CARBON-13 NMR ASSIGNMENTS OF BERBERINE AND SANGUINARINE

Gábor Blaskó[1], Geoffrey A. Cordell*, Sutatip Bhamarapravati, and Christopher W.W. Beecher

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

Abstract - Unambiguous proton and carbon-13 nmr assignments for the protoberberine alkaloid, berberine (1) and for the benzo[c]phenanthridine alkaloid, sanguinarine (2) were accomplished through the judicious use of APT, CSCM 1D and selective INEPT spectroscopy.

INTRODUCTION

Among the isoquinoline alkaloids, the protoberberines and the benzo[c]phenanthridines are the most important and well-known quaternary species. Recently, both groups of alkaloids have been the target of increased chemical and biological interest because some of their members have been found to possess potent anticancer activity[2]. Moreover, benzo[c]phenanthridines occupy a position at the end of a long and intriguing biosynthetic pathway in which protoberberines play a pivotal role as key intermediates derived directly from norcoclaurine, a universal precursor of a large number of different isoquinoline alkaloids[3]. As a prelude to anticipated biological and biosynthetic studies in this area we became interested in the ^{13}C nmr spectral data of quaternary protoberberine and benzo[c]phenanthridine alkaloids. Several monographs[4,5] and reviews[6,7] have reported detailed ^{13}C nmr studies on isoquinoline alkaloids. However, we were very surprised to find no previous ^{13}C nmr data on either berberine and related quaternary protoberberines or on any of the quaternary benzo[c]phenanthridines[8,9].

We wish to report the first unambiguous assignment of the proton and carbon-13 nmr spectra of the protoberberine alkaloid, berberine (1) and of the benzo[c]phenanthridine alkaloid sanguinarine (2).

DISCUSSION

The unambiguous assignments of the ^{13}C nmr spectra of the representative alkaloids, berberine (1) and sanguinarine (2), were based on two recently developed pulse programming sequences; the CSCM 1D[10] and the selective INEPT[11] techniques. In the CSCM 1D experiment, a carbon satellite either upfield or downfield of a particular proton is irradiated, magnetization is transferred to the attached carbon and then a corresponding signal, either positive (upfield irradiation) or negative (downfield irradiation) is observed. In the selective INEPT experiment, a particular proton is irradiated with a soft pulse resulting in thorough magnetization transfer and enhancement of carbon atoms three bonds away from the irradiated proton. The above two techniques were used, to assign the protonated and non-protonated carbons respectively. It should be noted that both the CSCM 1D and selective INEPT experiments require a well resolved and unambiguously assigned proton spectrum. The ^{1}H nmr data of berberine (1) and sanguinarine (2) are summarized in Table 1.

Table 1. ^{1}H NMR DATA OF BERBERINE (1) AND SANGUINARINE (2)[a]

Berberine (1)			Sanguinarine (2)		
Proton	Chemical shift		Proton	Chemical shift	
1	7.79 s		1	7.72 s	
4	7.09 s		4	8.24 s	
5	3.22 t	(5.6)	6	10.11 s	
6	4.95 t	(5.6)	9	8.70 d	(8.7)
8	9.91 s		10	8.09 d	(8.7)
11	8.20 d	(8.7)	11	8.56 d	(9.2)
12	8.01 d	(8.7)	12	8.23 d	(9.2)
13	8.96 s		5-CH_3	4.91 s	
2,3-OCH_2O	6.17 s		2,3-OCH_2O	6.35 s	
9-OCH_3	4.07 s		7,8-OCH_2O	6.61 s	
10-OCH_3	4.10 s				

[a] All spectra were recorded in DMSO-d_6 at 361.07 MHz. Chemical shifts (δ) are given in ppm using TMS as internal standard. Coupling constants are given in Hz in parentheses. The assignments are similar to the previous data for berberine (1)[12] and sanguinarine (2)[13] obtained at 60 MHz.

As shown in its APT spectrum (Figure 1), berberine (1) has two methyl, three methylene, six methine and nine quaternary carbon atoms. The three methylene signals at δ26.35, 55.19 and 102.07 were assigned to C-5, C-6 and 2,3-methylenedioxy carbon, respectively. CSCM 1D irradiation of the

^{13}C satellites of H-1, H-4, H-8, H-11, H-12 and H-13 resulted in the magnetization transfer to their carbon atoms appearing at δ105.43, 108.43, 145.42, 126.67, 123.53 and 120.18, respectively. The nine quaternary carbon atoms could be subdivided as either ring-junction (δ120.42, 121.37, 130.64, 132.94 and 137.41) on oxygen-substituted (δ143.61, 147.63, 149.76 and 150.36). Selective INEPT irradiation of H-8 enhanced the carbon signals at δ55.19 (C-6), 132.94, 137.41 and 143.61, of which the latter could be assigned to C-9. The two other quaternary carbon enhancements C-12a and C-13a, were distinguished when H-11 was irradiated resulting in enhancements at δ143.61 and 132.94 confirming the attribution of C-9 and permitting the assignment of C-12a, respectively. Irradiation of H-12 enhanced a methine carbon signal at δ120.18 (C-13) as well as an oxygenated quaternary carbon (δ150.36) and a ring-junction carbon (δ121.37), which were assigned to C-10 and C-8a, respectively. Polarization transfer from H-13 resulted in enhancement at δ121.37 (C-8a), and 120.42, which should be the signal of C-13b. Irradiation of the protons in the methylenedioxy group enhanced two oxygenated quaternary carbon signals (δ147.63 and 149.76). These were distinguished by the two following experiments (Figure 2). Irradiation of H-1 enhanced the signal at δ149.76, which must be C-3, at δ137.41 (C-13a) and 130.64 which consequently should be assigned to C-4a. Finally, irradiation of H-4 permitted the assignment of C-2 and C-13b (δ147.63 and 120.42, respectively). The complete assignment of the ^{13}C nmr spectra of berberine (1) is shown in Table 2.

Figure 1

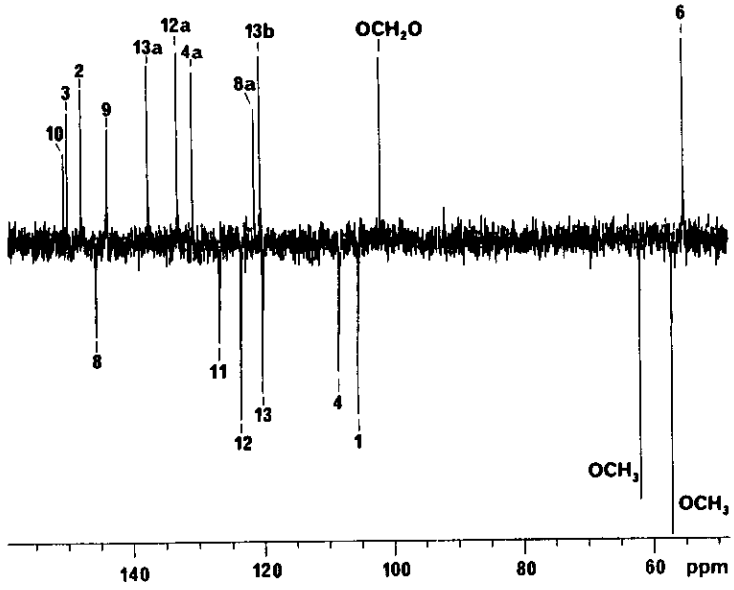
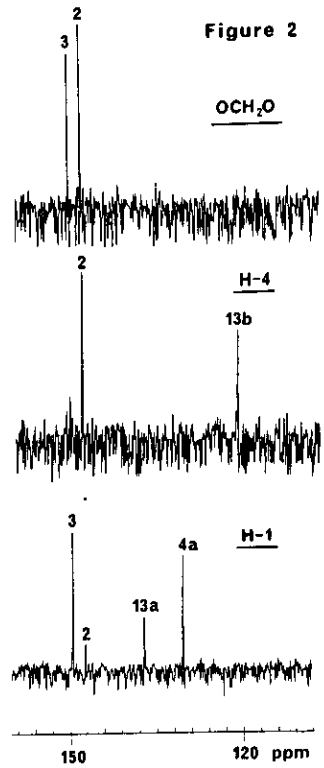


Figure 2



The benzo[c]phenanthridine alkaloid sanguinarine has twenty carbon atoms, but in its ^{13}C nmr spectrum two of these overlapped. The ten quaternary carbons could be assigned as either oxygenated (δ 146.00, 147.27, 148.50, 148.52) or ring-junction (δ109.15, 119.90, 125.28, 126.78, 131.00, 131.87) carbon atoms, respectively. The remaining carbon atoms from the APT spectrum (Figure 3) were observed as seven methine carbons (δ103.96, 105.54, 117.05, 118.53, 119.68, 131.00, 149.49) and a methyl group (δ52.09). Initially, only the latter signal could be assigned unambiguously. CSCM 1D irradiation of the ^{13}C satellites of H-1, H-4, H-6, H-9 and H-10, as well as the two methylenedioxy groups permitted magnetization transfer to their carbon atoms. But it was not until the SINEPT irradiations of H-6 and the methylenedioxy protons were conducted that the proton assignments of the methylenedioxy groups could be firmly established through the attribution of C-7 (Figure 4). Two other signals, at δ131.00 and 126.78, were also enhanced through polarization transfer on irradiation of H-6, and these should be C-4b and C-10a. They were distinguished when the quaternary methyl group was irradiated to enhance C-6 (δ149.49) and C-4b (131.00). Irradiation of H-10 enhanced the signals at δ147.27, assigned to C-8, and the signals at 131.87 and 125.28. The latter two signals, C-6a and C-10b, were distinguished through the irradiation of H-12. Irradiation of H-1 permitted the assignment of C-3 and C-4a (δ148.52 and 119.90, respectively) and the remaining carbon, C-12a, was attributed through the irradiation of H-11. With this result, assignment of all carbons of sanguinarine (2) was complete and is shown in Table 2.

Figure 3

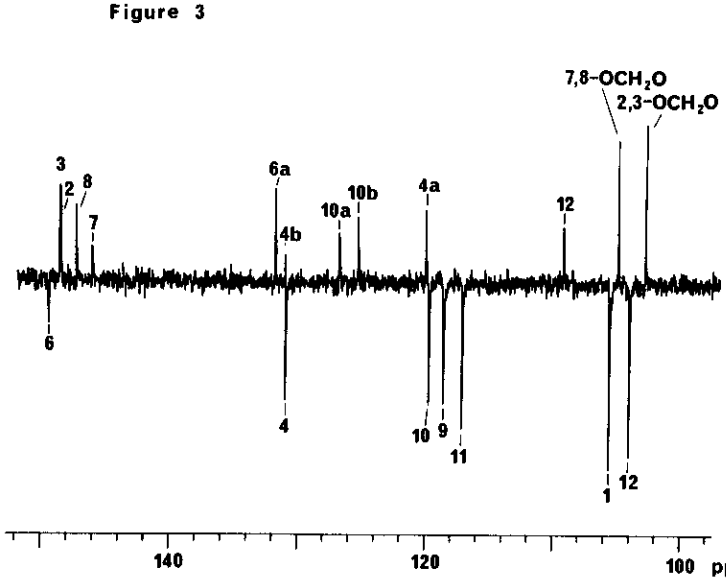
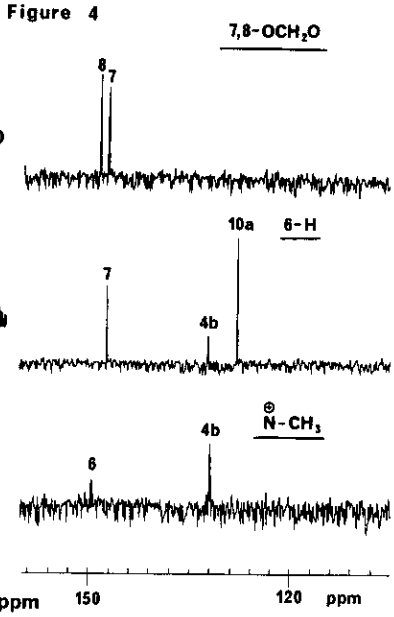
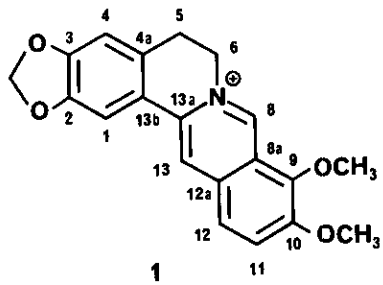


Figure 4





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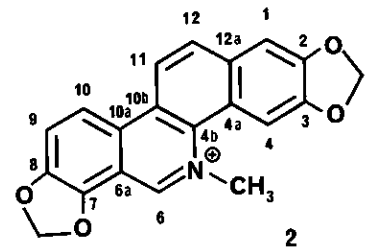

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Table 2. ^{13}C NMR DATA OF BERBERINE (1) AND SANGUINARINE (2)[a]

Berberine (1)		Sanguinarine (2)	
Carbon	Chemical shift	Carbon	Chemical shift
1	105.43	1	105.54
2	147.63	2	148.50
3	149.76	3	148.52
4	108.43	4	131.00
4a	130.64	4a	119.90
5	26.35	4b	131.00
6	55.19	6	149.49
8	145.42	6a	131.87
8a	121.37	7	146.00
9	143.61	8	147.27
10	150.36	9	118.53
11	126.67	10	119.68
12	123.53	10a	126.78
12a	132.94	10b	125.28
13	120.18	11	117.05
13a	137.41	12	103.96
13b	120.42	12a	109.15
2,3-OCH_2O	102.07	2,3-OCH_2O	102.71
OCH_3	57.06	7,8-OCH_2O	104.84
OCH_3	61.95	N-CH_3	52.09

[a] All spectra were recorded in DMSO-d_6 at 90.54 MHz. Chemical shifts (δ) are given in ppm using TMS as internal standard (δ_{TMS} = 0 ppm).

EXPERIMENTAL

The ^{1}H and ^{13}C nmr spectra were obtained on a Nicolet NMC 360 spectrometer operating at 361.07 and 90.54 MHz, respectively. The one-dimensional heteronuclear ^{1}H-^{13}C shift correlation (CSCM 1D) and selective INEPT experiments were performed with data sets of 16K covering a spectral width of 10,000 Hz. A value of 160 Hz was applied as ^{1}H-^{13}C one bond coupling constant and of 8Hz for the three bond coupling constant throughout the CSCM 1D and SINEPT experiments, respectively.

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