Total Assignment of the Proton and Carbon NMR Spectra of 5'-NOR-Anhydrovinblastine

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Total spectral assignment of the bis-indole alkaloid Navelbine®, 5'-nor-anhydrovinblastine, was accomplished using a combination of two-dimensional nmr techniques. Conventional homonuclear and inverse-detected experiments were supplemented with the HMQC-TOCSY experiment to unequivocally assign carbons directly bound to overlapped protons. In addition, the recently introduced GEM-COSY experiment was evaluated as a technique for the identification of geminal methylene pairs. Partial assignments proposed by Potier and co-workers, based on chemical shift considerations, are largely in agreement with the unequivocal assignments determined in this study.

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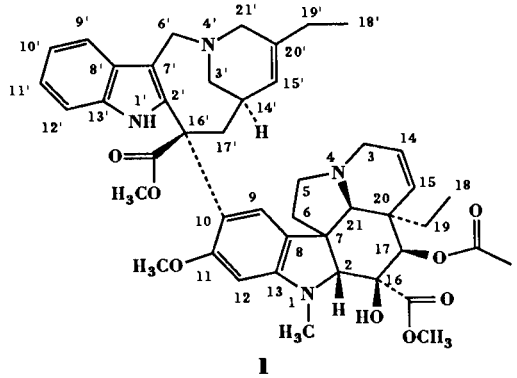
Navelbine®, 5'-nor-anhydrovinblastine (**1**), is one of the more recent of the bis-indole alkaloids to be found effective in the treatment of cancer. The structural complexity of this class of compounds has proved challenging to both the synthetic chemist and nmr spectroscopist alike. The value of two-dimensional nmr techniques in the spectral assignment of complex indole alkaloids has been amply demonstrated [1-14]. In the present study we wish to report the total assignment of the proton and carbon nmr spectra of **1** through the concerted application of a variety of two-dimensional techniques. In the process of making these assignments, COSY and proton-detected heteronuclear chemical shift correlation data (HMQC) [15] were supplemented with HMBC [16] (long-range proton-detected heteronuclear correlation) and HMQC-TOCSY [17,18] (heteronuclear correlation with proton homonuclear relayed coherence through isotropic mixing) data. In addition, the GEM-COSY (GEMinal-filtered COrrelation SpectroscopY) experiment, very recently reported by Freeman and co-workers [19], was evaluated as an alternative means of identifying geminal methylene pairs in congested spectra.



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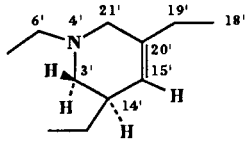
Navelbine® was first prepared and characterized in 1979 by Potier and co-workers in the course of their investigation of the modified Polonovski reaction in the synthesis of bis-indole alkaloids [20]. They demonstrated that anhydrovinblastine *N*-oxide is converted to 5'-nor-anhydrovinblastine (Navelbine®) on treatment with trifluoroacetic anhydride with subsequent hydrolysis. Using the work of Wenkert, *et al.* [21], Potier and co-workers were able to assign nearly all of the carbon resonances of this new vinblastine analogue on the basis of chemical shift comparisons and considerations. The complex proton spectrum, however, was only partially assigned. The investigation was performed before the widespread availability of two-dimensional nmr on a 250 MHz spectrometer, so this is hardly unexpected.

Assignment of the proton nmr spectra of bis-indole alkaloids like Navelbine® must be approached cautiously when chemical shift arguments are to be utilized. Protons in the "upper" or velbanamine half of these molecules are subject to the influence of conformation and chemical shift anisotropy. As an example, consider the variability of the chemical shift of the 17'a proton. In Navelbine®, this proton resonates at 2.89 ppm, while in vinblastine it resonates at 4.00 ppm [22]. In similar fashion, the H-15' olefinic proton resonates at 5.76 ppm in Navelbine® and at 5.00 ppm in an analogue in which the eight-membered ring has been opened [20]. Chemical shift variability is also observed in the protons of the "lower" or vindoline half of bis-indole alkaloids. A number of investigators have reported the chemical shift variability of the 18-methyl protons. The methyl resonance is shifted roughly 0.2-0.4 ppm upfield in the case of 16'(*R*) diastereomers [23,24]. This observation is especially remarkable, considering that the 18-methyl group is

eight bonds removed from the 16' position!

A reasonable place to start the spectral assignment of Navelbine® is at the 18'/18 methyl protons. Navelbine® possesses two ethyl substituents, so first it is necessary to distinguish the resonances which belong to their respective spin systems. The proton spectrum contains two methyl triplets which resonate at 0.54 ppm and 1.02 ppm. The COSY spectrum correlates the latter to a resonance at 2.02 ppm. Based on chemical shift considerations, we may tentatively assign the protons at 1.02 ppm as 18', and the protons at 2.02 ppm as 19'. The chemical shift of the 19' methylene is consistent with its allylic character. To further support this argument, we observe that the methyl resonance at 0.54 ppm correlates with a pair of anisochronous geminal methylene protons which resonate at 1.31 and 1.46 ppm. These chemical shifts are reasonable for assignment as the 18/19 ethyl group. The observation that the H-19 protons are non-equivalent is consistent with the assignment, considering the sterically crowded region where the 19-methylene attaches to the vindoline framework.

Continuing from the 19' methylene resonating at 2.02 ppm, the COSY spectrum shows allylic coupling of the H-19' protons with H-15' at 5.76 ppm. Unfortunately, H-15' overlaps a second proton, presumably one of the olefinic protons on the vindoline half of the molecule. This complicates the task of tracing out the velbanamine resonance assignments. The COSY spectrum provides the chemical shifts of resonances coupled to the two 5.76 ppm protons, but it is not immediately obvious which are correlated with H-15'. The COSY spectrum shows a correlation between one of the protons at 5.76 ppm and a proton at 5.24 ppm, which must be the remaining olefinic proton in the vindoline moiety. The latter is correlated to protons resonating at 3.21 and 2.62 ppm. These four protons in the vindoline half constitute an isolated spin system, which we may verify by analysis of the HMQC-TOCSY spectrum, discussed below. It follows that all other resonances which correlate with the protons at 5.76 ppm must be coupled to H-15'. Thus, the protons which resonate at 3.90, 3.58, and 1.69 ppm are all located in the velbanamine half of the structure. The two downfield protons may be assigned as the H-21' protons, which is supported by the observation that there are weak cross peaks indicating long-range coupling of these protons to the H-19' protons. The upfield proton resonating at 1.69 ppm must be H-14'. The rest of the spin system may be traced from the H-14' proton resonance. The H-14' resonance at 1.69 ppm is correlated with protons resonating at 2.52 and 2.86 ppm, and to a third proton at 2.69 ppm. The latter is, in turn, coupled to a resonance at 3.51 ppm. If these two protons are indeed a geminal pair, then from their chemical shifts we may infer that they are the H-3' protons, while the protons resonating at 2.52 and 2.86 ppm must be the H-17' protons. The portion of the molecular framework just delineated is summarized in **2**.



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The preceding discussion points to the value of methods which permit the unequivocal subgrouping of geminal methylene proton resonances. The methylene protons of Navelbine® were located using a GEM-COSY spectrum. GEM-COSY (GEMinally filtered COrrelation SpectroscopY) [19] is a pulse sequence proposed by Freeman and co-workers which utilizes a heteronuclear multiple quantum "filtering step" which selectively allows only magnetization from geminal methylene resonances to be detected. Thus, the final spectrum contains only responses from geminal pairs. This method potentially complements the identification of geminal methylenes from heteronuclear correlation spectra such as HMQC. For this reason, we were interested in evaluating GEM-COSY as an alternative to HMQC. The GEM-COSY pulse sequence schematic is shown in Figure 1 and the GEM-COSY spectrum of

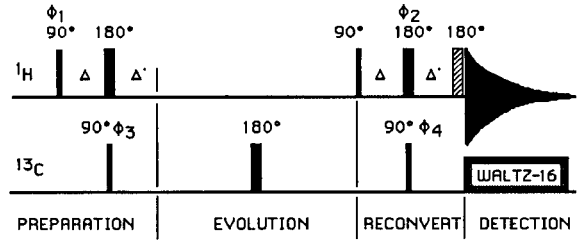


Figure 1. GEM-COSY pulse sequence described by Freeman and co-workers [19]. The first 90° proton pulse creates single quantum coherence in xy-plane which evolves during the delay, Δ; the first 90° carbon pulse creates heteronuclear multiple quantum coherence. During the delay Δ' coherence between the ^{13}C and the second of the geminal protons is active. The evolution period (t_1) encodes proton chemical shift and H-H couplings; C-H couplings are refocused by the 180° carbon pulse and C-C coupling can be neglected. The first 90° proton pulse following the evolution period involves coherence from the second of the geminal protons attached to the carbon being observed; the second Δ delay permits evolution under the one-bond heteronuclear coupling. Conversion of the heteronuclear multiple quantum coherence to observable proton magnetization is achieved by the final 90° carbon pulse followed by the final Δ' delay which refocusses one-bond heteronuclear couplings. The 180°y pulse denoted by the shaded bar serves as a purging pulse and, after even numbers of transients, suppresses undesired responses from methine protons in the molecule. Phases are cycled as: ϕ_1 = 00002222; ϕ_2 = 11111111; ϕ_3 = 02020202; ϕ_4 = 00220022; receiver = 13313113.

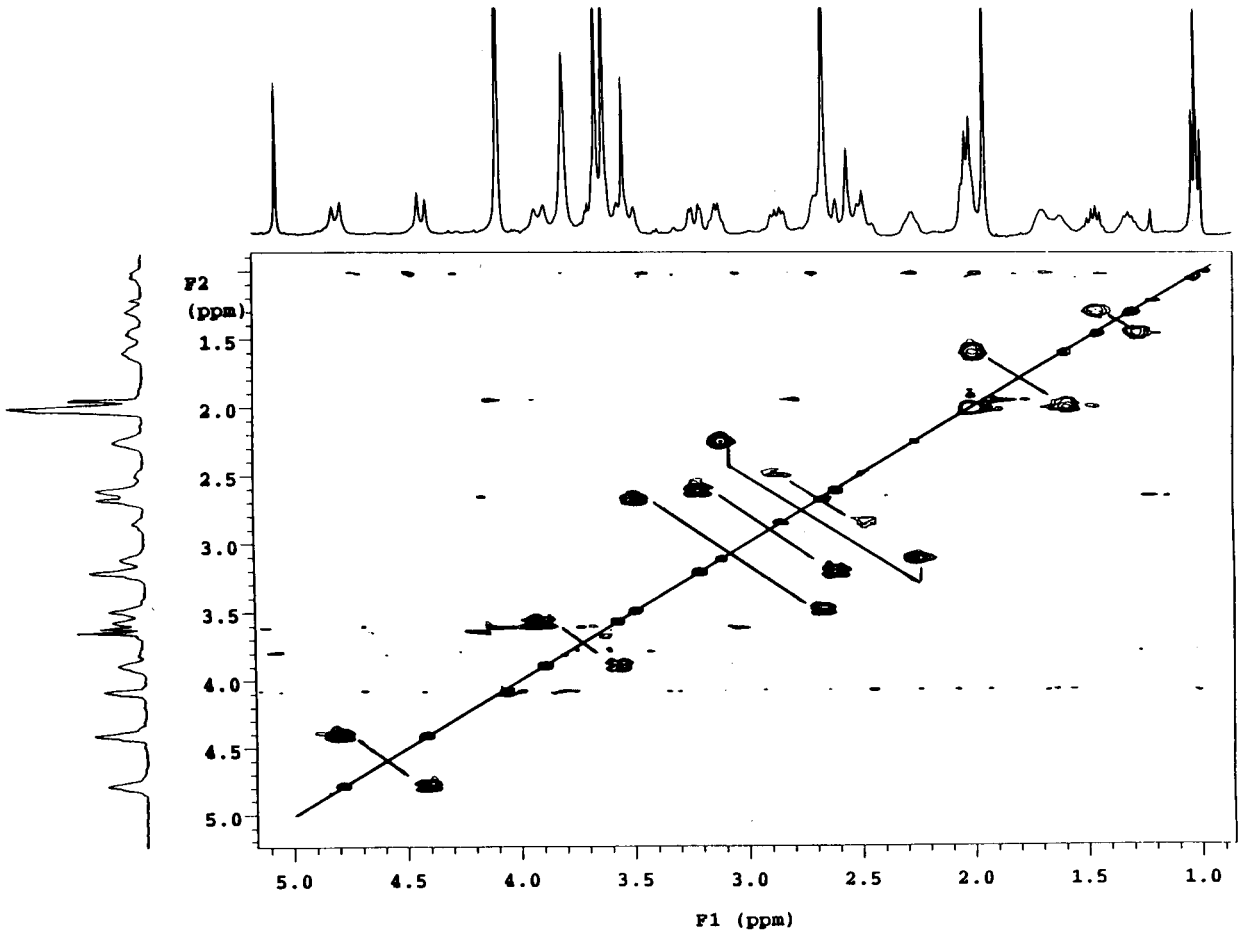


Figure 2. GEM-COSY spectrum of 5'-nor-anhydrovinblastine in d_6-DMSO recorded at 500 MHz. Cross peaks correlate geminal protons exclusively. The pulse sequence and the function of the individual pulses in the sequence are described in Figure 1.

Navelbine® is shown in Figure 2; correlations are delineated by bars which are perpendicular to the diagonal. All of the eight geminal methylene pairs in Navelbine® are identified by the GEM-COSY spectrum. The HMQC spectrum, Figure 3, confirmed that each observed correlation did arise from geminal protons. The 17' protons, resonating at 2.52 and 2.86 ppm, gave weak but discernible responses in the GEM-COSY spectrum. The HMQC spectrum also showed that the protons resonating at 2.69 and 3.51 ppm, tentatively assigned as the H-3' protons, are, in fact, correlated to the same carbon, at 43.1 ppm. The HMQC spectrum also correlates the H-17' protons, resonating at 2.52 and 2.86 ppm, with a carbon at 35.1 ppm. The H-17' protons resonate slightly downfield from where one might anticipate, but C-17' resonates at its expected chemical shift.

The remaining proton spin systems on the velbanamine half of Navelbine® are the H-6' protons, the indole-derived aromatic protons, and the methyl ester. The H-6' protons would be expected to resonate downfield from the other geminal protons. The GEM-COSY spectrum reveals a geminal pair resonating at 4.80 and 4.42 ppm, consistent with this expectation. The COSY spectrum confirms that these protons are isolated, with no scalar couplings observed; the HMQC spectrum correlates the 4.80/4.42 ppm protons with a carbon resonating at 46.3 ppm, which is reasonable for C-6'. The aromatic protons H-9' through H-12' constitute a four-spin system, easily identified from the COSY spectrum. We may trace the spin system from the observed vicinal couplings, starting with the proton resonating at 7.69 ppm and ending with the proton resonating at 7.40 ppm. Clearly, one of these two protons is H-9', while the other is H-12'. The simplest means to distinguish these protons is by the chemical shifts of their directly attached carbons, obtained from the HMQC spectrum. C-12' is beta to the indole

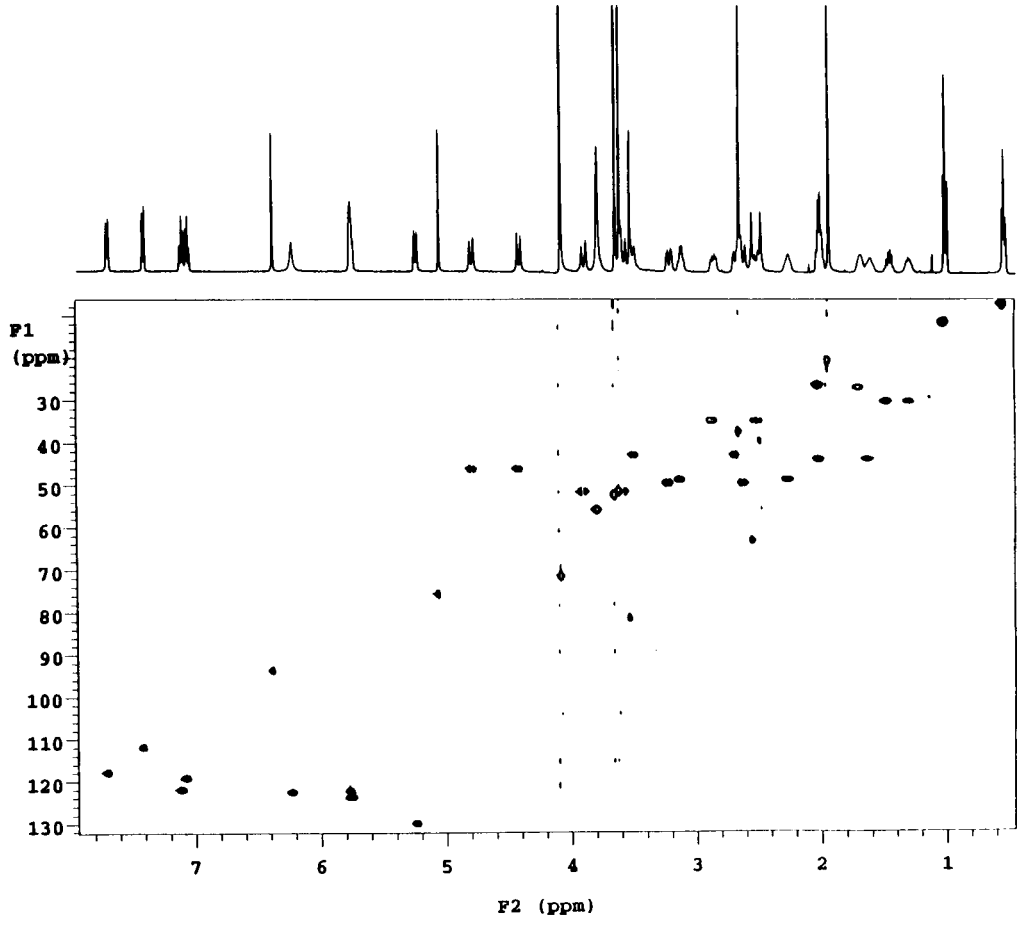


Figure 3. Inverse-detected heteronuclear chemical shift correlation spectrum (HMQC) of 5'-nor-anhydrovinblastine in d_6-DMSO recorded at 500 MHz.

nitrogen, so it should be shifted upfield. The proton resonating at 7.40 ppm is correlated with a carbon at 112.1 ppm, while the proton resonating at 7.69 ppm is correlated with a carbon at 118.1 ppm. This suggests that H-12' must be the proton resonating at 7.40 ppm. Long-range correlations support the proposed assignments (discussed below). Long-range heteronuclear correlation also permits the unequivocal assignment of the 16' methyl ester, resonating at 3.65 ppm, completing assignment of the velbanamine portion of the molecule.

The proton spectrum of the vindoline half of Navelbine® consists of the ethyl group, two four-spin systems, and a number of singlets. As noted previously, the ethyl resonances are comprised of the methyl group resonating at 0.54 ppm and two anisochronous methylene protons resonating at 1.31 and 1.46 ppm. One of the four-spin systems consists of the H-5/H-6 ethylene bridge protons. While there are a number of approaches by which these protons may be located, the most straightforward is by examination of the HMQC-TOCSY spectrum. During the TOCSY portion of this pulse sequence, magnetization which has been labeled during the HMQC step is propagated through the proton spin system. The extent to which propagation occurs is a function of the duration of the mixing time [18]. The HMQC-TOCSY spectrum correlates all four protons in the spin system with both C-5 and C-6. The downfield protons are the H-5 protons, which is easily verified from the HMQC or GEM-COSY spectrum. It is interesting to note that the H-5/H-6 spin system does not produce the expected pattern of cross peaks in the COSY spectrum. This problem arises because two of the pairs of vicinal protons are orthogonal. The remaining four-spin system consists of the two olefinic protons H-14 and H-15, and the H-3 methylene protons. In our analysis of the COSY spectrum, we located the olefinic protons at 5.76 and 5.24 ppm, and the H-3 protons at 3.21 and 2.62 ppm. The HMQC-TOCSY spectrum confirms this. Evidently, the TOCSY step was not sufficiently long to propagate the magnetization throughout the spin system, but all

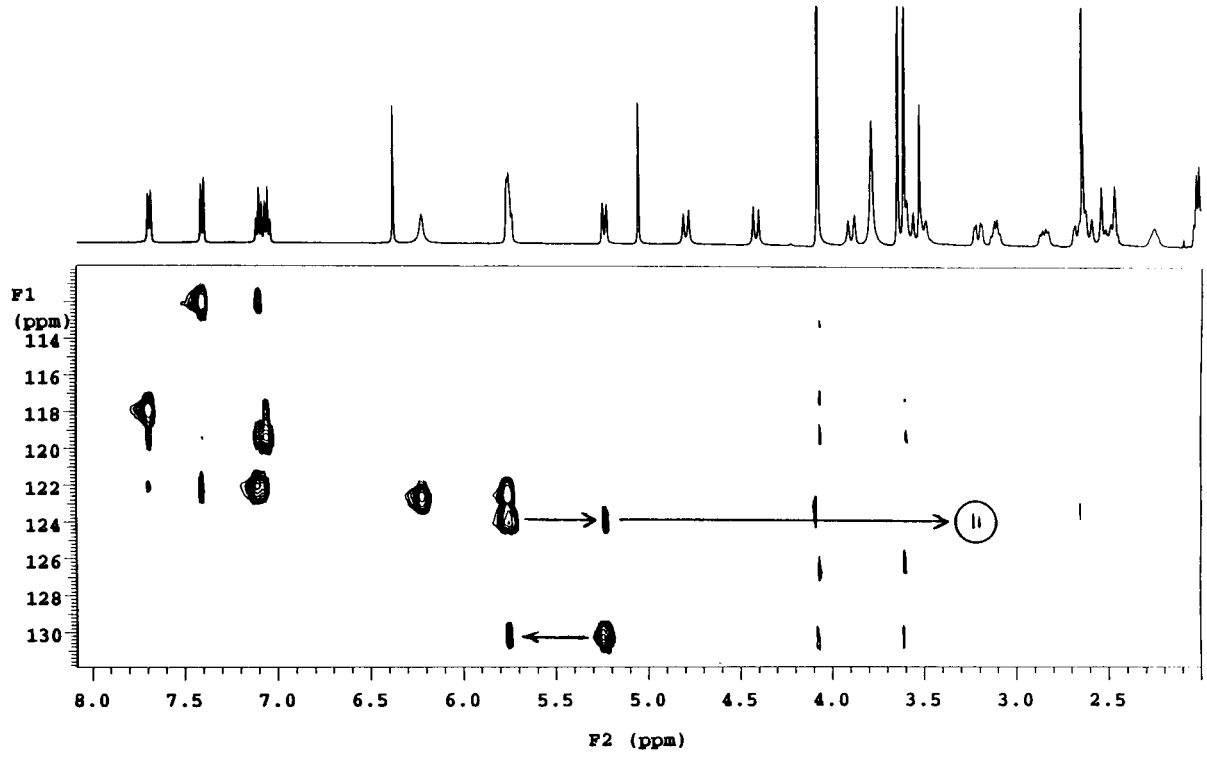


Figure 4. HMQC-TOCSY spectrum of 5'-nor-anhydrovinblastine in d_6-DMSO recorded at 500 MHz. The spectrum was acquired with a TOCSY mixing time of 14 msec. Heteronuclear decoupling was initiated with data acquisition to retain the direct responses [18]. The H-15/C-15 direct response (5.24/130.3 ppm) exhibits a relay to H-16 (5.76 ppm). The H-16/C-16 direct response (5.76/124.0 ppm) shows relay responses to H-15 and also to one of the geminal 3-methylene protons resonating at 3.21 ppm. These responses confirm the assignment of the proton/carbon pair resonating at 5.76/124.0 ppm as H-16/C-16 and allow the unequivocal assignment of the proton/carbon pair resonating at 5.76/122.5 ppm as H-15'/C-15'.

correlations arising from geminal and vicinal coupling are observed. The HMQC-TOCSY spectrum demonstrates its value in the disentanglement of overlapping proton connectivity patterns with the two vinyl protons resonating at 5.76 ppm. Despite the fact that the proton resonances are overlapped, their carbon resonances, as shown by the HMQC spectrum, are not. Hence, connectivities from each of the protons are resolved in the second frequency domain of the HMQC-TOCSY spectrum by virtue of the directly bound carbon's chemical shift.

The remainder of the proton resonances of the vindoline half of Navelbine® are singlets. A number of these may be assigned unambiguously on the basis of their chemical shifts. The 17-acetoxyl methyl group, for example, resonates at 1.94 ppm, while the N-1 methyl resonates at 2.65 ppm. Similarly, the one-proton singlet resonating at 5.05 ppm must be H-17. The singlets resonating at 3.53 ppm and 2.55 ppm must be H-2 and H-21, respectively, which we may verify by analysis of the HMBC spectrum. Proton chemical shifts are less helpful in assigning H-9 and H-12, but in this case the HMQC spectrum provides a solution. The same argument which we used to distinguish H-9' from H-12' applies here: C-12 is beta to nitrogen, and beta to the oxygen of the methyl ether as well, so it should be shifted upfield. The HMQC spectrum correlates the proton resonating at 6.38 ppm with a carbon at 93.9 ppm, and the proton resonating at 6.22 ppm with a carbon at 118.5 ppm. The former are clearly H-12/C-12, while the latter must be H-9/C-9. The 11-methoxyl methyl and the 16-carbomethoxyl methyl may be assigned by a similar line of reasoning. While the methyl ether protons resonate slightly downfield from where one might predict, at 3.79 ppm, the directly bound carbon resonates at 56.2 ppm, which is as expected. The 16-carbomethoxy methyl carbon resonates at 51.7 ppm, reasonable for a methyl ester. Long-range heteronuclear correlations confirm these assignments.

Unambiguous assignment of the quaternary carbon resonances depends on the long-range heteronuclear correlations in the HMBC spectrum (Table 2). The velbanamine portion of the molecule contains seven quaternary carbons: C-20', C-16', the 16' carbomethoxy carbonyl, and four of the indole carbons. Long-range coupling with the H-18' methyl protons identifies C-20', resonating at 131.9 ppm. Only H-14'

would be expected to exhibit three-bond coupling to C-16', but any cross peaks which might be associated with this proton are below the noise level. Fortunately, both C-17' protons are correlated with a quaternary carbon resonating at 54.2 ppm, which is a reasonable chemical shift for C-16'. There is evidence of coupling between the methyl ester resonance at 3.64 ppm and a carbon at 173.5 ppm. This carbon is also coupled to the H-17' proton which resonates at 2.87 ppm. These couplings assign the carbon resonating at 173.5 ppm as the 16' carbomethoxy carbonyl, and confirm the identity of the methyl ester protons. The assignment of the remaining indole carbons is also straightforward. The HMBC spectrum shows that the carbon resonating at 135.4 ppm is coupled to the H-17' proton resonating at 2.87 ppm as well as both H-6' protons, at 4.42 and 4.80 ppm. Thus, the carbon resonating at 135.4 ppm is clearly C-2'. Both H-6' protons are also coupled to two other quaternary carbons: one resonating at 105.0 ppm, the other resonating at 128.0 ppm. The upfield carbon is also correlated with one aromatic proton, resonating at 7.69 ppm, while the downfield carbon is correlated with two aromatic protons, one at 7.05 ppm and the other at 7.40 ppm. Based on the correlations just delineated, the carbon resonating at 105.0 ppm must be C-7', while the carbon at 128.0 must be C-8'. The observed correlations also support our previous assignments of H-9' (at 7.69 ppm) and H-12' (at 7.40 ppm). The remaining indole carbon, resonating at 135.4 ppm, may be identified as C-13' by its correlations with H-9' and H-11' (at 7.10 ppm).

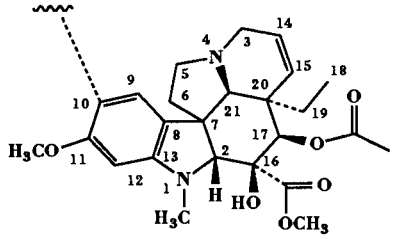
The quaternary carbons in the vindoline half of Navelbine® may be assigned from the HMBC spectrum in a similar manner. H-12, resonating at 6.40 ppm, exhibits correlations with four quaternary carbons, clearly by two- and three-bond coupling. Each of these carbons is easily assigned: the carbon resonating at 152.7 ppm, coupled to the *N*-methyl, must be C-13. The carbon at 122.2 ppm, coupled to H-2, must be C-8; the carbon at 157.9 ppm, coupled to the methoxyl methyl protons, must be C-11; the remaining carbon at 118.5 ppm must be C-10. Long-range correlations identify the aliphatic quaternary carbon resonating at 42.1 ppm as C-20, based on its coupling with the H-18 methyl protons resonating at 0.57 ppm, and the aliphatic quaternary carbon resonating at 52.9 ppm as C-7, based on its coupling with the H-6 proton which resonates at 2.03 ppm. The remaining quaternary aliphatic carbon is C-16. Unfortunately, three-bond heteronuclear coupling to C-16 cannot occur. We may still locate C-16, for the only remaining quaternary carbon with the appropriate chemical shift resonates at 79.5 ppm. This leaves the two carbonyl carbons to be assigned. The 16-carbomethoxyl carbonyl resonates at 171.0 ppm, based on correlations with H-17 and the 16-carbomethoxyl methyl protons. The 17-acetoxyl carbonyl, resonating at 170.1 ppm, is also coupled to H-17, and to the 17-acetoxyl methyl protons.

The HMBC spectrum of Navelbine® also serves to link together the various spin systems. In the velbanamine half, for example, the H-6' proton resonating at 4.42 ppm correlates with C-21', at 51.8 ppm. The other H-6' proton, resonating at 4.80 ppm, correlates with C-3', at 43.1 ppm. The H-17' proton which resonates at 2.86 ppm also correlates with C-3'. On the vindoline half, a number of spin systems may be tied together via C-21, at 63.4 ppm. C-21 is coupled to the H-5 proton resonating at 3.12 ppm, the H-3 proton resonating at 3.21 ppm, and the H-19 proton resonating at 1.49 ppm. In addition, H-21 is long-range coupled to C-2, at 81.7 ppm, and C-17, at 75.9 ppm, as

Table 1

Comparison of the Chemical Shifts of Navelbine® Determined in this Study with Those reported by Potier and Co-workers [6]

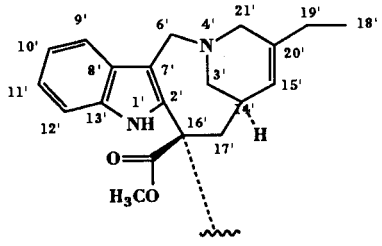
Resonance Assignments for the Vindoline Portion of 5-Nor-anhydrovinblastine



	Present study		Reference 6	
	^1H	^{13}C	^1H	^{13}C
2	3.53	81.7	-	83.1
3	2.62,3.21	49.8	-	49.8
5	2.27,3.12	48.9	-	50.3
6	1.61,2.01	44.3	-	44.6
7	-	52.9	-	53.4
8	-	122.2	-	-
9	6.22	122.9	6.08	-
10	-	118.5	-	-
11	-	157.9	-	157.9
11-OCH_3	3.79	56.2	3.82	55.8
12	6.40	93.9	6.34	93.8
13	-	152.7	-	152.7
14	5.76	124.0	5.96	124.8
15	5.24	130.3	5.25	129.6
16	-	79.5	-	79.8
16-CO_2CH_3	3.62	51.7	(3.13)	52.9
16-CO_2CH_3	-	170.1	-	170.6
17	5.05	75.9	5.37	-
17-$OCOCH_3$	1.94	21.0	2.06	21.3
17-$OCOCH_3$	-	171.0	-	171.3
18	0.54	7.5	0.20	8.3
19	1.31,1.46	30.6	-	30.8
20	-	42.1	-	42.8
21	2.55	63.4	2.57	64.7

Table 1 (Continued)

Resonance Assignments for the Velbanamine Portion of 5-Nor-anhydrovinblastine



2'	-	135.4	-	132.5
3'	3.56,2.69	43.1	-	44.1
6'	4.80,4.42	46.3	4.58,4.41	53.6
7'	-	104.8	-	-
8'	-	128.0	-	128.3
9'	7.69	118.1	-	118.4
10'	7.05	119.5	-	122.7
11'	7.10	122.2	-	120.6
12'	7.40	112.1	-	110.4
13'	-	135.1	-	134.2
14'	1.69	27.2	-	28.2
15'	5.76	122.5	5.76	-
16'	-	54.2	-	54.7
16'-CO_2CH_3	3.65	52.8	3.68	52.1
16'-CO_2CH_3	-	173.5	-	173.9
17'	2.52,2.86	35.1	-	35.2
18'	1.02	11.7	1.07	12.2
19'	2.02	26.7	-	27.7
20'	-	131.9	-	134.2
21'	3.90,3.58	51.8	-	-

well as C-5, at 48.9 ppm. The long-range heteronuclear correlations with C-21 and H-21 are shown in 3. Finally, the HMBC spectrum ties together the two halves of the molecule. Although the correlation peak is weak, the H-17' proton at 2.87 ppm is coupled to C-10, at 118.5 ppm.

The analysis of complex alkaloids by nmr spectroscopy has advanced considerably since the time when Potier and co-workers first examined Navelbine®. The majority of our assignments could be made unequivocally through standard two-dimensional nmr techniques. We were able to resolve the few ambiguities through the HMQC-TOCSY experiment. We also evaluated the utility of the recently published GEM-COSY experiment. The present investigation confirms the utility of the GEM-COSY experiment, but the HMQC spectrum alone would have sufficed to identify the geminal methylene resonance pairs in the present study. The sensitivity of the GEM-COSY experiment is somewhat lower than that of the HMQC experiment but is at least comparable to the sensitivity of the HMBC experiment. The HMQC experiment was able to identify the geminal methylene pairs in the present study. However, had the HMQC data been ambiguous due to an unfortuitous carbon resonance overlap the GEM-COSY experiment could have been used to advantage. In this sense, the GEM-COSY experiment may find practical application in the study of terpenes, peptides, and other classes of molecules containing numerous methylene carbon resonances with closely similar chemical shifts where there is a significant chance of overlap.

EXPERIMENTAL

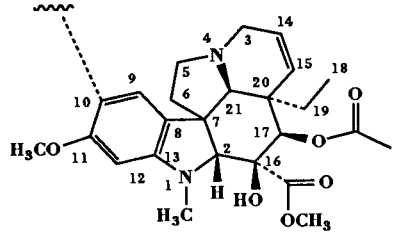
General

A 12 mg sample of 5'-nor-anhydrovinblastine ditartrate was dissolved in 0.8 ml 99.9% d_6-DMSO (MSD Isotopes, Merck and Co., Inc., Rahway, NJ). All nmr spectra were recorded on either a Varian Unity 400 or a Varian VXR-500S spectrometer, both equipped with Z-Spec indirect detection probes obtained from Nalorac Cryogenics Corporation, Martinez, CA. The observation frequencies for protons were 399.952 and 499.843 MHz, respectively. Typical 90° pulse widths for the Varian Unity 400 were 13.4 μsec for protons and 13.3 μsec for carbons. Typical 90° pulse widths for the Varian VXR-500S were 12.6 μsec for protons and 11.0 μsec for carbons.

Table 2

Long-range Heteronuclear Correlations Observed in the HMBC Spectrum of Navelbine®

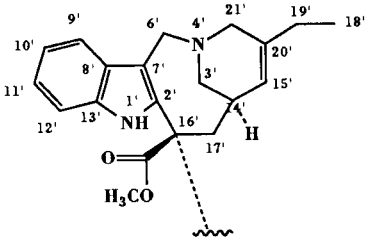
Long-range Correlations Observed in the Vindoline-half of the Molecule



Position	Chemical Shift δ^1H	Carbon(s) to which long-range correlations were observed
1-*N*-methyl	2.65	C2, C13
2	3.53	1-NMe, C6, C8, C16, C17
3	2.62	
	3.51	C14, C15, C21
5	2.27	
	3.12	C7, C21
6	1.61	C7, C8
	2.01	C7
9	6.22	
11-OMe	3.79	C11
12	6.38	C8, C10, C11, C13
14	5.76	C20
15	5.24	C20, C21
17	5.05	C15, C16, C19, C20
18	0.54	C19, C20
19	1.31	C15, C18, C20
	1.46	C15, C17, C18, C20, C21
21	2.55	C2, C5, C17, C19

Table 2 (continued)

Long-range Correlations Observed in the Velbanamine-half of the Molecule



Position	Chemical Shift δ^1H	Carbon(s) to which long-range correlations were observed
3'	2.69	C7', C8', C21'
	3.51	
6'	4.42	C7', C8', C21'
	4.80	C2', C3', C7', C8'
9'	7.69	C7', C11', C13'
10'	7.05	C8', C12'
11'	7.10	C9', C12', C13'
12'	7.40	C8', C10'
14'	1.69	
15'	5.76	
16' methyl ether	3.65	16'-C=O
17'	2.52	C10 [a], C16'
	2.86	C2', C3', C14', C16'
18'	1.02	C19', C20'
19'	2.02	C15', C18', C20', C21'
21'	3.58	C15', C20'
	3.90	C15', C20'

[a] Located in the vindoline subunit of the structure.

NMR Spectroscopy

The COSY spectrum of 5'-nor-anhydrovinblastine was recorded at 500 MHz using the pulse sequence and phase cycling of Freeman and co-workers [26]. The data were acquired as 3264 x 450 points with a spectral width of 5406.1 Hz in both frequency domains. The data were sine-bell apodized and zero-filled to 4096 x 2048 points during processing.

Heteronuclear chemical shift correlation data were obtained at 500 MHz by inverse-detection using the pulse sequence of Bax and Subramanian [15]. The spectrum was acquired as 2048 x 400 points with spectral widths of 5344.7 and 33435.4 Hz in F_2 and F_1, respectively. The data were processed using Gaussian apodization prior to both Fourier transforms and were zero-filled to 2048 x 1024 points.

Heteronuclear chemical shift correlation data with relayed coherence transfer were acquired using a modification of the HMQC-TOCSY pulse sequence originally described by Lerner and Bax [17]. Rather than delaying the onset of broadband heteronuclear decoupling by the interval 1/2J ($^1J_{CH}$ couplings were optimized for an assumed average coupling of 140 Hz) as in the original work of Lerner and Bax, we elected instead to initiate decoupling with the commencement of acquisition to retain the direct responses as described in the recent review of Martin and Crouch [18]. Acquisition and processing parameters were essentially the same as those employed in the heteronuclear chemical shift correlation experiment (above). The duration of the mixing period was 14 msec.

The long-range proton-detected heteronuclear multiple-bond correlation spectrum of 5'-nor-anhydrovinblastine was obtained at 500 MHz using the pulse sequence of Bax and Summers [16]. The data were acquired as 2560 x 440 points with spectral widths of 5344.7 and 45250.9 Hz in F_2 and F_1 (TPPI), respectively. The data were processed using Gaussian apodization prior to both Fourier transforms and were zero-filled to 4096 x 1024 points.

The GEM-COSY spectrum of 5'-nor-anhydrovinblastine was obtained at 400 MHz using the pulse sequence of Freeman and co-workers [19]. The spectrum was acquired as 1536 x 288 points with a spectral width of 3200 Hz in both dimensions. Delays for the multiple quantum step were the same as those employed for the HMQC experiment.

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