A Reassignment of the 13C-NMR Spectrum of the Alkaloid Ajmaline Through the Use of Two-Dimensional NMR Techniques

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The reassignment of the 13C chemical nmr spectrum of the alkaloid, ajmaline, is reported. An earlier assignment based on analogy to other alkaloids and deductions from molecular orbital theory was found to be partially in error. The present study uses broadband decoupled (both dimensions) proton-carbon shift correlation and long-range proton-carbon chemical shift correlation techniques. These experiments, in conjunction with earlier work by others on the assignment of the proton spectra, suffice to now unequivocably assign the 13C nmr spectrum of the title compound.

Introduction.

The structure of the alkaloid ajmaline (1), first isolated in the 1930's [1,2], was confirmed by Woodward in 1956 [3]. The carbon skeleton of this compound is common to a number of closely related alkaloids which were the subject of a 1985 proton nmr study reported by Lounasmaa, Jokela, and Kan [4]. Interestingly, that study disagreed with several of the proton resonance assignments of ajmaline (1) and isoajmaline made in an earlier study by Neukomm, Kletzhaendler, and Hesse [5]. More recently, a two-dimensional nmr (COSY) study by Koskinen and Lounasmaa [6] has confirmed the previous assignments of Lounasmaa and co-workers [4].
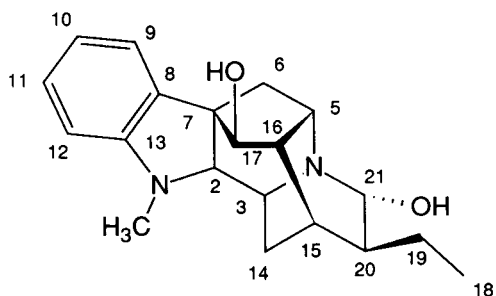
The 13C nmr studies of ajmaline and related alkaloids were done somewhat earlier, the most noteworthy appearing in publications by Wenkert, et al. in the 1970's [7,8]. Of particular interest here is the fact that, for ajmaline, some of the carbon assignments were reported as being possibly reversed or ambiguous. Since the ajmaline system must serve as the basis for the assignment of a rather large family of analogous alkaloids, it was felt that a totally unequivocal assignment of the 13C spectrum of this compound would be of considerable use. Such a study will now be discussed.

Results and Discussion.

Because of the availability of the assigned proton spectrum [4,6], the assignment of the 13C-nmr spectrum is relatively straight-forward--but not completely so. Proton frequencies as obtained earlier [6] and as obtained from the heteronuclear broad-band decoupled experiment are shown in Table 1. The directly-bound heteronuclear chemical shift correlations are shown in Figure 1. Except for

Table I

Proton Chemical Shifts for Ajmaline

Proton	Literature [a]	This Work [b]
H2	2.63	2.526
H3	3.58	3.424
H5	3.04	2.818
H6	2.05,1.93	ca. 1.75
H9	7.46	7.436
H10	6.80	6.660
H11	7.17	7.020
H12	6.68	6.608
H14	1.82,1.48	ca. 1.5
H15	2.24	2.105
H16	2.00	1.876
H17	4.43	4.221
H18(Me)	0.95	0.913
H19	1.48,1.36	1.342
H20	1.48	1.231
H21	4.26	4.028
N-Me	2.79	2.647

[a] Ref [6]. In deuteriochloroform relative to TMS. [b] In DMSO-d6 relative to TMS. Obtained from zero-filled slices of the H-C broadband-decoupled correlations. Shifts are accurate to ± 0.001 ppm. Only approximate values could be obtained for H6 and H14; these along with H19 are averaged values.
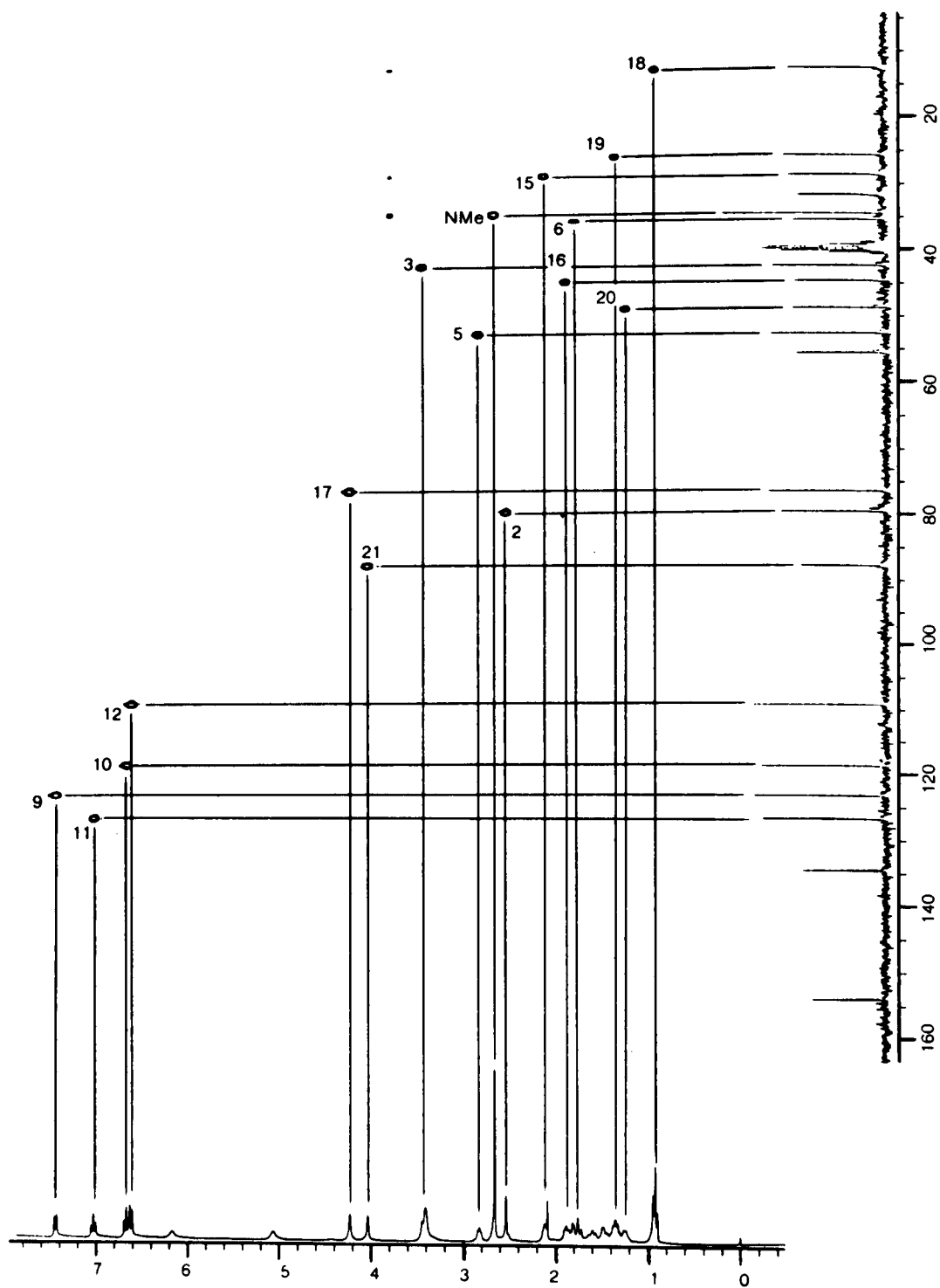
Figure 1. Direct proton-carbon correlations (decoupled in both dimensions). Note that the correlations for position 6 is very weak and that for position 14 is unobserved. The correlation for position 19, on the other hand, is quite strong and readily discerned.
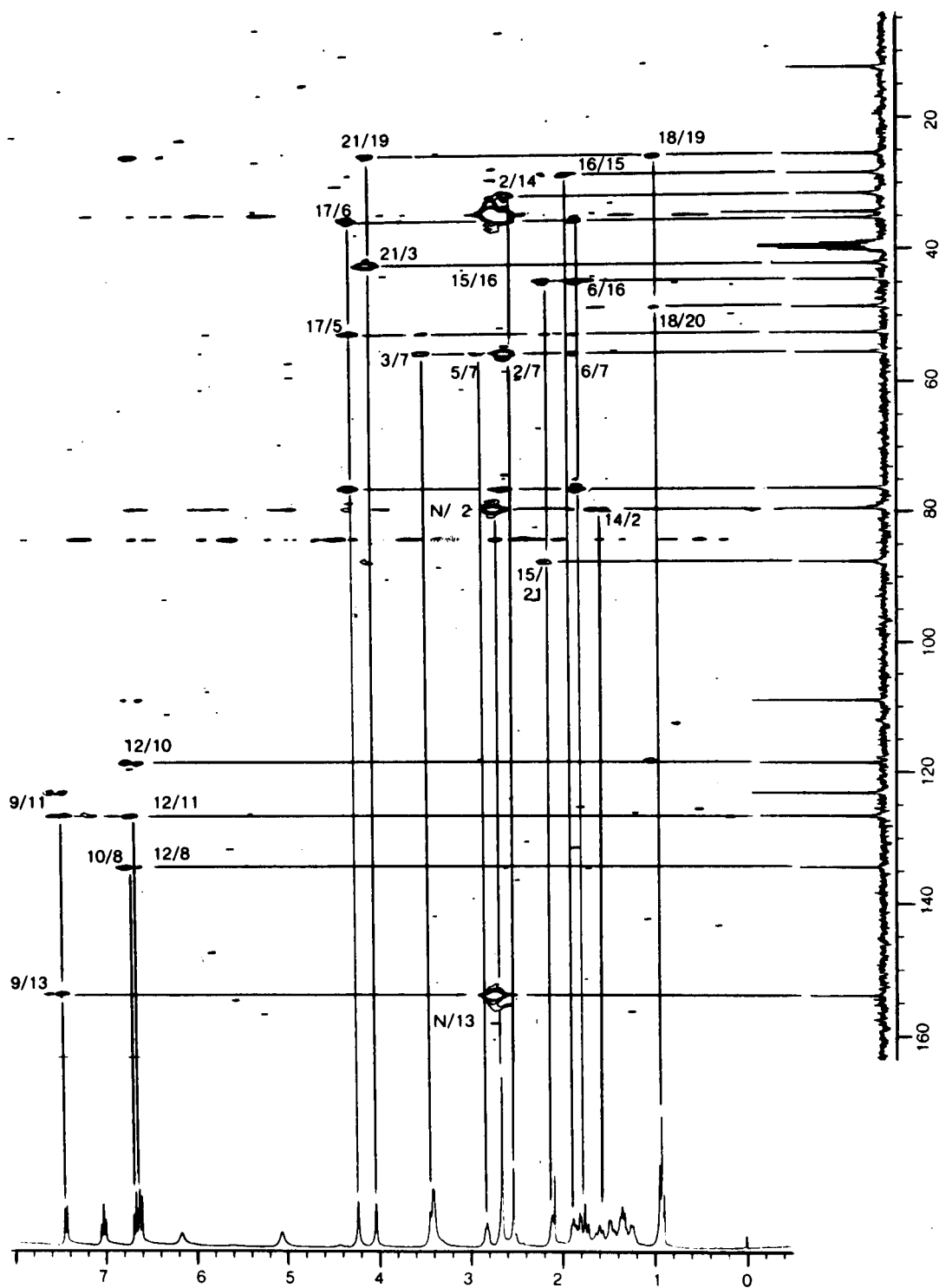
Figure 2. Long-range proton carbon correlations (with $^1J_{CH}$ modulation removed). All long-range correlations are labelled; those which are most germane to the assignment are discussed in detail in the text.

the anisochronous hydrogens attached to the carbons in positions 6 and 14, the proton shifts could be obtained to very high accuracy (*vs.* TMS) from reprocessing (with zero-filling) of the relevant slices in the F_1 frequency domain. Because of solvent and aromatic upfield shift effects, our proton frequencies are somewhat upfield of those previously reported [6]. This upfield shift causes no inordinate confusion, however. The spectral results shown in Figure 1 allow assignment of all carbons except for the quaternaries (carbons 7, 8, and 13) and for C14. In this latter case, the two bound protons are considerably separated in chemical shift and a direct correlation cannot be observed. In addition, the correlation at C6 is just barely visible and its assignment would not, at this point, necessarily be rigorously unambiguous. Finally, it should be noted that, although the two methylene protons in the ethyl group (bound to C19) have been reported to be anisochronous [6], this carbon gives a strong direct response as can easily be seen in Figure 1; in this case, the effect is probably due to a fortuitously benevolent solvent effect.

Completion of the ^{13}C-nmr assignments requires an examination of long-range proton-carbon correlations. The relevant two-dimensional spectrum is shown in Figure 2. All long-range connectivities are labelled here; labels were omitted for the direct connectivities (which, when they did appear, were weak in all cases). Of most import here are connectivities which complete the assignment. We look first at the unprotonated aromatic carbons, C8 and C13. The assignment of C13 is the more obvious of the two because of its very strong correlation to the *N*-methyl protons. The identity of C8 is then easily obtained from its correlations to the protons in the 10 and 12 positions. Also, even though the proton assignments are available, it is useful to note that the other aromatic carbon assignments are readily confirmed by the data at hand. Of particular use are the H9-C11 and H12-C10 correlations.

Assignment of the remaining quaternary carbon, C7, is readily carried out *via* its correlations with H2, H3, H5, and H6.

Finally, we complete our analysis by examining the carbons in positions 6 and 14. C6 has already been tentatively assigned from its direct but weak response to H6. However, H6 and H14 are very close to each other and it is not impossible for these two protons to have reversed assignments, especially since the two protons in position 14 have a fairly large chemical shift difference. Fortunately, the identity of C6 can be immediately reconfirmed *via* its correlation with H17. No direct connectivity is observed for C14; however, the H2-C12 correlation is observed and this confirms the identity of this carbon and completes our assignment. The complete list ^{13}C shifts is given in Table 2.

Table II

Carbon-13 Chemical Shifts of Ajmaline

Position	Literature [a]	Observed Shift [b]
C2	79.4	79.34
C3	44.6*	42.06
C5	52.5	52.43
C6	35.3	35.18
C7	55.5	55.40
C8	134.5	134.40
C9	123.1	123.07
C10	118.5	118.45
C11	126.7	126.60
C12	109.1	108.98
C13	154.0	153.88
C14	31.6	31.40
C15	28.4	28.32
C16	48.7*	44.42
C17	76.3	76.17
C18	12.3	12.21
C19	25.5	25.39
C20	42.2*	48.54
C21	87.6	87.56
C-NMe	34.3	34.22

[a] From ref [7] for purposes of comparison. Shifts marked with an asterisk have been reassigned in the paper.   [b] Defined relative to DMSO-d_6 at 39.5 ppm. Shifts precise to ± 0.01 ppm.

Summary and Conclusions.

The complete assignment of the ^{13}C spectrum of the alkaloid, ajmaline, was carried out by observation of both direct (one-bond) and long-range proton-carbon connectivities. The latter were particularly necessary in light of the fact that the protons in the 6 and 14 positions were anisochronous with either very weak (6) or unobservable (14) direct correlations. And, of course, the long-range correlation experiment was necessary to unambiguously identify the nonprotonated carbons.

EXPERIMENTAL

The sample used in these studies was prepared *via* dissolving 60 mg. of analytically pure ajmaline obtained from the Sigma Chemical Co. in 0.4 ml of perdeutero dimethylsulfoxide (DMSO-d_6).

All experiments were performed on a Nicolet NT-300 wide bore spectrometer operating at observation frequencies of 300.068 and 75.462 MHz for ^{1}H and ^{13}C, respectively. The instrument was controlled by a model 293-C pulse programmer. Proton and carbon 90° pulses were calibrated at 16.5 and 23.0 μsec, respectively. The ^{1}H 90° pulse from the decoupler coils was calibrated as 42.8 μsec using the technique described by Bax [9].

Direct proton-carbon chemical shift correlations were established using the semiselective broadband homonuclear proton-decoupled sequence described by Bax [10]. The fixed delays in the BIRD pulse located midway through the evolution period were optimized for 155 Hz. The defocusing and refocusing delays were set to 3.23 and 2.15 msec, respectively. Data were taken as 164 (zero-filled to 512) by 1024 complex points with 256 pulses per block. Double exponential apodization with a line width of 3 Hz was used in both dimensions. Accurate proton chemical shifts were determined by taking appropriate slices and zerofilling these to 64K prior to Fourier transformation. Aside from deviations to be expected from solvent effects, the proton chemical shifts were sufficiently in agreement with earlier work to warrant their use in assigning most of the carbon resonances [4,6]. The ^{13}C chemical shifts are referenced relative to DMSO-d_6 at 39.5 ppm (exactly). An 8K FID was zero-filled to 64K prior to the Fourier transform. Proton and carbon shifts reported here are accurate to ± 0.001 ppm and ± 0.01 ppm, respectively.

Long-range proton-carbon connectivities were established *via* a modified Freeman-Morris [11] pulse sequence incorporating BIRD pulse midway through the refocusing delay [12-15], as shown in Figure 3. The experiment was optimized for a 155 Hz $^1J_{CH}$ and a long-range coupling
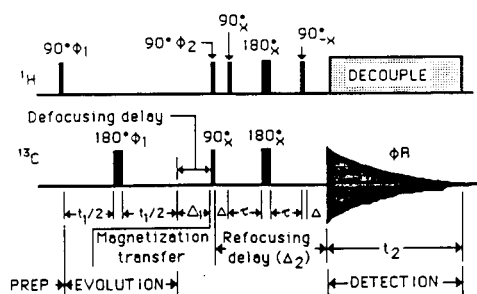

Figure 3. Modified Freeman-Morris [11] sequence incorporating a BIRD pulse midway through the refocusing delay, Δ, [12-14]. The duration of Δ_1 is optimized as $1/(2^nJ_{CH})$. In the case of a molecule such as ajmaline, with both aromatic and aliphatic carbons, Δ_2 should be set to 2/3 Δ_1. Finally, $\tau = 1/(2^1J_{CH})$, giving, $\Delta = [(\Delta_2 - 2\tau)/2]$.

($^nJ_{CH}$) of 10 Hz. Delays used here were 50.00 (Δ_1), 13.06 (Δ) and 3.44 (τ) msec. Note that this gives a total refocusing interval of $\Delta_2 = 33.33$ msec. Long-range data were collected as 164 (zero-filled to 512) by 1024 complex points with double exponential processing (with a constant of 3 Hz) used in both dimensions.

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