Conformational Study of C_2 Symmetrical Benzo[c]phenanthridine Alkaloid Derivatives

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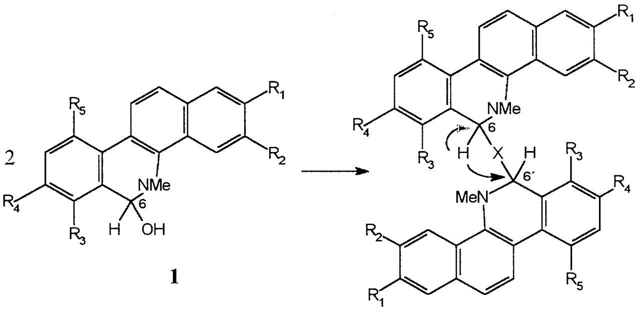
We report utilization of the phase-sensitive GSQMBC experiment for the study of benzo[c]phenanthridine derivatives. The technique allows determination of the C_2 symmetrical structures by observation of two different scalar interactions between H6 and C6 atoms as well as measurement of long range ^1H-^{13}C coupling constant. A prefered conformation determined using a dihedral angle constraint extracted from the measured $^3J_{H,C}$ coupling constant and AM1 calculations is discussed.

Formation of dimeric derivatives in benzo[c]phenanthridine alkaloids have been recently confirmed using gradient-enhanced HMBC experiments.[1] Monomers (**1**) and dimers (**2**) present during the reaction were unambiguously observed and distinguished (Figure 1). In continuation of our research on benzo[c]phenanthridine alkaloids we report now utilization of the phase-sensitive GSQMBC (Gradient-enhanced Single-Quantum Multiple Bond Correlation) experiment[2] for the study of their conformation.



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2: X = O
3: X = NH

Chelirubine: **a**: $R_1 + R_2 = R_3 + R_4 = \text{-OCH}_2\text{O-}$, $R_5 = \text{-OCH}_3$
Sanguinarine: **b**: $R_1 + R_2 = R_3 + R_4 = \text{-OCH}_2\text{O-}$, $R_5 = H$
Chelerythrine: **c**: $R_1 + R_2 = \text{-OCH}_2\text{O-}$, $R_3 = R_4 = \text{-OCH}_3$, $R_5 = H$

Figure 1.

Quaternary benzo[c]phenanthridine alkaloids reacting in alkaline environment are converted to the species called pseudobases (**1**), and to O or NH bridged species **2**, **3** (Figure 1).[3-5]

All ^1H- and ^{13}C-NMR signals of compounds specified in Figure 1 were assigned[5] using NOESY,[6] HSQC[7] and HMBC[8,9] experiments. The chemical shifts of H6, C6 and N5 atoms as well as the three-bond coupling constants $^3J_{H6,C6'}$ are summarized in Table 1.

The compounds **2** and **3** bear two stereogenic atoms (C6, C6′) and therefore mixtures of two diastereomers (racemate 6*S*,6′*S* + 6*R*,6′*R* and meso-form 6*R*,6′*S* ≡ 6*S*,6′*R*) with slightly different NMR chemical shifts are expected. For chelerythrine and sanguilutine dimeric structures no evidence of other diastereomer signals has been found. However, for compounds **2a**, **2b** and **3b** the NMR spectra showed that the amount of the minor isomer ranged between 5-15%. Considering thermodynamic-controlled formation of dimeric structures, the AM1 calculations indicate that the racemate (6*S*,6′*S* or 6*R*,6′*R*) is favored by 2.7 kcal over the meso-form in case of compound **2b**. Calculated energy difference for other alkaloids is even larger.

Table 1. Selected NMR chemical shifts (ppm) and coupling constants (Hz) for compounds **1-3** in $CDCl_3$ at 303K

	$^1H_6$	$^{13}C_6$	$^{15}N_5$	$^1J_{H6,C6}$	$^3J_{H6,C6'}$
1a	5.76	79.01	49.7	157	-
2a	6.20	78.97	40.1	156	2.8
1b	5.82	78.69	50.3	157	-
2b	6.33	79.09	41.2	156	3.5
3b	5.75	65.41	38.4	149	3.8
2c	6.60	77.38	39.0	160	2.3
3c	5.98	64.05	35.8	153	2.5

The three-bond ^1H-^{13}C interactions were detected using the HMBC and GSQMBC experiments with pulsed field gradients[2] for the selection of coherence transfer pathways.[10]

The three-bond coupling constants $^3J_{H6,C6'}$ are small and comparable in size to the natural line-width of the H6 protons. While in HMBC experiment only unresolved signals positioned at the proton chemical shifts of H6 were observed,[1] the GSQMBC spectra of compounds **2** and **3** showed clearly antiphase doublets of H6 signal at C6 chemical shifts with $^3J_{H6,C6'} = 2.3 - 3.8$ Hz (Figure 2). In addition, a large splitting of the H6 signals by single-bond interactions $^1J_{H6,C6} = 149 - 160$ Hz was detected in both experiments.

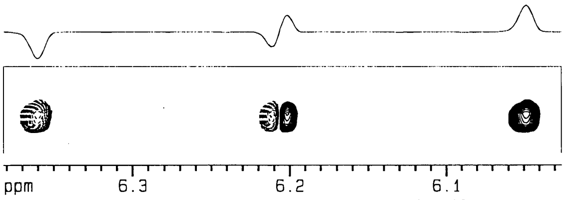

Figure 2. The H6-C6 correlation in the ^1H-^{13}C GSQMBC spectrum of **2a**.

The values of coupling constants summarized in Table 1 were obtained from separations of antiphase patterns in absorptive and dispersive part of the spectrum using analytical approach of Kim and Prestegard.[11] The coupling constants $^3J_{H6,C6'}$ for structures

2 and **3** range between 2.3 and 3.8 Hz (nearly the same for both diastereomers). This coupling constant provides information about the dihedral angle subtended by the coupled atoms. The Karplus equation[12,13]

$$^3J = A\cos^2\Phi + B\cos\Phi + C$$

parametrized for ^{1}H-C-O-^{13}C fragments[14,15] indicates either synclinal or anticlinal conformation as a preferred orientation of the O-linked aminal structure.

In order to determine unambiguously the prefered conformation, AM1 calculations[16] were performed. Two resulted structures[17] with synclinal and synperiplanar conformation differed in energy by more than 4.5 kcal. This difference points to the synclinal conformation (45°±10°NMR, 50±20°AM1) as the prefered one (Figure 3).

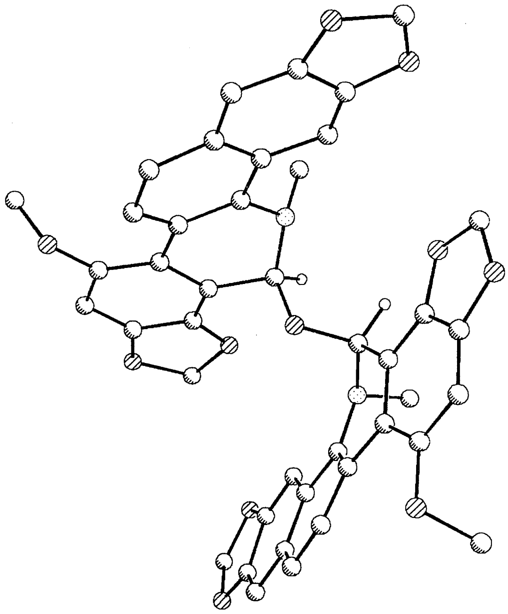

Figure 3. An ORTEP plot of AM1 minimized[17] structure of compound ***6S,6′S*-2a** (hydrogen atoms with exception of H6 and H6′ are omitted for clarity).

Further research will be conducted in order to complete a detailed study on benzo[c]phenanthridine alkaloids and their chemical behaviour.

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References and Notes

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10 The spectra were obtained on a 500 MHz Bruker DRX spectrometer using the pulse sequence: D1-90°(^{1}H)-D6--180°(^{1}H)/180°(^{13}C)-D6-90°(^{1}H)/90°(^{13}C)-t_1/2-180°(^{1}H)-t_1/2-G1-D16-180°(^{13}C)-G2-D16-D20-90°(^{1}H)/90°(^{13}C)-ACQ(t_2)/G3 with following parameters: D1 = 2.1 s, D6 = 33 ms, D16 = 100 ms, G = 1 ms, G1 : G2 : G3 =12 : 36 : ± 6 G/cm, echo - antiecho, spectral windows of 3 kHz in f_2 and 15.5 kHz in f_1, block size 8k x 0.8k. The ^{13}C and ^{1}H spectra were referenced to the solvent signal of $CDCl_3$ and signal of TMS at 77.00 ppm (^{13}C) and 0.00 ppm (^{1}H), respectively. ^{1}H - ^{15}N correlations were recorded by sequence desribed above using: D1 = 2.5 s, D6 = 50 ms, G1 : G2 : G3 = 4.8 : 52.8 : ± 4.8 G/cm, a 3250 Hz f_2 spectral window and an 2500 Hz f_1 spectral window, 32 - 128 scans acquired per increment, block size 4k x 0.5k. The ^{15}N spectra were referenced to the signal of liquid ammonia used as an external standard at 298 K.

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17 The quantum chemistry calculations were performed by the semiempirical AM1 method.[15] The heat of formation was calculated for varied values of dihedral angles H6-C6-O-C6′ and H6′-C6′-O-C6. The conformer with lowest energy is presented on Figure 3 with dihedral angles for fragment H-C-O-C almost in + synclinal conformation (*6S,6′S*-**2a** - +33°, +35°; **2b** - +69°, +69°; **2c** - +68°, +73°).

Dedicated to Professor Jaroslav Jonas on the occasion of his 60th birthday.