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Graham E. Jackson^a

^a Department of Inorganic Chemistry, University of Cape Town, Rondebosch, SOUTH AFRICA

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2D NMR STUDY OF VINDOLINE

Key Words: Vindoline, NMR, HETCOR, COSY

Graham E. Jackson

Department of Inorganic Chemistry, University of Cape Town,
Rondebosch 7700 SOUTH AFRICA

ABSTRACT:

Two Dimensional COSY and HETCOR experiments have been performed on the alkaloid vindoline which allow the complete ^1H and ^{13}C spectral assignments to be made. Proton-proton and proton-carbon coupling constants are also given.

INTRODUCTION:

Vindoline is the major alkaloid in the leaves of *Vinca rosea*, Linn¹. (*Catharapthus roseus* G. Don.) While vindoline occurs naturally it is also found bound to many indolic varieties, giving rise to the indole-indoline alkaloids. These alkaloids are powerful oncolytic agents². One such alkaloid, vincaleukoblastine, has been introduced clinically for the treatment of Hodgkins' disease, P1534 leukemia, Erlich ascites carcinoma and Freund ascites².

Both the ^1H and ^{13}C spectra of vindoline have been reported before^{3,4,5}. Initial proton resonance assignments were based on

chemical shift arguments but later corrected using a powerful, but tedious, decoupling difference spectroscopic technique³. On the other hand the ^{13}C resonance assignments were based on comparisons with model compounds. In view of the importance of this alkaloid it was felt necessary to confirm these assignments using the more modern 2D NMR approach.

Because of the large number of quaternary carbons it was found necessary to perform a long range HETCOR⁶ ($J_{\text{CH}} = 6 \text{ Hz}$) experiment in addition to the more normal homonuclear correlation (COSY)⁷ and heteronuclear correlation (HETCOR)⁸ experiments.

RESULTS AND DISCUSSION

^1H Assignments of vindoline

The proton spectrum of vindoline is shown in figure 1. Based on the coupling pattern the aromatic protons 9, 10 and 12 are readily assigned as are protons 14, 15, 17, 19 and 18. Resonance assignment in the range $\delta 2\text{--}4$ are not as simple as these are due to non equivalent methylene protons. The COSY spectrum (Fig 1) allows assignment of these signals. The coupling constants allow assignment of H-3_α and H-3_β as given in Table 1. Similarly assignment of H-5_α and β are possible.

Of all the proton resonances the hardest to assign are those of H-2 and H-21. This is because these two protons are in similar environments and do not couple to any other protons. Without explanation Gorman *et al*³ assign H-2 and H-21 to resonances at $\delta 3.75$ and 2.65 respectively. This assignment is confirmed by correlation with the ^{13}C spectrum (see later) but also by NOE difference spectroscopy. Irradiation of H-6 led to enhancement of resonances corresponding to H-2, 9 and 5. The final set of chemical shifts and coupling constants are given in Table 1.

^{13}C Assignment of vindoline

The HETCOR spectrum of vindoline is shown in figure 2. From this the assignment of the protonated carbons follows directly.

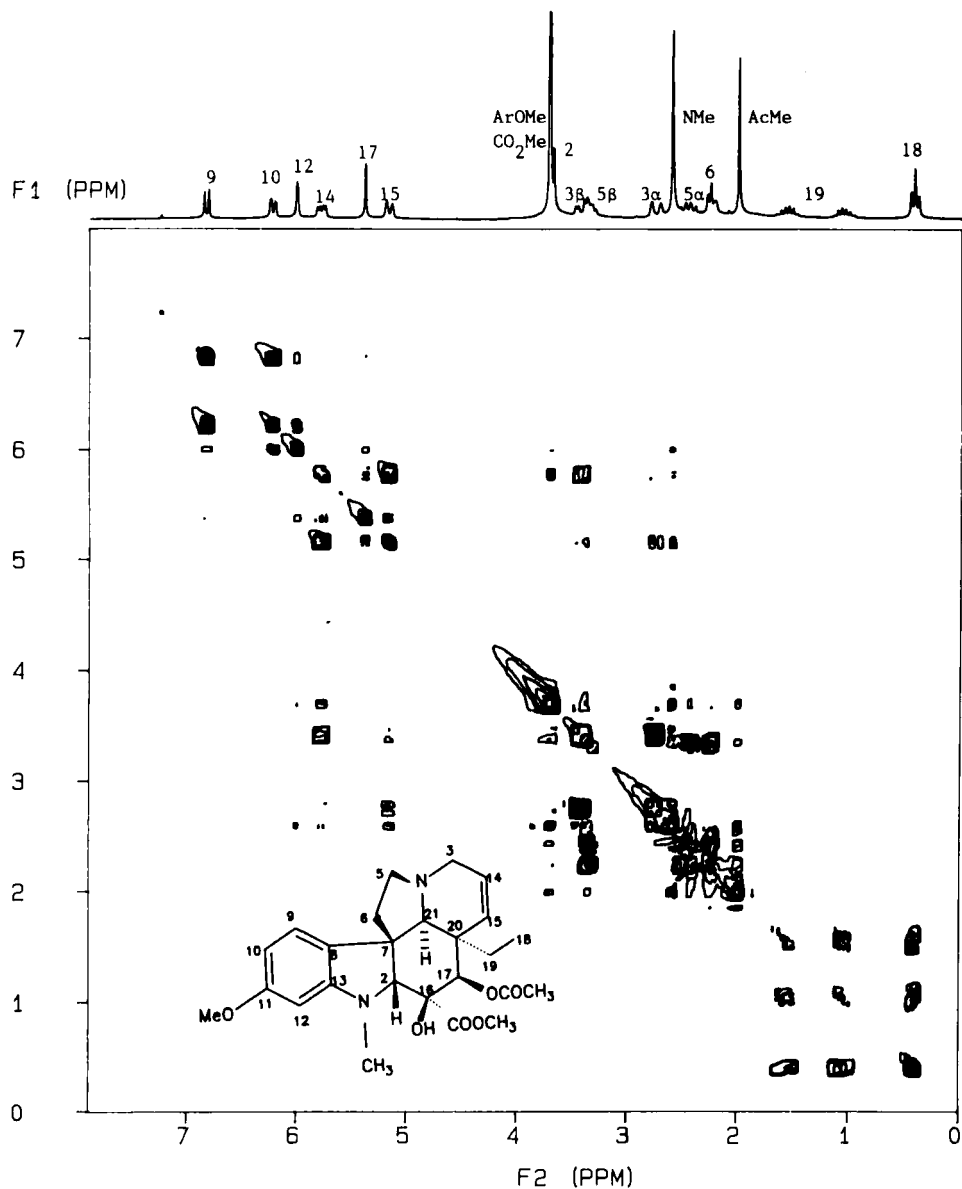


FIG. 1 200MHz COSY spectrum of vindoline.

TABLE 1
¹H Nmr Chemical Shifts and Coupling Constants for Vindoline

Proton	(ppm)	Coupling Constants (Hz)	
2	3.69		
3 _α	2.78	J _{3α,3β} = 16.9	J _{3α,14} = 1.4
3 _β	3.45	J _{3β,14} = 4.7	J _{3β,15} = 1.7
5 _α	2.49	J _{5α,5β} = 11.0	J _{5α,6α} = 10.0
5 _β	3.36	J _{5β,6α} = 4.0	J _{6β,6β} = 9.0
6 _α	2.21	J _{6α,6β} = 13.0	
6 _β	2.27	J _{5α,6β} = 7.0	
9	6.85	J _{9,10} = 8.2	
10	6.25	J _{10,12} = 2.2	
12	6.03		
14	5.81	J _{14,15} = 10.15	
15	5.19		
17	5.41		
18	0.43	J _{18,19} = 7.5	
19	1.59	J _{19,19'} = -14.5	
19'	1.08		
21	2.61		
OH	9.5		
NMe	2.62		
COCH ₃	2.02		
COOCH ₃	3.73		
OMe	3.74		

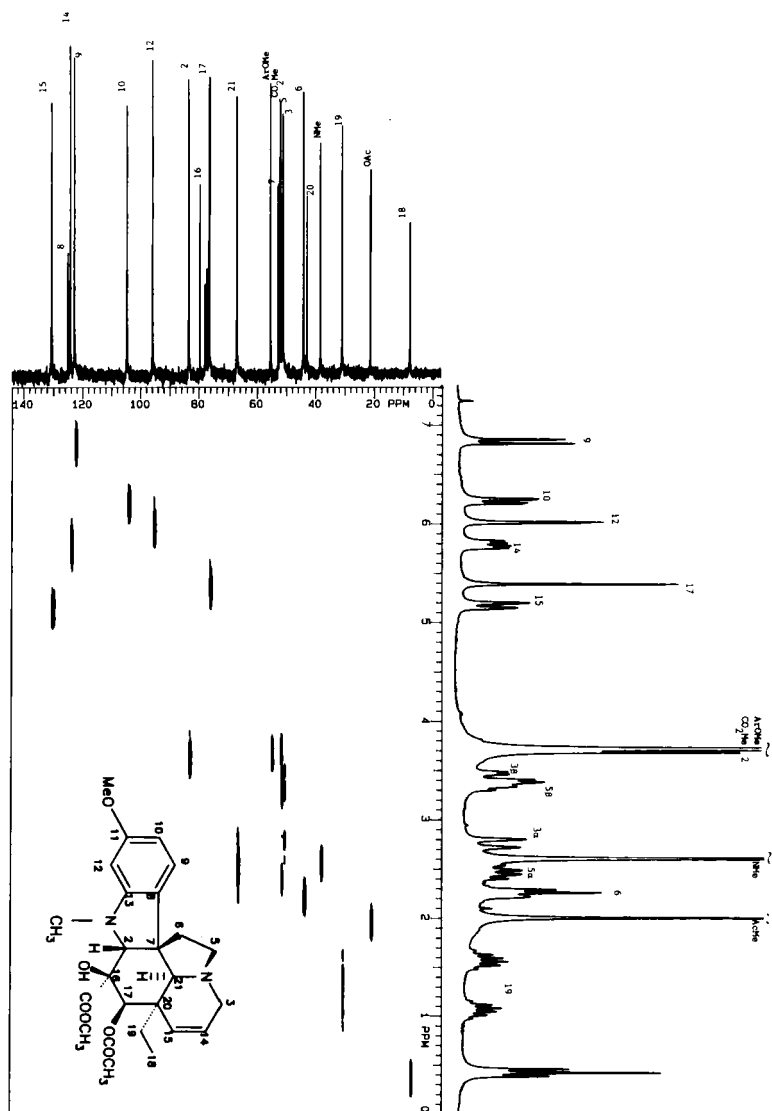


FIG. 2 Two dimensional heteronuclear shift correlated spectrum (HETCOR) of vindoline, using $J_{C,H} = 140$ Hz.

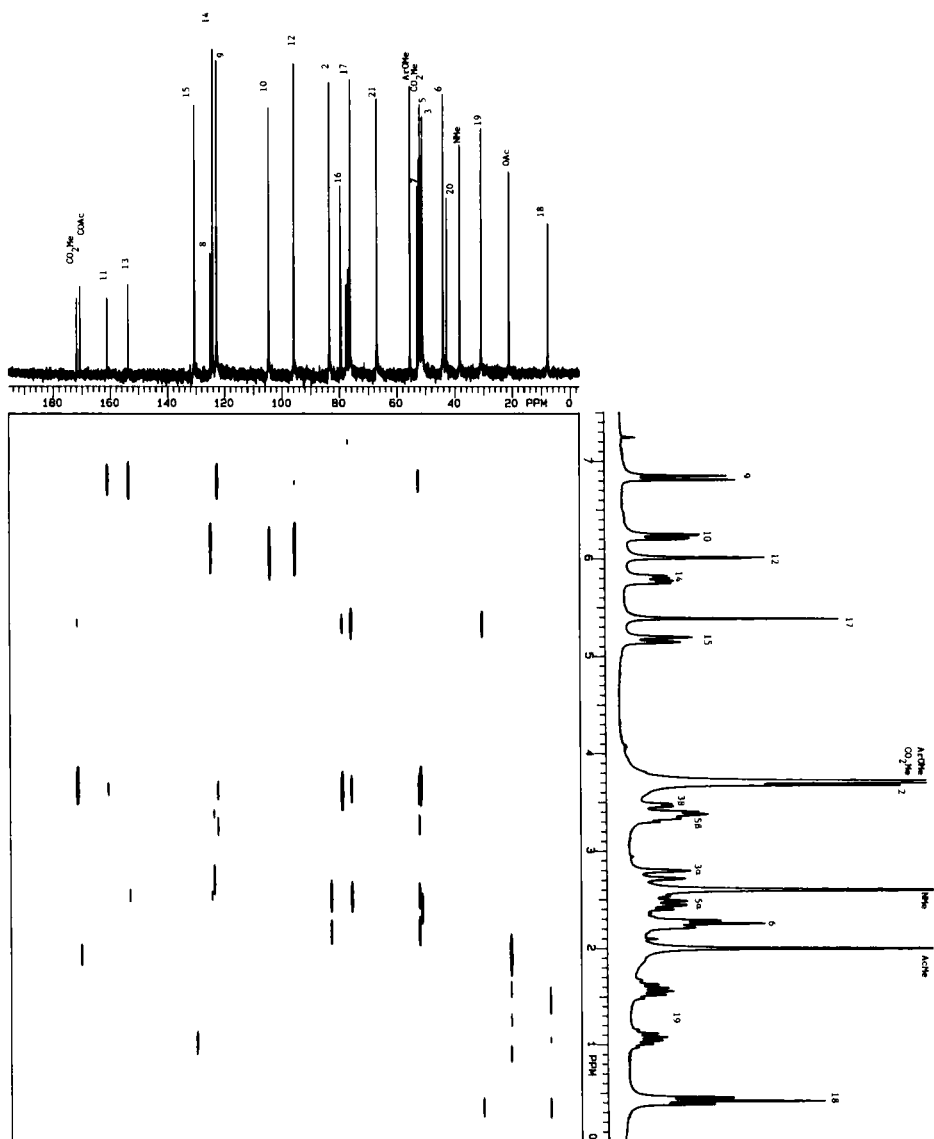


FIG. 3 Two dimensional long range heteronuclear shift correlated spectrum of vindoline, using $J_{\text{C,H}} = 140$ Hz and $J_{\text{C,H}}$ (long range) ≈ 6 Hz.

TABLE 2

¹³C Nmr Chemical Shifts and J_{CH} Coupling Constants for Vindoline

Carbon	(ppm)	J _{CH} (Hz)	Carbon	(ppm)	J _{CH} (Hz)
2	83.21	145	15	130.27	160
3	50.87	147	16	79.39	
5	51.74	140	17	76.17	147
6	43.87	133	18	7.48	125
7	52.60	-	19	30.60	128
8	124.81	-	C*OAc	170.53	-
9	122.49	155	C*O ₂ Me	171.70	-
10	104.40	160	NMe	38.07	138
11	160.94	-	ArOMe	55.15	144
12	95.59	160	CO ₂ Me*	51.98	151
13	153.51	-	20	42.70	-
14	129.92	161	21	66.82	135
			OAc	20.87	130

Note the coupling of C-3 to both H-3_α and H-3_β and C-5 to H-5_α and H-5_β which supports the proton assignments.

In order to assign the quaternary carbons a long range HETCOR (J_{CH}=6Hz) was performed (Fig 3). While some of the one bond H-C interactions are still present many three bond H-C correlations are now apparent. C-13 shows cross peaks with H-9 and NMe, C-11 is correlated to H-9 and Ar-OMe, C-8 is correlated to H-12, C-7 is correlated to H-6, C-16 is correlated to H-17 and H-2, and the acetate and acetoxyl quaternary carbons are correlated to their respective methyl groups.

The ambiguity with regard to H-2 and H-21 and hence C-2 and C-21 is now lifted because C-2 is correlated to N-CH₃ and H-6.

Unfortunately, under the conditions of this experiment C-21 shows no correlation. The final set of ^{13}C chemical shifts are given in table 2. With the exception of the acetate and acetoxy assignments these chemical shifts are in good agreement with the literature.

In conclusion we have shown that, using modern nmr pulse techniques, it is possible to make complete unambiguous assignments of complex spectra without recourse to the synthesis of model compounds.

EXPERIMENTAL

Vindoline was a kind donation from Fine Chemicals Pty Ltd. All experiments were performed as a CDCl_3 solution in 5 mm sample tubes on a Varian VXR200 spectrometer. Chemical shifts are relative to deuteriochloroform (7.24 ppm for ^1H and 77.0 ppm for ^{13}C .)

Two dimensional COSY spectra were recorded using the standard pulse sequence with a 90° observe pulse. 128 increments were recorded with a sweepwidth of 2057 Hz and an acquisition time of 0.249 sec. Data were processed as a 1024 x 1024 data matrix with apodization functions RE = 0.016, AF = 0.062, RE2 = 0.008 and AF2 = 0.031 for the two dimensions respectively.

Two dimensional HETCOR spectra were recorded using 128 increments, a sweep width of 10000 Hz, and an acquisition time of 0.102 sec. The final data matrix of dimensions 2048 x 256 was used with apodization functions RE = 0.006, AF = 0.026 and RE2 = 0.004, AF2 = 0.016 respectively. For the standard HETCOR spectra a C-H coupling of 140 Hz was used while for the long range HETCOR $J_{\text{CH}} = 6\text{Hz}$

NOE difference spectra were recorded using the DOCYCLE pulse sequence⁹ which allowed for low power irradiation of multiplets.

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