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Note

Sanguinarine pseudobase: re-examination of NMR assignments using gradient-enhanced spectroscopy

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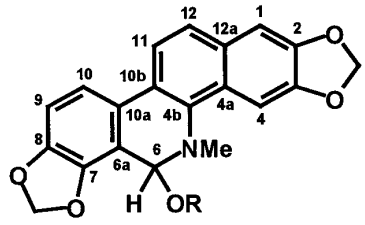
ABSTRACT: The formation of sanguinarine pseudobase (6-hydroxydihydrosanguinarine) was studied by 1D and 2D NMR spectroscopy. The unequivocal evidence of a hemiaminal OH group and unambiguous ^{1}H, ^{13}C and ^{15}N NMR assignments of this compound are discussed. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; 2D NMR; NMR assignment; ^{1}H NMR; ^{13}C NMR; ^{15}N NMR; sanguinarine pseudobase; 6-hydroxydihydrosanguinarine; 6-methoxydihydrosanguinarine; 6-ethoxydihydrosanguinarine; benzophenanthridine alkaloids

INTRODUCTION

Sanguinarine is a quaternary benzo[*c*]phenanthridine alkaloid, widely distributed in the plants of the *Papaveraceae* and other families,[1] displaying considerable susceptibility towards nucleophiles.[2] Upon action of hydroxide anion, quaternary sanguinarine is converted into its free base. The covalent adduct of OH^- to the iminium bond is 6-hydroxydihydrosanguinarine (**1a**), which is often called by a historical term, pseudobase[3] or alkanolamine.[4] Pseudobase formation is assumed to be the first step in a transformation leading to bis[6-(5, 6-dihydrosanguinarinyl)] ether, which is the real free base of sanguinarine.[5] We have found[5] that sanguinarine pseudobase, **1a**, was formed by spontaneous hydrolysis of sanguinarine free base, due to moisture present in a solid sample and $CDCl_3$. Continuing our research on free bases of benzophenanthridine alkaloids, we have re-examined the previously published[5,6] NMR spectrum of **1a**. Using a more sophisticated approach based on gradient-enhanced NMR experiments, additional data were obtained. In order to facilitate the ^{13}C NMR assignment of **1a**, the NMR spectra of sanguinarine alkoxy adducts **1b** and **1c** were also recorded. These derivatives are easily prepared by treatment of sanguinarine free base with an appropriate alcohol.[2]

The aim of this study was to prove unequivocally the structure of sanguinarine pseudobase and the existence



1a: R = H
1b: R = Me
1c: R = Et

of a hemiaminal OH group. Until now no direct evidence[2] for an OH group covalently bound to carbon C-6 has been presented. As will be shown, under favourable conditions this covalent bond is readily detected in an NMR experiment by observation of the scalar coupling between the hydrogen of the OH group and the H-6 atom.

RESULTS AND DISCUSSION

The ^{1}H, ^{13}C and ^{15}N chemical shifts of sanguinarine pseudobase **1a** and compounds **1b** and **1c** are listed in Table 1. The assignment of ^{1}H NMR signals was obtained using the two-dimensional NOESY experiment.[7] The ^{13}C NMR signals were assigned using phase-sensitive gradient-enhanced HSQC experiments for observation of direct[8] and long-range connectivities.[9]

The chemical shifts of the H-6 (5.83 ppm) and C-6 (78.91 ppm) atoms are typical markers of the hemiaminoacetal **1a**. The pseudobases of biogenetically related alkaloids show analogous chemical shifts for the H-6 (C-6) atoms: chelirubine 5.76 (79.01), chelerythrine 6.04 (79.31), sanguilutine 6.00 (79.03) and chelilutine

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Table 1. NMR data for compounds **1a–c** ($CDCl_3$)

	^{1}H NMR				^{13}C NMR			
Atom	**1b**	**1c**	**1a**	**1a**[a]	**1b**	**1c**	**1a**	**1a**[a,b]
1	7.12	7.12	7.11	7.71	104.66	104.63	104.54	108.5
2					147.44	147.40	147.58	
3					148.10	148.02	148.23	102.2
4	7.69	7.66	7.65	7.13	100.63	100.68	100.77	104.4
4a					126.87	126.92	127.11	
4b					138.21	138.53	138.00	141.9
6	5.37	5.48	5.83	5.40	85.92	84.26	78.91	89.7
6a					113.18	113.40	113.88	116.8
7					145.28	145.24	145.27	
8					147.25	147.31	147.34	
9	6.93	6.92	6.92	6.94	108.84	108.72	108.90	112.8
10	7.41	7.40	7.42	7.42	116.36	116.39	116.51	
10a					125.87	125.90	125.24	
10b					122.81	122.97	122.31	
11	7.75	7.76	7.75	7.77	120.23	120.31	120.09	127.6
12	7.47	7.47	7.49	7.49	123.74	123.60	124.06	124.0
12a					131.10	131.04	131.06	
7,8-OCH_2O	6.05	6.05	6.07	6.06	101.12	101.69	101.83	105.0
	6.11	6.11	6.11	6.07				
2,3-OCH_2O	6.05	6.05	6.04	6.13	101.06	101.04	100.92	105.7
	6.05	6.05	6.04					
NMe	2.79	2.76	2.74	2.80	40.88	40.87	40.63	44.8
OH			2.15	7.97				
				4.25				
OMe	3.45				54.10			
CH_2		3.68				61.68		
		3.92						
Me		1.10				15.06		

	^{15}N NMR		
	1b	**1c**	**1a**
5	40.3	41.3	50.3

[a] Data from Ref. 6.
[b] Only explicit ^{13}C assignments are quoted.

5.97 (79.06) ppm.[10–13] The signal of the hemiaminal OH group emerged as a doublet at 2.15 ppm ($J = 3.8$ Hz) with the same splitting appearing for the signal of the H-6 atom. In order to prove unequivocally the interaction between H-6 and the hydrogen of the OH group in a complex NMR spectrum, a gradient-enhanced DQF-COSY spectrum was measured. To minimize the measuring time, the experiment was arranged as one-dimensional using selective 90° pulse excitation.[14] When the signal of the OH group was excited by an EBURP2-shaped pulse,[15] only one interaction with the H-6 atom (5.83 ppm) was detected (Fig. 1). Alternatively, selective excitation of H-6 resulted in spectra showing only the signal of the OH group. For a sample containing *ca.* 100–300 μg of **1a**, unambiguous evidence for an H6—C6—O—H three-bond scalar interaction was obtained within 3 min.

Revising our previous results,[5] the assignment of the signals of carbon atoms C-2, C-3, C-8, C-10a and C-10b for compound **1a** was made in the following way. The signals at δ 145.27 and 147.34 ppm showed long-range interactions with the H-9 signal at 6.92 ppm. The resonance at 145.27 ppm was assigned to C-7 because of a long-range interaction with the H-6 atom. Consequently, the signal at δ 147.34 ppm corresponds to the C-8 atom. The assignment of signals at δ 147.58 and 148.23 ppm is based on the observed heteronuclear long-range coupling constants. In general, the larger couplings in aromatic systems belongs to three-bond interactions and smaller couplings to two-bond interactions. The constants were obtained from the antiphase doublets-observed in the HSQC experiment[9] and analysed by the approach of Kim and Prestegard.[16] For the H4–C2 and H4–C3 interactions; the coupling constants ^3J(H-4,C-2) = 8 Hz and ^2J(H-4,C-3) = 5.5 Hz were obtained, and similarly, ^3J(H-1,C-3) = 8 Hz and ^2J(H-1,C-2) = 5.5 Hz. Therefore, the resonance at 147.58 ppm was assigned to C-2 and that at 148.23 ppm to C-3. The signal at 125.24 ppm belongs to C-10a, due to correlation with the H-6 atom.

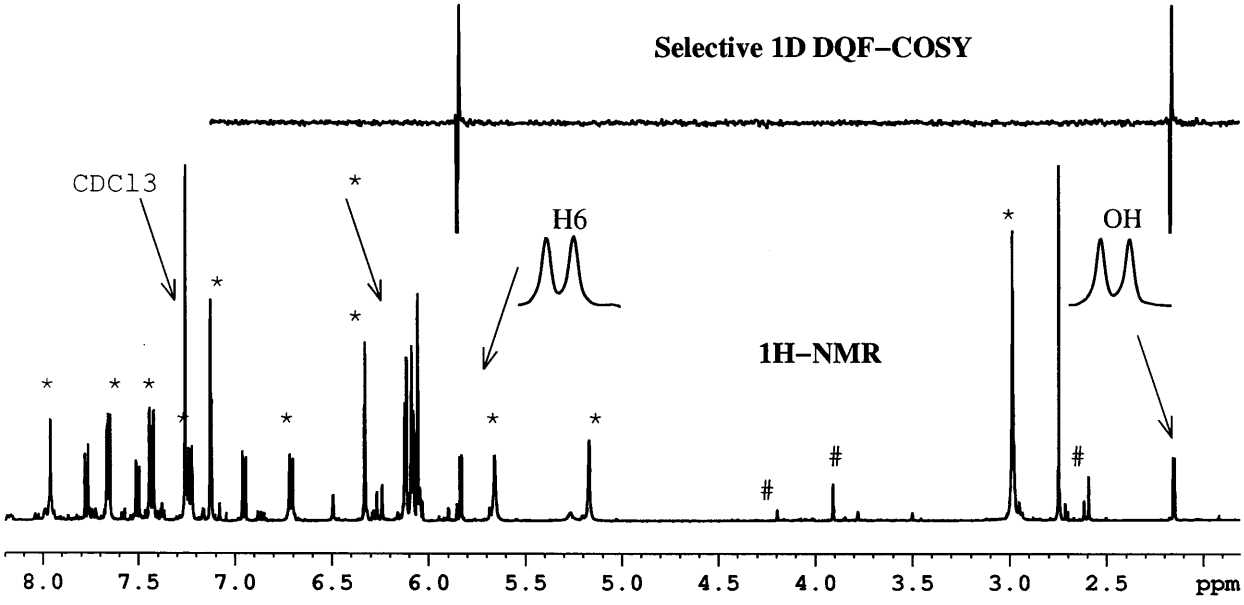

Figure 1. The ^{1}H NMR spectrum (bottom) and the selective 1D DQF-COSY spectrum (top) of a mixture of 6-hydroxydihydrosanguinarine (**1a**) and bis(dihydrosanguinarinyl) ether (marked by asterisks).[5] Other minor signals belong to the second diastereomer[5,12] of bis(dihydrosanguinarinyl) ether and the products of **1a** disproportionation (dihydrosanguinarine, oxysanguinarine)[5] (marked by #).

Recently, we published the first ^{15}N NMR data on benzo[*c*]phenanthridine alkaloids and their derivatives.[12,17] For the investigation of ^{15}N chemical shifts and ^{1}H–^{15}N coupling pathways, long-range HMBC[17–20] and HSQC[9,20] experiments were applied. For sanguinarine pseudobase (**1a**), 6-methoxydihydrosanguinarine (**1b**) and 6-ethoxydihydrosanguinarine (**1c**), N-5 chemical shifts of 50.3, 40.3 and 41.3 ppm, respectively, were recorded. The coupling constants ^{2}J(H-6,N-5) = 3.7 Hz and ^{2}J(NMe,N-5) = 3.5 Hz were measured for compound **1a**.

In the literature, oxygen-nucleophile adducts to sanguinarine, namely 6-methoxydihydrosanguinarine (**1b**) and 6-ethoxydihydrosanguinarine (**1c**), are frequently claimed to be isolated from plants as genuine alkaloids.[21–24] Recently, 6-hydroxydihydrosanguinarine (**1a**) has been reported to be obtained from *Dactylicapnos torulosa* Hook f. et Thoms.[6] However, in plants, owing to an acidic environment of their tissues, only the quaternary benzophenanthridine alkaloids can be present. In the reported extraction procedure,[6,25] the crude alkaloid extract was treated with 5% HCl. If compound **1a** had occurred in plants as a natural alkaloid, the strong acid used during the extraction procedure would have converted it immediately into a quaternary cation of sanguinarine.[2] Similarly, in other earlier reports,[21–24] the acids were used in the early steps of extraction procedures. As discussed above, also in these cases compounds **1b** and **1c** were probably produced during the chemical manipulations and cannot be classified as genuine alkaloids.

Zhang *et al.*[6] described compound **1a** as a yellow powder and presented its m.p., optical rotation, UV, IR, ^{1}H, ^{13}C NMR and HR-EIMS data. In the IR spectrum, a broad absorption at 3450 cm^{-1} in KBr was attributed to the OH group. However, the HR-EI mass spectrum with a value of *m/z* 335 does not confirm the structure **1a**. The molecular mass of sanguinarine pseudobase **1a** is 349 ($C_{20}H_{15}NO_5$) and the mass of the quaternary ion ($M^+ - OH$) is 332. The peak at *m/z* 332 is usually a prominent peak in the mass spectra of 5,6-dihydrosanguinarine derivatives, corresponding to the relatively stable quaternary cation of sanguinarine.[1,5] The chemical shifts of the H-6 and C-6 atoms published by Zhang *et al.*[6] also differ significantly from our data (Table 1). A proton NMR signal of the OH group is inconsistently reported in the Discussion (δ = 4.25, br s) and in the Experimental section (δ = 7.97, s) in Ref. 6. Also, the signals of H-1 and H-4 are incorrectly assigned. We have obtained clear evidence for the assignment of the H-1 and H-4 atoms in **1a** from two-dimensional NOESY experiment.[7] The H-1 atom at 7.11 ppm displayed a significant cross peak with H-12 at 7.49 ppm and also the NOE interaction between H-4 (7.65 ppm) and NMe (2.74 ppm) was clearly detected.

Our data unambiguously confirm the structure of the title compound, **1a**. The proof of 6-hydroxydihydrosanguinarine (**1a**) is based on the observation of the coupling between the OH proton and the H-6 atom. The data reported previously[6] do not correspond to the structure discussed and probably relate to another derivative of sanguinarine.

EXPERIMENTAL

Spectra

All the spectra were recorded in $CDCl_3$ using a Bruker

Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^{1}H), 125.76 MHz (^{13}C) and 50.68 MHz (^{15}N). The temperature was 303 K. Sample concentrations ranged from 500 μg ml^{-1} to 40 mg ml^{-1}. TMS was used as an internal standard for ^{1}H and ^{13}C spectra and liquid ammonia as external standard for ^{15}N spectra. The pulse conditions were a 90° pulse, duration 7.7 μs for ^{1}H, 16 μs for ^{13}C and 21 μs for ^{15}N (5 mm triple-resonance inverse probehead {^{1}H/BB/^{13}C} equipped with a self-shielded *z*-gradient coil) and 6.3 μs for ^{13}C (5 mm probehead for direct observation of heteronuclei). Computer processing was performed with Bruker XWINNMR software. Gradient pulses used in this study were shaped to a 1% truncated sine envelope with the exception of GSQMBC experiments, where square gradients were applied. For optimization of ^{1}H–^{15}N GSQMBC experiments, see Refs 9, 12, 17 and 20.

The following conditions were applied: ^{1}H spectra, spectral width 4500 Hz and size of data block 16K; ^{13}C spectra, spectral width 27 670 Hz and size of data block 48K; and selective 1D gradient-enhanced DQF-COSY,[14] evolution delay 60 ms, 90° selective EBURP2 pulse 40 ms, gradient pulse duration 800 μs, post-gradient recovery 300 μs, gradient ratio 15:30 G cm^{-1}, spectral width 5000 Hz and size of data block 16K.

Compounds

Sanguinarine chloride was isolated from *Sanguinaria canadensis* L.[26] Bis[6-(5,6-dihydrosanguinarinyl)] ether (sanguinarine free base) was prepared using the following procedure. Sanguinarine chloride was dissolved in H_2O and the solution was made alkaline with saturated Na_2CO_3 in aqueous solution. The precipitate was filtered off and dried. M.p. 243–246 °C. IR (Nujol), 1251, 1189, 1040, 942, 862 cm^{-1}. DCI MS (CH_4–N_2O), *m/z* $[M + H]^+$ 681 (0.9), 665 (3), 362 (19), 348 (17), 332 (100), 318 (23); MS/MS of *m/z* 681, 332 (100). ^{1}H and ^{13}C NMR: see Ref. 5. 6-Hydroxy-5,6-dihydrosanguinarine (**1a**) (sanguinarine pseudobase) is a product of hydrolysis of sanguinarine free base observed in an NMR tube. Compounds **1b** and **1c** were prepared by repeated crystallization of sanguinarine free base from methanol and ethanol, respectively.

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