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TWO DIMENSIONAL NMR STUDIES OF CHAKSINE

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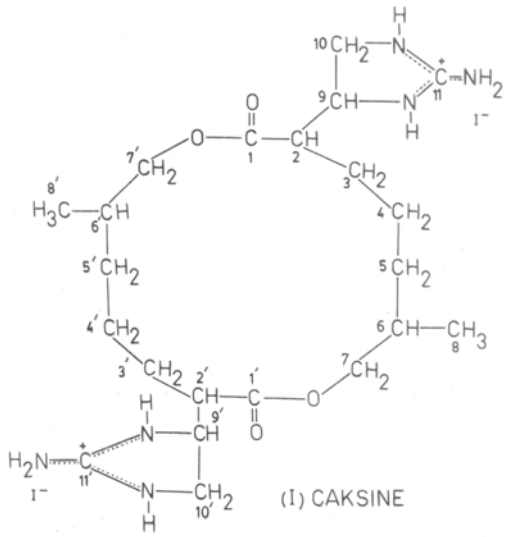
ABSTRACT

Assignments of ^{1}H and ^{13}C-nmr signal of chaksine were made with the help of two dimensional ^{1}H-^{1}H homonuclear and ^{1}H-^{13}C heteronuclear shift correlation.

Introduction

Chaksine, an alkaloid isolated from *Cassia absus* Linn. by Siddiqui and Ahmad in 1935 has been subjected to chemical investigation during the course of which it was assigned various functional groups and structures. Wiesner et, al. in 1958 ultimately reported a structure based on chemical data and its degradation studies. Subsequently, Fowler, Valenta and Wiesner in 1962 supported the structure of chaksine on the basis of NMR spectra of some of its derivatives. Recently our group has revised the structure of chaksine (I) based on X-ray crystallography and has shown that it consists of 16 membered dilactone ring. This is also indicated by FAB and FD mass spectrometery of



(I) CAKSINE

Table 1: ^{1}H and ^{13}C chemical shifts for chaksine iodide

Carbon No.	^{13}C-Assign-ments ppm	Jc-H Hz	APT	^{1}H Atta-ched in carbon No. δ	^{1}H Assign-ments chemi-mical shifts	Multiplicity	JH-H (Coupling Constants) Hz.
1/1′	171.88(s)	–	C	2/2′	2.52	m	–
2/2′	48.87(d)	133.6	CH	3A/3A′	1.41	m	–
3/3′	28.10(t)	126.8	CH_2	3B/3′B	1.53	m	–
4/4′	24.07(t)	124.5	CH_2	4/4′	1.05	m	–
5/5′	32.74(t)	127.4	CH_2	5/5′	1.35	m	–
6/6′	31.96(d)	127.0	CH	6/6′	1.70	m	–
7/7′	67.79(t)	147.9	CH_2	7A/7′B	3.96	dd	$J_{gem} = 10.7\ J_{7\text{-}6} = 3.0$
8/8′	16.65(q)	125.2	CH_3	7B/7′B	3.64	dd	$J_{gem} = 10.7\ J_{7\text{-}6} = 3.0$
9/9′	56.34(d)	149.38	CH	8/8′	0.83	d	$J_{8\text{-}6} = 6.7$
10/10′	45.76(t)	146.0	CH_2	9/9′	4.10	ddd	$J_{9\text{-}2} = 9.7\ J_{9\text{-}10A} = 6.5$
10/11′	158.87(s)	–	C	10A/10′A	3.68	d	$J_{gem} = 10.0\ J_{9\text{-}10B} = 6.5$
				10B/10′B	3.25	dd	$J_{gem} = 10.0\ J_{9,\ 10} = 6.5$
				NH_2	7.56(2H)	br(s)	
				NH	7.78	br(s)	
				NH	8.10	br(s)	

chaksine iodide which showed a prominent peak at m/z 451 (MH^+). With the revision of structure of chaksine it has became imminent to furnish NMR data of the alkaloid. We present in this paper the assignments of ^1H and ^{13}C signals based on ^1H-^1H homonuclear and ^1H-^{13}C heteronuclear NMR shift correlations, off resonance and Gated Spin Echo (GASPE) spectra of chaksine.

Experimental

^1H and ^{13}C NMR data were obtained from spectra determined at 9.4 Tesla NMR spectrometer (Bruker WM 400), using DMSO-d_6 as solvent and TMS as internal standard.

The heteronuclear two dimensional ^1H and ^{13}C chemical shift correlation diagram was obtained using a 5 mm dual probehead. Data acquisition was performed taking a spectral width of 8000 Hz (^{13}C, F_2) and of ± 2000 Hz (^1H, F_1). 32 scans times 512 increments were accumulated to provide a matrix of 2K x 512W (t_2, t_1), which was transformed into (F_2, t_1) and then into (F_2, F_1) employing shifted sine bell function of 11/4. The resulting digital resolution in the F2 domain was 3.9 Hz/point, in the F1 domain 3.2 Hz/point. The refocusing delay was 1.9 ms, the mixing delay 3.7 ms. the relaxation delay 3 s and quadrature phase cycline was used.

The homonuclear ^1H-^1H chemical shift correlated two-dimensional diagram was obtained using the decoupling coil of the dual probehead. A COSY 45 experiment was performed with the phase program for N-type selection. The spectral widths were 2000 Hz (F_2) and ± 1000 Hz (F_1). 16 scans times 512 increments were accumulated to provide a matrix of 2Kx512W (t_2, t_1,), which was transformed in both dimensions as in the previous case by weighting with sine bell function. The resulting digital resolution in both dimensions was 2Hz/point. Quadrature phase cycling was performed using a relaxation delay of 4 s.

An spin echo experiment (Gated Spin Echo, GASPE), where methyl and methine carbons provide positive peaks and methylene and quarternary carbon negative signals was carried out on a 1.9 Tesla NMR spectrometer (Bruker WP 80).

Results and Discussion

Since chaksine is a symmetrical dilactone the signals of corresponding pairs of protons and carbons (e.g. C-1/1' and H-2/H2' etc.) absorb at the same position in all cases.

CH_3 groups:

The two methyl groups absorb as a doublet in ^{1} H-NMR spectrum at δ 0.83 ppm ($J_{8,6}$ = 6.7 Hz). The 2D heteronuclear ^1H-^{13}C NMR spectrum clearly shows that C-8/8' absorb at δ 16.65 in the carbon domain, this signal appears, as expected, as a quartet in the off resonance spectrum.

CH_2, groups:

The protons of the methylene groups adjacent to oxygen atom (C-7/7') are magnetically non equivalent and therefore show a pair of double doublets at δ 3.64 and 3.94 (J_{gem} = 10.7 Hz, $J_{7,6}$ = 3.0 Hz). In the ^{13}C NMR spectrum C-7/7' absorb at 67.79 ppm and this assignment is supported by the 2D spectra. The methylene groups adjacent to nitrogen (C-10/10') likewise show in ^{1}H-NMR spectrum a double doublet (J_{gem} = 10.0 Hz) at δ 3.25 due to the non-equivalence of the protons. The carbons of these two methylene groups absorb at 45.76 ppm, appearing as a triplet in the off resonance spectrum. The NMR signals of the protons of methylene groups which are adjacent to carbon atoms, viz H-3/3', H-4/4' and H-5/5', are complicated and appear as multiplets. The signals of H-3/3' present at a relatively lower field δ 1.41 and 1.53 (each m) because of the presence of a carbonyl group in the β-position. The carbon of these two methylene groups absorb at 28.10 ppm. The methylene groups at C-4/4' and C-5/5' give multiplets at δ 1.05 and 1.35 and these signals appears at 24.07 and 32.74 ppm in ^{13}C NMR spectra respectively. The assignments are supported by heterononuclear correlated 2D spectrum as these are not very distignuishable in homonuclear proton-proton connectivity plot.

CH-group:

The C-2/2' signals in the ^{1}H-NMR spectrum is a multiplet at δ 2.52, its relatively downfield position being due to the proximity of the lactone carbonyl. The 2D heteronuclear spectrum shows the C-2/2' absorb at 48.87 ppm. The protons attached to C-6/6' absorb at δ 1.70 giving a rise to a multiplet at this position, whereas the corresponding carbons give a signal at 31.96 ppm. The C-9/9' is directly attached to one nitrogen atom of the guanidine ring and consequently the H-9/9' absorb as a doublet of doublet of doublet (ddd) at a lower field (4.10). The coupling constant between H 9/9' and H-2/2' is higher (9.7 Hz) than between H-9/9' and H10A/10'A and H 10B/10B' both of the latter being equal to 6.5 Hz. The shape of the H-9/9' signal consequently resembles a double triplet. It can be seen from the (Fig. 1) that C-9/9' absorb at 56.34 ppm.

Quaternary carbons:

The carbon (C-1/1') of the lactone absorbs in the normal range i.e. at 171.88 ppm, whereas the quaternary carbon of the guanidine ring gives a signal at 158.87 ppm,

Protons attached to nitrogen:

The protons of the NH_2 group absorb as a broad singlet at δ 7.56 whereas the NH protons show two broad singlets at δ 7.78 and 8.10. All of these three signals disappear when the sample solution is shaken with D_2O.

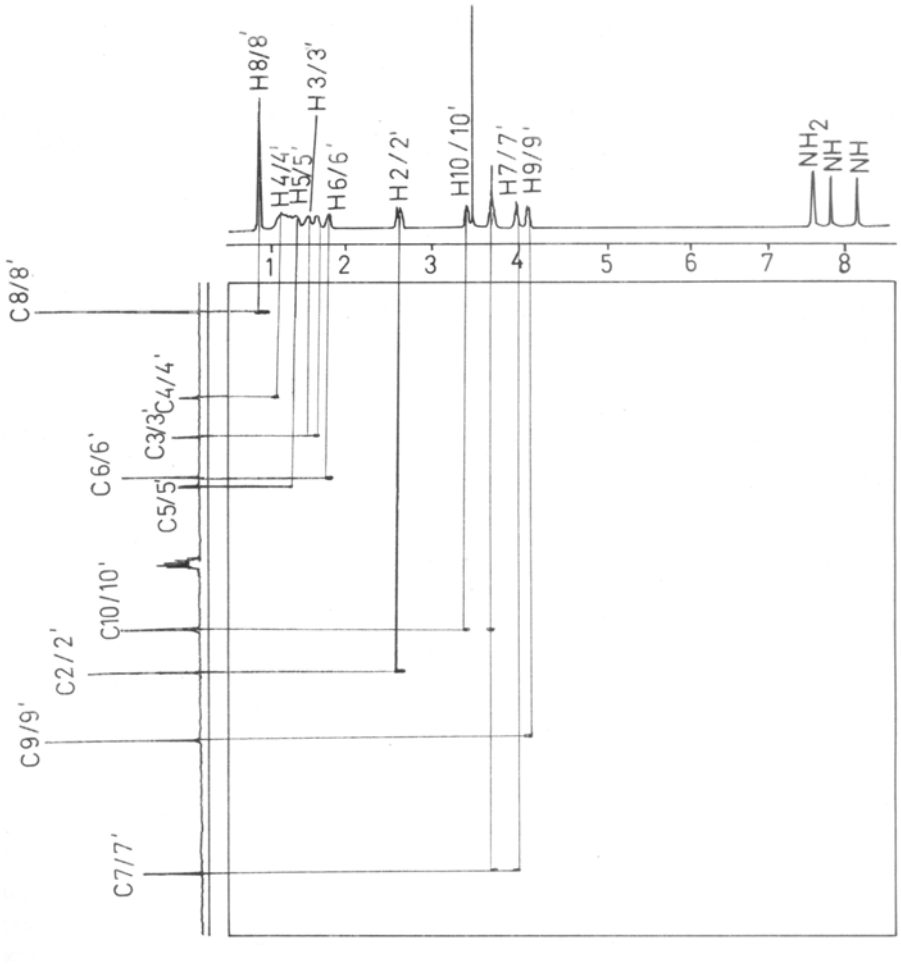


Fig. 1: Contor plot ^{1}H-^{13}C shift correlation of Chaksine iodide in DMSO-d_6

Acknowledgements

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