Density Functional Theory Calculation of ^{13}C NMR Shifts of Diazaphenanthrene Alkaloids: Reinvestigation of the Structure of Samoquasine A
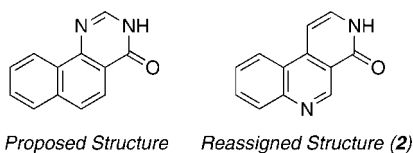
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Proposed Structure   Reassigned Structure (**2**)

The ^{13}C NMR shifts of 48 diazaphenanthrene-type alkaloids have been calculated at the B3LYP/6−311+G(2d,p) level of theory. The results support the structural reassignment of samoquasine A as **2**.

Benzo[*h*]quinazoline alkaloids are a small group of natural products of varying biological activities, including antitumor[1] and antidepressive[2] properties. In 2000, Kobayashi and co-workers isolated an alkaloid from the seeds of the custard apple tree *Annona squamosa*.[3] This alkaloid exhibited cytotoxic activity against murine lymphoma L1210 cells. The structure was proposed to be 3,4-dihydrobenzo[*h*]quinazolin-4-one (**1**) and was given the trivial name samoquasine A. In 2002, the authors reported that samoquasine A was actually identical to perlolidine, a known compound with a different structure, and withdrew the assigned trivial name.[4]

Since that time, several groups have reported synthetic studies of the putative structure of samoquasine A and related compounds. These syntheses have called into question whether samoquasine A is indeed perlolidine. Wu[5] and Chakrabarty[6] have independently reported chemical syntheses of the originally proposed structure of samoquasine A and have unambiguously confirmed the nonidentity of samoquasine A with 3,4-dihydrobenzo[*h*]quinazolin-4-one. However, the definitive proof of the identity or nonidentity of samoquasine A to perlolidine

has been an elusive endeavor. In their 2003 report on the synthesis of the putative structure of samoquasine A, Wu and co-workers also published the isolation of perlolidine from the stem extracts of *A. squamosa* and identified its structure as **2**. Due to its poor solubility in CDCl$_3$, the authors were unable to directly compare the NMR data of their isolated compound with the data originally published for samoquasine A. However, due to the differing solubilities, as well as different melting points, the authors concluded that the two compounds were different. On the basis of a reinvestigation of the HMBC spectrum of samoquasine A, Wu and co-workers proposed benzo[*f*]phthalazin-4(3*H*)-one (**3**) as a possible structure consistent with the spectroscopic data. Mátyus, Maes, and co-workers subsequently reported a chemical synthesis of **3** and the isomeric benzo[*f*]phthalazin-1(2*H*)-one (**4**).[7] The NMR spectroscopic data for both of these new compounds were found to be inconsistent with the reported data for samoquasine A.

The computational prediction of ^{13}C NMR chemical shifts has become an increasingly popular tool for the structural assignment or reassignment of natural products.[8−10] ^{13}C NMR is particularly useful for the evaluation of natural products because resonances are not greatly dependent upon solvent effects and are spread out over a relatively wide range of frequencies.[8h,9] Some notable recent successes in this field include the structural revision of hexacyclinol by Rychnovsky[9] and our own reassignment of TAEMC161 as viridiol.[10] The controversy surrounding the identity of samoquasine A prompted us to examine the NMR data from a computational standpoint. We identified 48 isomeric alkaloids, including **1**−**4**, for study and comparison to the experimental data for samoquasine A. Herein, we report the results of our computations and confirm that the structure of samoquasine A is identical to **2**.

Calculations were performed using the Gaussian 03[11] software package. Geometry optimizations and frequency calculations were performed at the B3LYP/6−31G(d) DFT level. All geometries obtained were optimized to true minima as evidenced by the absence of imaginary frequencies. GIAO-based ^{13}C NMR chemical shifts were calculated at the B3LYP/6−311+G(2d,p) DFT level. Reported shifts are based on a benzene reference (128.5 ppm) calculated at the same level of theory.[12] To determine the accuracy of this computational model as applied to this general class of compounds, ^{13}C NMR shifts were initially calculated for **1**, **3**, and **4** and were compared to the literature reports (Table 1). These compounds have all been synthesized by unambiguous routes.

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TABLE 1. Comparison of the Calculated[a] vs. Experimental Carbon Resonances for Compounds 1, 3, and 4

carbon	$\delta_{calcd.}$ 1 (ppm)	$\delta_{expt.}$ 1 (ppm)[5]	$\delta_{calcd.}$ 3 (ppm)	$\delta_{expt.}$ 3 (ppm)[7]	$\delta_{calcd.}$ 4 (ppm)	$\delta_{expt.}$ 4 (ppm)[7]
1			132.9	134.7	161.0	161.5
2	142.2	147.1				
4	160.1	161.7	159.6	160.8	137.9	139.1
4a	121.2	119.8	129.4	127.8	131.2	130.0
5	123.2	122.0	124.0	121.7	122.7	122.7
6	127.9	127.5	132.3	132.6	136.0	135.6
6a	137.7	136.4	136.6	135.3	135.1	134.4
7	127.8	128.8	129.8	129.3	127.7	128.6
8	129.2	130.0	128.2	129.2	128.9	128.9
9	126.4	127.9	127.5	128.4	129.7	129.4
10	127.3	125.4	123.4	123.3	131.1	128.5
10a	132.3	130.2	129.6	127.2	133.0	130.4
10b	149.9	148.0	128.1	127.8	124.5	123.8

[a] Calculated chemical shifts based on a benzene reference (δ 128.5 ppm).

TABLE 2. Comparison of Samoquasine A and Calculated 2[a]

carbon	$\delta_{calc.}$ 2 (ppm)	δ_{exp} samoquasine A[3] (ppm)	Δ(2 − samoquasine A) (ppm)
1	98.2	101.6	−3.4
2	133.0	132.8	0.2
4	161.0	164.0	−3.0
4a	118.2	118.7	−0.5
5	152.2	150.5	1.7
6a	152.1	148.5	3.6
7	133.2	136.0	−2.8
8	130.5	130.4	0.1
9	126.2	128.7	−2.5
10	123.1	125.0	−1.9
10a	122.7	123.6	−0.9
10b	142.9	144.3	−1.4

[a] Calculated chemical shifts based on a benzene reference (δ 128.5 ppm).

FIGURE 1. Previously proposed structures of samoquasine A.

As can be seen from Table 1, the calculated chemical shifts were in excellent agreement with those determined experimentally. For **1**, the maximum absolute difference between predicted and observed shifts was 4.9 ppm (C-2), and the mean absolute difference was 1.7 ppm. The computed shifts for **3** and **4** were of even greater accuracy than those for **1**. The maximum absolute difference and the mean absolute difference for **3** were found to be 2.4 and 1.1 ppm, respectively, whereas those calculated for compound **4** were found to be 2.6 and 0.9 ppm, respectively.

Upon validating this computational model, attention turned to the calculation of the ¹³C shifts of other diazaphenanthrene isomers of the same molecular formula. For the purposes of this study, structures calculated all possessed the amide in various positions in the A-ring. The position of the nonprotonated nitrogen was varied between the A-, B-, and C-rings.

Inspection of the predicted shifts reveals that the carbonyl carbon shifts of most compounds are in good agreement with the value reported for samoquasine A (164.0 ppm), although predicted shifts were consistently of somewhat lower magnitude. This underestimation of this shift was also observed for **1**, **3**, and **4** (Table 1). The upfield signal reported for samoquasine A (101.6 ppm) is readily assigned as the carbon γ to the carbonyl (**2**, C-1). Similar shifts were predicted for the enamide termini in **6–9**, and **12–16** (see the Supporting Information). This upfield signal would appear as a distinguishing characteristic of the terminal carbon of the enamide moiety in this molecular scaffold.
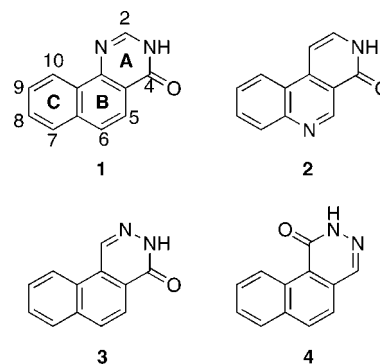
Performing a direct comparison of the predicted shifts with the data reported for the isolation of samoquasine A shows that only one compound, **2**, is in agreement based on chemical shifts, multiplicities, and 2-dimensional NMR correlations.[3,13] The summary of the ¹³C NMR shifts for **2** and samoquasine A is shown in Table 2.

The maximum absolute deviation in ¹³C NMR shifts between the data reported for samoquasine A and those calculated for **2** was found to be 3.6 ppm. The average deviation and the average absolute deviation, respectively, were found to be −0.9 ppm and 1.8 ppm. This accuracy is well within the margin of error observed for the known compounds **1**, **3**, and **4**.

It is worthwhile to comment on the literature discrepancies that have contributed to previous assertions that samoquasine A is not identical to perlolidine. A primary argument has been that the compound isolated by Wu and co-workers and identified as perlolidine possesses a different melting point (280−282 °C) and chloroform solubility than reported for samoquasine A (Mp reported: >300 °C). However, the melting point for the compound Wu identified as perlolidine also disagrees with several prior reports for perlolidine (Mp: 337−341 °C).[14] The most upfield signal in the ¹³C NMR spectrum of Wu's compound was at δ 118.6 ppm, which is in sharp contrast to the calculated shifts of the enamide terminus in **2** and other

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(13) If reported multiplicities and 2-dimensional correlations (ref 3) are disregarded and shifts are compared in numerical order, then one structure, **9** (see Supporting Information), is identified as having a smaller maximum absolute shift difference (3.4 ppm) than **2** (3.6 ppm). However, due to differences in multiplicities and 2-dimensional correlations between **9** and samoquasine A, structure **9** can definitively be precluded from further consideration.

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compounds in the present study. UV/vis spectral data are not included in Wu's isolation report. In the absence of extensive NMR data, UV/vis has historically been a key piece of information used in the identification of perlolidine. The UV/vis data of samoquasine A are in close agreement with literature reports.[14a,b] On the basis of the poor agreement between the available experimental data for perlolidine and the compound identified by Wu and co-workers as perlolidine, a strong argument can be made that the two are structurally different.

In conclusion, the structures and ^{13}C NMR shifts for a number of diazaphenanthrene alkaloids have been calculated. All exhibited amide carbonyl resonances that correlate well with those observed for samoquasine A. In all compounds possessing an enamide moiety, the most upfield signal in the spectrum was identified to be the carbon γ to the carbonyl. Furthermore, in comparison with multiplicities and 2-dimensional NMR cor-relations,[3] the only compound in this series that matches the reported data is **2**. The strong correlation between the chemical shifts reported for samoquasine A and those calculated for **2** supports the assertion that they are indeed identical.

Supporting Information Available: Structures, optimized coordinates, energies, and predicted ^{13}C NMR shifts for all 48 compounds calculated in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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