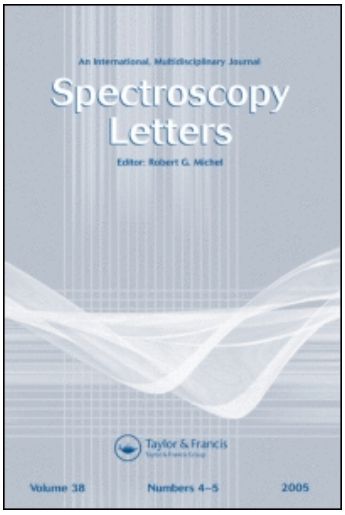

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The Application of 2D NMR Techniques in the Structural Assignment of the Diterpenoid Alkaloid, Delphinine

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THE APPLICATION OF 2D NMR TECHNIQUES IN THE STRUCTURAL ASSIGNMENT OF THE DITERPENOID ALKALOID, DELPHININE

Key Words: Delphinine, Natural products, NMR

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ABSTRACT

The two dimensional inverse detected heteronuclear correlation experiment HMQC and the homonuclear correlation experiments COSY and ROESY were performed on the natural alkaloid, delphinine enabling complete assignments of the ^{1}H and ^{13}C spectra. The stereochemistry of ring A and B have been determined in solution. The results suggest that the application of direct correlation multipulse NMR techniques allows for unambiguous structural assignment of delphinine.

INTRODUCTION

Delphinine [I] is a diterpenoid alkaloid, extracted from the seeds of *Delphinium staphisagria L.*, Ranunculaceae.[1] The alkaloid is poisonous leading

to respiratory paralysis and there may be concomitant cardiac damage.[2] Pelletier and Djarmati[3] have assigned the ^{13}C NMR spectrum of delphinine using chemical shift substituent effects generated by modification of the structure of the alkaloid. The authors established the degree of substitution for the individual carbon resonances using SFORD (single frequency off resonance decoupling) and assignments were made on the basis of chemical shifts calculated using additivity relationships and confirmed where possible by comparing the effects of specific structural changes. While assignments in general based solely on chemical shifts are often correct, in the past, we have found significant errors using this approach.[4] For this reason we have repeated the ^{13}C NMR assignment of delphinine using modern 2D pulse techniques. In addition we report the complete ^{1}H NMR assignment of delphinine as well as an analysis of the stereochemistry of the molecule. The ^{13}C assignments presented here are in agreement with literature values.

DISCUSSION

The ^{1}H (FIG. 1) and ^{13}C (FIG. 2) spectra of delphinine were fully assigned with the aid of various 2D multipulse NMR sequences viz COSY[5], HMQC[5] (FIG. 3), HETCOR[5] (long range) and ROESY[5] (FIG. 4). An edited DEPT analysis for protonated carbons confirmed that there are six CH_3 groups and six CH_2 groups as well as fourteen CH groups. Seven quaternary carbon atoms are present.

H_{14} (δ 2.89,*d*) was easily assigned since it was the most deshielded proton. The COSY spectrum confirmed the observed splitting pattern via correlation of H_{14} to H_9 and H_9 to H_{10}. In the long range HETCOR spectrum the OH group (δ 3.18) correlates with a quaternary carbon signal C_{13} which also shows three bond correlation to H_{10}. H_{16} reveals many correlations in the various 2D spectra.

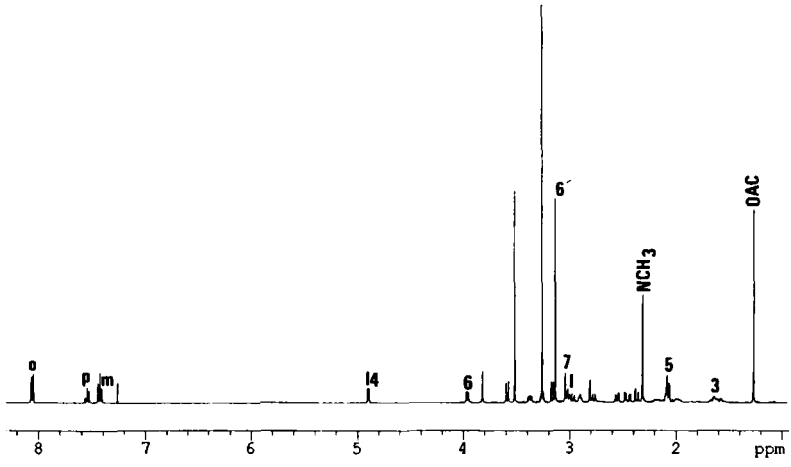

FIG. 1 ^{1}H nmr spectrum of delphinine showing assignments.

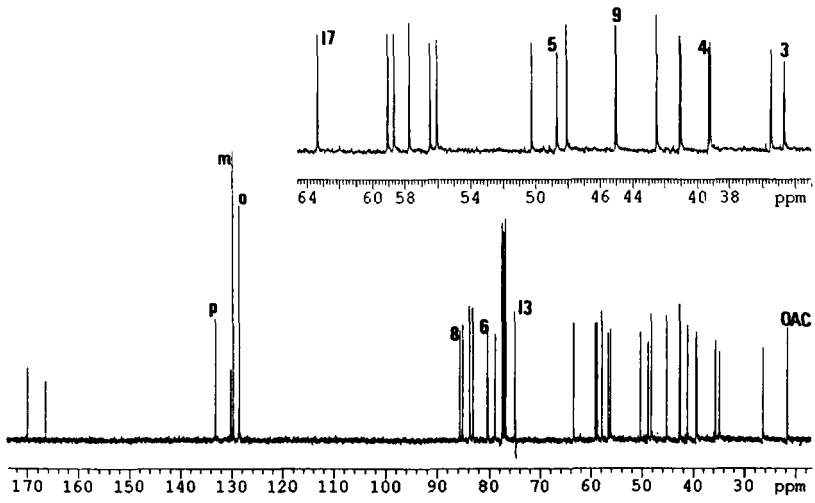

FIG 2. ^{13}C nmr spectrum of delphinine showing some of theassignments. For clarity, the δ 30-64 region has been expanded.

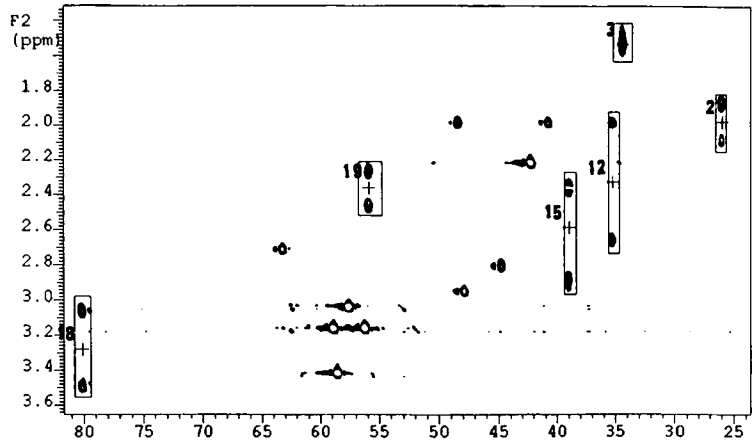

FIG 3. Expanded region of the HMQC spectrum showing the methylene groups.

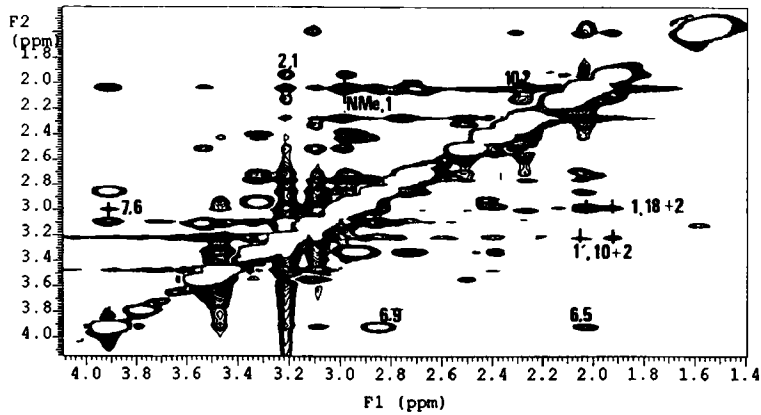

FIG 4. Expanded region of the ROESY spectrum showing through space correlations of the A and B ring protons.

Dipolar correlation of H_{14} to H_{16} (δ 3.37, *m*) as well as H_{14} to H_{10} correlation is observed in the ROESY spectrum thus indicating the nature of the stereochemistry about H_{10}, H_{14} and H_{16}. The long range HETCOR reveals coupling of H_{16} to $C_{16'}$ and $C_{16'}$ to $H_{16'}$ (δ 3.51). In the COSY spectrum H_{16} correlates to $H_{15\alpha\beta}$.

The methine protons of the benzoxy group have been assigned according to their splitting patterns and correlations. In the long range HETCOR spectrum a three bond correlation of the quaternary phenyl carbon to the meta protons is observed. The ortho protons appear further downfield. Pelletier and Djarmati[3] assigned the aromatic carbons of the benzoxy group attached to C_{14} by comparison with methyl benzoate. Other methine carbon resonances were separated into high and low field resonances with the latter believed to consist of hydroxy and methoxy substituted resonances.

Due to the inductive effect of the methoxy group, $H_{18\alpha\beta}$ (δ 3.57, *d* and 3.14ppm, *d*) is the most deshielded methylene group. These protons also correlate with C_{18} (δ 80.25) (HMQC spectrum) which shows coupling to methyl protons $H_{18'}$ (δ 3.26) in the long range HETCOR spectrum. Also observable in this spectrum is the correlation of $C_{18'}$ to $H_{18'}$ and H_{18}.

The correlation of the $H_{19\alpha\beta}$ methylene protons (δ 2.55 and 2.37) is evident in the COSY spectrum. Deshielded due to the influence of the nitrogen atom, C_{19} (δ 56.09) was assigned by analysis of the HMQC spectrum.

H_6 (δ 3.96, *d*) is deshielded by the oxygen atom of the methoxy group and in the ROESY spectrum a dipolar correlation to H_9 is observed. Additional confirmation of H_6 is the dipolar correlation to H_5. Assignment of C_6 (δ 82.93) follows directly from the observed one band C-H correlation in the HMQC spectrum. Pelletier and Djarmati[3] assigned C_6 and C_{16} tentatively as the ^{13}C chemical shift values are separated by only 0.7ppm.

In the long range HETCOR experiment , C_9 to H_7 and H_7 to C_8 correlations confirm the assignment of the H_7 resonance. Other quaternary groups assigned from the long range HETCOR experiment include C_4 and C_{11}. The influence of the oxygen substitution on quaternary carbons C_8 and C_{13} was used to differentiate these assignments from those of C_4 and C_{11}.

The assignment of the methylene carbons C_2 and C_3 was not an easy task but the increased sensitivity of the HMQC experiment was of assistance in assigning H_2 (δ 2.17 and 1.98) and H_3 (δ 1.63) as well as H_{12} (δ 1.88,1.98). The final set of assignments appear in Table 1 and Table 2.

Considering the stereochemistry of delphinine, there has been considerable controversy over the location of the ring A methoxy substituents. Birnbaum *et al*[6] have reported on the basic uncertainty about the conformation of ring A in alkaloids of the delphinine type. Using X-ray structure determination they concluded that ring A and B occur in a chair conformation, the five membered ring C is in an envelope conformation and ring D is a distorted half chair. They also reversed the stereochemistry of the C_1 methoxy group, placing it equatorial rather than axial (Fig 5).

Pelletier *et al*[7] have also reported on the X-ray crystallographic analysis of delphinine and pyrodelphinine, an alkaloid with a similiar ring system. Rings A, C, E and F have the same conformation in both molecules whilst the conformation of rings B and D differ due to a C_8-C_{15} double bond effect in pyrodelphinine. The D ring of delphinine is in a bent chair conformation with atoms C_8, C_9, C_{13}, C_{15}, C_{16} nearly coplanar and C_{14} forming the flap.

Confirmation of some aspects of the stereochemistry of ring A of delphinine was attempted via 2D dipolar through space correlation. The ROESY experiment provides transverse nuclear Overhauser effect and the dynamics of the

TABLE 1
^{1}H chemical shifts and assignments of Delphinine

Atom	δ_H	Multiplicity
1	2.96	d
2	2.17,1.98	m,m
3	1.60,1.63	m,m
5	2.07	m
6	3.96	d
7	3.04	s
9	2.90	m
10	2.09	m
12	2.55,2.36	dd,d
14	4.89	d
15	3.01,2.45	dd,dd
16	3.37	m
17	2.80	s
18	3.58,3.16	d,d
19	2.02,2.76	m,d
1′	3.26	s
6′	3.13	s
16′	3.51	s
18′	3.26	s
N-CH3	2.30	s
-O(C=O)CH_3	1.27	s
Phenyl	8.05 (*o*)	m
	7.42 (*m*)	m
	7.54 (*p*)	m

experiment results in positive nOe values for all correlation times.[8] From the ROESY experiment (FIG. 4) through space correlations were observed between H_5 and H_6, H_6 and H_9, and H_6 and H_7 indicating that ring B has a chair conformation in solution. On ring A, both H_1 and the attached methoxy group correlates with H_2 and H_{10} but a weaker correlation is observed for the methoxy group. Also N-Me correlates with H_1. This seems to indicate that the C_1 methoxy is equatorial and that H_1 is axial. Also H_{10} correlates with H_2, placing ring A in a chair conformation.

TABLE 2
^{13}C chemical shifts and assignments of delphinine

Atom	δ_C	δ_C Lit.[3]
1	84.98	84.9
2	26.35	26.3
3	34.69	34.7
4	39.31	39.3
5	48.72	48.8
6	82.93	83.0#
7	48.10	48.2
8	85.51	85.4
9	45.05	45.1
10	41.07	41.0
11	50.26	50.2
12	35.50	35.7
13	74.86	74.8
14	78.80	78.9
15	39.21	39.3
16	83.59	83.7#
17	63.38	63.3
18	80.25	80.2
19	56.09	56.1
N-CH3	42.51	42.3
1′	56.52	56.1
6′	57.77	57.6
16′	58.71	58.6
18′	59.10	58.9
C=O-CH3	169.82	169.4
	21.56	21.4
C=O-C_6H_5	166.36	166.0
	130.22(*q*)	130.4
	133.06(*p*)	132.8
	129.69(*o*)	129.6
	128.50(*m*)	128.4

Interchangeable literature assignments

EXPERIMENTAL

Delphinine was a kind donation from E. Merck (SA).

Experiments were performed in $CDCl_3$ solution at 25°C on a Varian Unity400MHz spectrometer using a 5mm inverse detection probe. Chemical shifts are relative to deuterochloroform at 7.25ppm for ^1H and 77.0ppm for ^{13}C spectra.

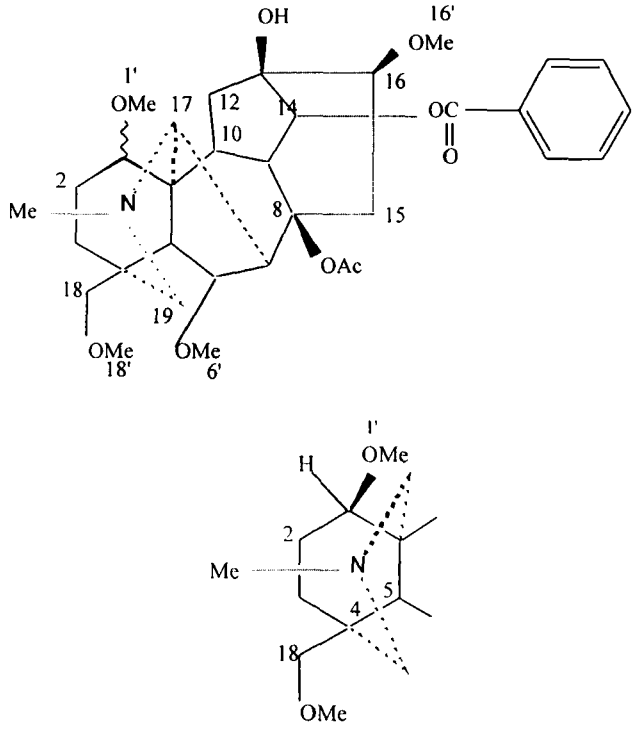

FIG 5. Stereochemical conformation of the ring A methoxyl of Delphinine.

The COSY spectrum was recorded using a 90° observe pulse with an acquisition time of 0.138s. 32 transients and 128 increments as well as a relaxation delay of 1s were applied. Data were processed as a 1024*1024 matrix using a sinebell of 0.064s in F1 and 0.017s in F2. Four transients with an acquisition time of 0.138s were recorded for each of the 512 increments of the HMQC spectrum. A relaxation delay of 2s was applied and the data were processed as a 1024*2048 matrix using a sinebell and shifted sinebell in F1 of -0.138s and in F2 a sinebell of 0.016s and a shifted sinebell of -0.014s. 256 increments and 128 transients were recorded for the long range HETCOR using an

acquisition time of 0.081s and a relaxation delay of 1s. Data were processed as a 4096*512 matrix with the application of a sinebell of 0.041s in F1 and 0.020s in F2. The ROESY spectrum was recorded using 4 transients for each of the 256 increments with an acquisition time of 0.138s. A mixing time of 150ms was applied and data were processed using a 1024*1024 matrix with a sinebell and shifted sinebell of -0.138s in F2 and a sinebell and shifted sinebell of -0.0037s in F1.

ACKNOWLEDGEMENTS

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