UNAMBIGUOUS CARBON-13 NMR ASSIGNMENTS OF SOME BIOLOGICALLY ACTIVE PROTOBERBERINE ALKALOIDS

Raouf A. Hussain,[1] Jinwoong Kim,[2] Christopher W.W. Beecher, and A. Douglas Kinghorn*

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

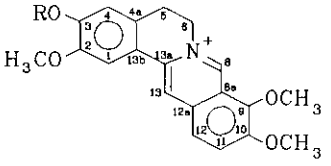
Abstract - Unambiguous proton and carbon-13 nmr chemical shifts of the protoberberine quaternary alkaloids palmatine (1) and jatrorrhizine (2), their tetrahydro derivatives (3, 4), and tetrahydroberberine (5) have been determined through the use of 2D, CSCM 1D and selective INEPT nmr techniques.

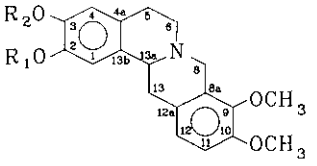
INTRODUCTION

Sphenocentrum jollyanum Pierre (Menispermaceae) is a woody herb or shrub growing in West Africa from Ivory Coast to Nigeria.[3] Its roots are acidic and bitter, and are used in traditional medicine as an aphrodisiac, appetizer, laxative, stimulant and wound healer.[3] It has also been reported that, when chewed, the roots of this species impart a sweet sensation to food chewed thereafter.[3,4] However, such activity was not substantiated by Inglett and May,[4] nor by observation in our laboratory. In the present study, chromatographic work-up of the chloroform-soluble fraction of an aqueous methanol extract of S. jollyanum furnished two protoberberine alkaloids, palmatine (1) and jatrorrhizine (2), as previously reported,[5] which were identified as their chloride salts, and by conversion to their tetrahydro derivatives, (3) and (4), respectively, with sodium borohydride.

Plants that contain protoberberine alkaloids are reported to be used as analgesics, antiseptics, sedatives and stomatics in Chinese folkloric medicine.[6] In Indian and Islamic folkloric medicine, such plants are used for bleeding disorders and eye diseases, and as antiseptics, sedatives, stomatics and uterine muscle depressants.[7] Both quaternary alkaloids and their tetrahydro derivatives possess many substantiated biological and therapeutic effects, e.g., palmatine (1), jatrorrhizine (2) and tetrahydropalmatine (3) have been reported to show in vitro anti-malarial activity.[8] In China, tetrahydropalmatine (3) is used as an analgesic, and has also been reported to exhibit bradycardial, hypotensive and sedative activities.[9,10]

As part of our interest in the application of modern nmr techniques in the structural elucidation and biosynthesis of natural products,[11] we have determined the first unambiguous ^{13}C nmr assignments for palmatine (1), jatrorrhizine (2) and tetrahydrojatrorrhizine (4), with especial use of two modern pulse techniques, CSCM 1D (Carbon Spectrum Contour Mapping 1D) and selective INEPT (Selective Insensitive Nuclear Enhancement Polarization Transfer),[12,13] in addition to conventional ^{13}C and 2D nmr methods.[14] We have been able to clarify certain tentatively assigned carbon signals of tetrahydropalmatine (3) and tetrahydroberberine (5).[15-17] The present investigation will serve as a basis upon which complete ^{13}C chemical shifts of any protoberberine alkaloid may be readily determined. Unambiguous ^{13}C nmr assignments have so far been published for only one other protoberberine alkaloid, namely, berberine.[18]





	R			R1	R2	
1	CH_3	Palmatine	3	CH_3	CH_3	Tetrahydropalmatine
2	H	Jatrorrhizine	4	CH_3	H	Tetrahydrojatrorrhizine
			5	-CH_2-		Tetrahydroberberine

RESULTS AND DISCUSSION

Data obtained using conventional ^{13}C nmr [proton-noise decoupled, Attached-Proton Test (APT) and Single Frequency Off-Resonance Decoupled (SFORD)], 2D ^{1}H-^{13}C HETeronuclear shift CORrelated (HETCOR) and CSCM 1D techniques for tetrahydropalmatine and tetrahydroberberine (3 and 5) provided useful information concerning the ^{13}C nmr chemical shifts of the protonated carbons. These findings (Tables 1 and 2) agreed well with values previously published.[15-17] However, the utilization of these techniques was insufficient to resolve ambiguities among the non-protonated carbons, especially C-2, C-3, C-4a, C-8a, C-12a and C-13b. The advantage of using the selective INEPT technique to clarify this problem is illustrated in Figure 1, where the irradiation of H-1 (δ 6.79, J_{CH}= 6 Hz) of 3 selectively enhanced C-4a at δ 126.69 as well as an oxygenated quaternary carbon, C-3 at δ 147.34. In addition, irradiation of H-4 (δ 6.66, J_{CH}= 6 Hz) led to a selective enhancement of C-13b at δ 129.59 and C-2 at δ 147.53. In the same fashion, irradiation of H-11 (δ 6.77) enhanced carbons at a three-bond distance, namely C-12a (δ 128.61) and C-9 (δ 150.21). Similarly, two quaternary carbons (δ 127.66 and 144.98) were polarized upon irradiating H-12 (δ 6.92), and could therefore be assigned as C-8a and C-10, respectively. In the same manner, C-4a, C-8a and C-12a of compound 5 were assigned to resonances observed at δ 127.47, δ 127.40 and δ 128.04, respectively, following the irradiation of H-1, H-11 and H-12 (δ 6.73, 6.83 and 6.87, J_{CH}= 6 Hz).

Table 1. ^{1}H Nmr Assignments for Compounds 1-5[a]

Proton	Compound				
	1	2	3	4	5
1	7.66 s	7.58 s	6.73 s	6.70 s	6.73 s
4	7.02 s	6.98 s	6.66 s	6.65 s	6.59 s
5	3.30 t (5.7)	3.25 t (5.6)			
5 ax.			3.10 m	3.10 m	3.15 m
5 eq.			2.65 m	2.64 m	2.60 m
6	4.97 t (5.7)	4.90 t (5.6)			
6 ax.			2.68 m	2.66 m	2.64 m
6 eq.			3.23 m	3.18 m	3.20 m
8	9.79 s	9.83 s			
8 ax.			3.54 d (15.7)	3.55 d (15.7)	3.49 d (15.5)
8 eq.			4.31 d (15.7)	4.27 d (15.6)	4.22 d (15.5)
11	8.08 d (8.2)	8.01 d (8)	6.77 d (8)	6.78 d (8)	6.83 d (8.2)
12	7.86 d (8.2)	7.90 d (8)	6.92 d (8)	6.86 d (8)	6.87 d (8.2)
13	8.86 s	8.82 s			
13 ax.			2.91 dd (15.8,12.2)	2.88 dd (15.6,12)	2.81 dd (15.7,12)
13 eq.			3.27 dd (15.8,4)	3.25 dd (15.6,4)	3.23 dd (15.7,4)
13a			3.49 dd (12.2,4)	3.46 dd (12,4)	3.40 dd (12,4)
OCH_2O					5.88 s
$2OCH_3$	3.88 s	3.82 s	3.85 s[b]	3.84 s	
$3OCH_3$	3.88 s		3.86 s[b]		
$9OCH_3$	3.96 s	3.91 s	3.88 s	3.85 s	3.83 s
$10OCH_3$	3.99 s	4.01 s	3.90 s	3.89 s	3.81 s

[a] Chemical shifts (δ) are given in ppm using TMS as internal standard. Coupling constants (Hz) are shown in parentheses.

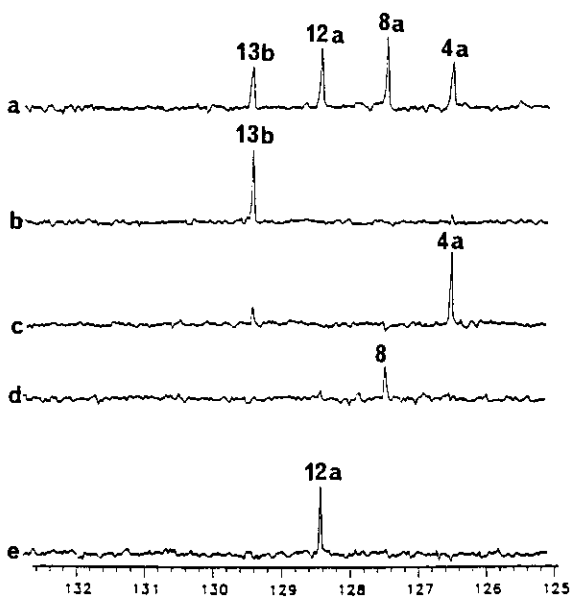
[b] Values interchangeable.

Table 2. ^{13}C Nmr Assignments for Compounds 1-5[a]

Carbon	Compound				
	1	2	3	4	5
1	108.67	110.90	108.47	107.70	105.54
2	149.57	149.36	147.53	147.11	146.44
3	150.59	146.46	147.34	146.97	146.27
4	111.12	116.26	111.23	114.19	108.46
4a	133.97	130.21	126.69	127.41	127.47
5	27.23	29.06	29.09	28.83	29.14
6	56.76	57.58	51.49	51.46	51.54
8	145.03	145.19	53.98	53.95	53.91
8a	119.08	119.22	127.66	127.62	127.40
9	152.41	151.51	150.21	150.18	150.43
10	144.53	145.19	144.98	144.96	144.98
11	123.60	122.89	110.84	110.82	111.30
12	126.82	125.68	123.81	123.77	124.19
12a	128.26	128.16	128.61	128.59	128.04
13	120.52	119.25	36.32	36.35	35.96
13a	138.28	134.92	59.30	59.34	59.78
13b	122.14	120.94	129.59	129.08	130.36
OCH_2O					100.96
$2OCH_3$	57.21	58.30	55.79	55.81	
$3OCH_3$	57.21		55.79		
$9OCH_3$	62.50	62.98	60.13	60.17	60.22
$10CH_3$	56.43	57.03	56.01	55.99	55.87

[a] Chemical shifts (δ) are given in ppm using TMS as internal standard. (δ_{TMS} = 0 ppm)

Figure 1. Downfield region of the ^{13}C nmr spectra of tetrahydropalmatine (3). (a) Proton-noise decoupled spectrum, (b-e) selective INEPT spectra obtained by irradiation of H-4, H-1, H-12 and H-11, respectively (J_{CH}= 6 Hz).



Data collected after performing CSCM 1D nmr experiments for compounds 3 and 5 showed very good agreement with values obtained from ^{1}H-^{13}C HETCOR nmr pulse sequence. All of the above-mentioned nmr techniques, excluding ^{1}H-^{13}C HETCOR, were utilized to obtain the ^{13}C nmr chemical shifts of compounds 1, 2 and 4 (Table 2). Thus, the value and importance of the modern nmr pulse techniques in obtaining unambiguous ^{13}C chemical shift assignments for a group of biologically important alkaloids are clearly demonstrated in this study.

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2. Present address: College of Pharmacy, Seoul National University, Seoul, Korea.
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