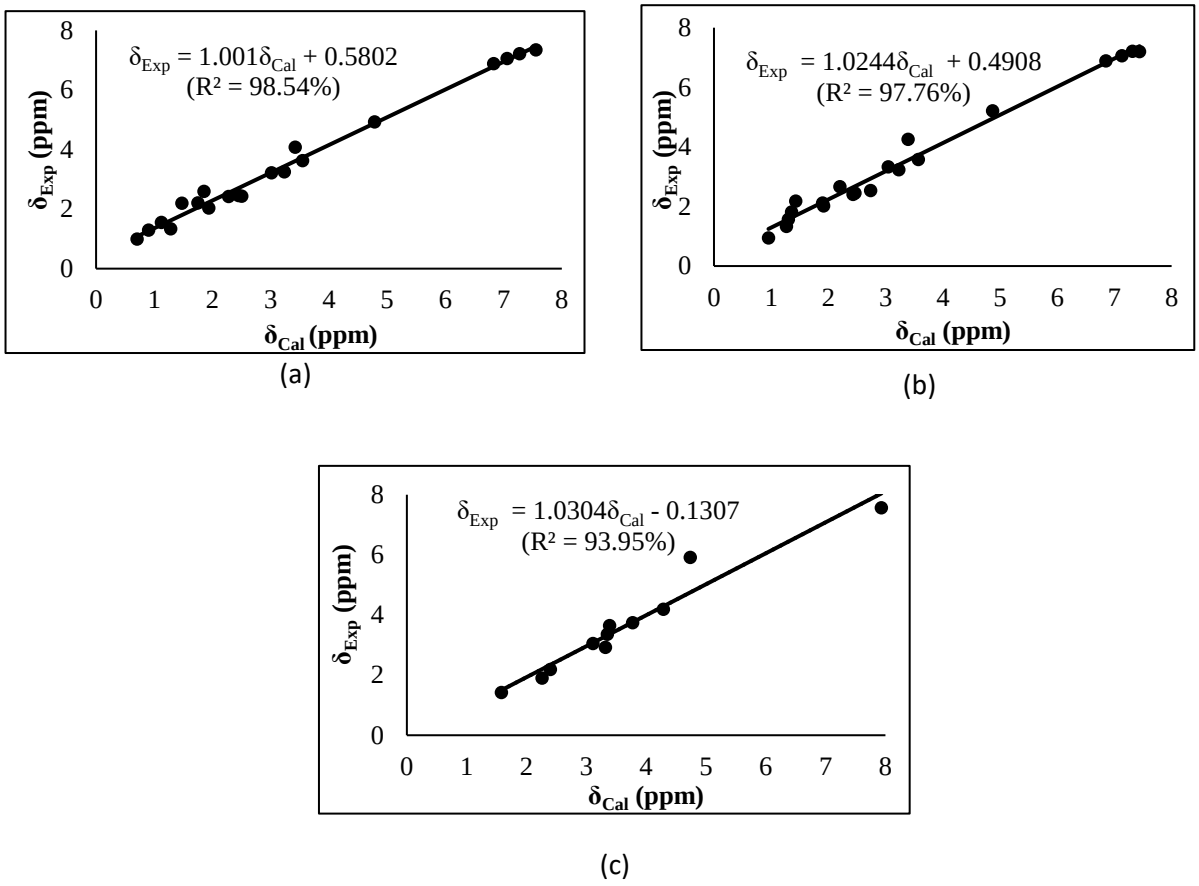


Figure 3. Correlation curves between experimental and calculated BW ^{1}H-NMR chemical shifts of alkaloids (a) **1**, (b) **2**, and (c) **3**.

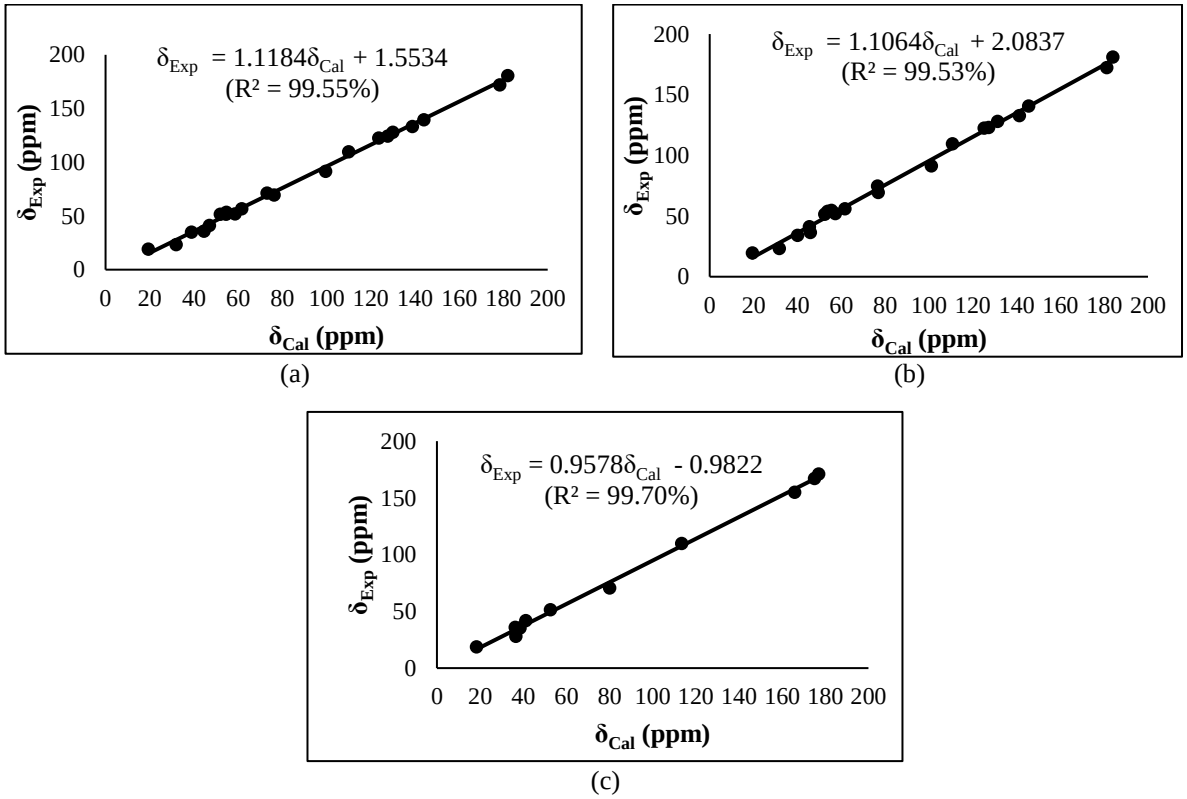


Figure 4. Correlation curves between experimental and calculated BW ^{13}C-NMR chemical shifts of alkaloids (a) **1**, (b) **2**, and (c) **3**.

CONCLUSION

It was found that NMR data, which is the most critical spectroscopic analysis in the structural elucidation of natural products, would be excellently calculated by using DFT-GIAO method. This is very useful to ascertain absolute structural elucidation in easing ambiguity of chiral molecules. The present findings provide insights on the usefulness of integrating experimental and theoretical NMR data in structural elucidation of complex polyatomic organic systems.

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