Model of Interaction of Ajmaline with Polyvinylpyrrolidone

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Abstract □ The chemical shifts in the Fourier transform ^{13}C-NMR spectrum of ajmaline were assigned from consideration of the acetylation shifts and multiplet splitting in the off-resonance decoupled spectrum. On the basis of the assigned signals, the changes of chemical shifts in ajmaline induced by polyvinylpyrrolidone were investigated, and an interaction model of ajmaline with polyvinylpyrrolidone in chloroform was proposed.

Keyphrases □ Ajmaline—model of interaction with polyvinylpyrrolidone □ Polyvinylpyrrolidone—model of interaction with ajmaline □ ^{13}C-NMR spectroscopy—ajmaline, chemical shifts assigned

Tachibana and Nakamura (1) reported a new method for enhancing the dissolution rates of poorly soluble drugs by using water-soluble polymers such as polyvinylpyrrolidone. The coprecipitate of ajmaline with polyvinylpyrrolidone was prepared (2) in the same manner as the coprecipitate of reserpine (3) and griseofulvin (4), and the enhancement of the dissolution rate of ajmaline from this coprecipitate was ascertained. This effect seemed to be caused largely by some interaction between polyvinylpyrrolidone and ajmaline in chloroform, the solvent in which this coprecipitate was prepared. The interaction sites of polyvinylpyrrolidone and ajmaline in chloroform were investigated. The hydrogen bond formed between the C-21 hydroxyl group in ajmaline and the carbonyl groups in polyvinylpyrrolidone was confirmed (2), and complexation between the aromatic ring in ajmaline and the amide groups in polyvinylpyrrolidone was suggested (5).

This study was conducted to confirm these interactions using Fourier transform ^{13}C-NMR spectroscopy and to propose a model of interaction of polyvinylpyrrolidone with ajmaline in chloroform.

EXPERIMENTAL

The ajmaline, 17,21-diacetylajmaline[1], and polyvinylpyrrolidone[2] (K-15) employed were of the same origin and were purified as described previously (2). ^{13}C-NMR spectra[3] were obtained in deuterochloroform with tetramethylsilane as the internal reference.

RESULTS AND DISCUSSION

To clarify the behavior of the electron density of the carbon atoms in ajmaline caused by the interaction with polyvinylpyrrolidone, the influence of polyvinylpyrrolidone on the ^{13}C-NMR spectrum of ajmaline was investigated. Since there were no previous reports on the ^{13}C-NMR spectrum of ajmaline, the signal assignment was performed initially. The number of signals observed in the complete proton-decoupled ^{13}C-NMR spectrum was equal to the number of carbon atoms constituting ajmaline (Fig. 1). Therefore, no overlap of signals was detected.

From consideration of the chemical shift rules (6) and the multiplet splitting in ^1H-signal frequency off-resonance decoupling experiments, the carbons constituting ajmaline were classified as aromatic carbons (C-8–C-13), carbons adjacent to hydroxyl groups (C-17 and C-21), a quaternary carbon (C-7), methyl carbons (C-18 and C-22), methylene carbons (C-6, C-14, and C-19), and methine carbons (C-2, C-3, C-5, C-15, C-16, and C-20).

The signals of the aromatic carbons in ajmaline were assigned according to previous reports about various compounds containing an indole ring (7, 8) (Table I).

Three signals were observed in the magnetic field of the carbon atoms adjacent to hydroxyl groups, except for the signals derived from deuterochloroform. The signal of δ 88.10 ppm was derived from C-21 because it was adjacent to the nitrogen atom and a hydroxyl group. Generally, the carbon atoms at the α- and β-positions are shifted downfield and upfield, respectively, due to the substituent effects produced by replacement of the hydroxyl group by the acetoxy group (9). Therefore, it seems reasonable that the signal of δ 88.10 ppm was shifted downfield by diacetylation (Table I, $\delta_b - \delta_a$).

On the other hand, the signal of either δ 79.19 or 77.72 ppm corresponded to C-17. The C-17 signal was expected to be shifted significantly downfield by diacetylation because of its α-position to the hydroxyl group. Accordingly, the δ 77.72-ppm signal was determined to be C-17, in spite of the interchangeability of the C-17 assignment with C-2 in the diacetate (δ_b).

The quaternary carbon atom, C-7, was assigned easily from multiplicity.

In the two signals of the methyl carbon atoms (quartet), the δ 34.11-ppm signal was determined to be C-22 because of its adjacency to the nitrogen atom.

For the methylene carbons (C-6, C-14, and C-19), the side-chain carbon (C-19) was attributed to δ 25.39 ppm. Although the assignments of C-6 and C-14 were attempted, the discrimination remained uncertain because one of these signals exhibited the large downfield shift (+1.50 ppm) from diacetylation in spite of the distance from the hydroxyl groups, and this large shift could not be explained by the inductive effect of the acetoxy groups. Further investigation involving an additional steric effect is required to assign these signals.

The signals of the methine carbons (C-2, C-3, and C-5) adjacent to the nitrogen atom were downfield. The signal of C-2 adjacent to the aromatic nitrogen atom in the indole ring was expected to appear considerably downfield (10), and the signal of δ 79.19 ppm was assigned to C-2. The large shift of the δ 77.72-ppm signal by diacetylation also provided convincing evidence that this signal was not derived from C-2.

The signals due to C-15, C-16, and C-20 were expected to be farther upfield than those of C-2, C-3, and C-5. Since C-20 is located at the β-position to C-18 in the aliphatic side chain, this signal seemed to appear significantly upfield. The signal of δ 28.12 ppm was assigned to C-20 and exhibited a reasonable upfield shift by diacetylation (−0.85 ppm). The C-15 and C-16 carbons exist in the ring system, so these signals appear relatively downfield compared with that of C-20. It appears that C-16 is almost as susceptible to influence as C-7 because it is located at the same β-position as C-7 to C-17. The C-15 signal is not influenced by diacetylation due to the long distance between C-15 and either hydroxyl group.

On the basis of these chemical shift rules and acetylation shifts, the possibility that the signals of C-15 or C-16 in ajmaline occur at δ 52.78 or 47.95 ppm is excluded. Consequently, it was concluded that the C-16 signal corresponds to δ 45.22 ppm and that the δ 43.08-ppm signal corresponds to C-15 because the latter was not influenced appreciably by diacetylation. These assignments remained unaltered in spite of the interchangeability of δ 43.65 and 43.01 ppm in the diacetate.

Subsequently, on the basis of these assignments, the change of signals in 0.34 *M* ajmaline by the addition of various concentrations of poly-

[1] Mass spectrum: *m/e* 410 (M^+). *Anal.*—Calc. for $C_{24}H_{30}N_2O_4$: C, 70.37; H, 7.29; N, 6.70. Found: C, 70.16; H, 7.31; N, 6.82.

[2] The mean molecular weight of polyvinylpyrrolidone (K-15) was evaluated to be ~7500 by viscosity measurements at 30°.

[3] JEOL-FX-100 spectrometer operating at 25.0 MHz in the Fourier transform mode.

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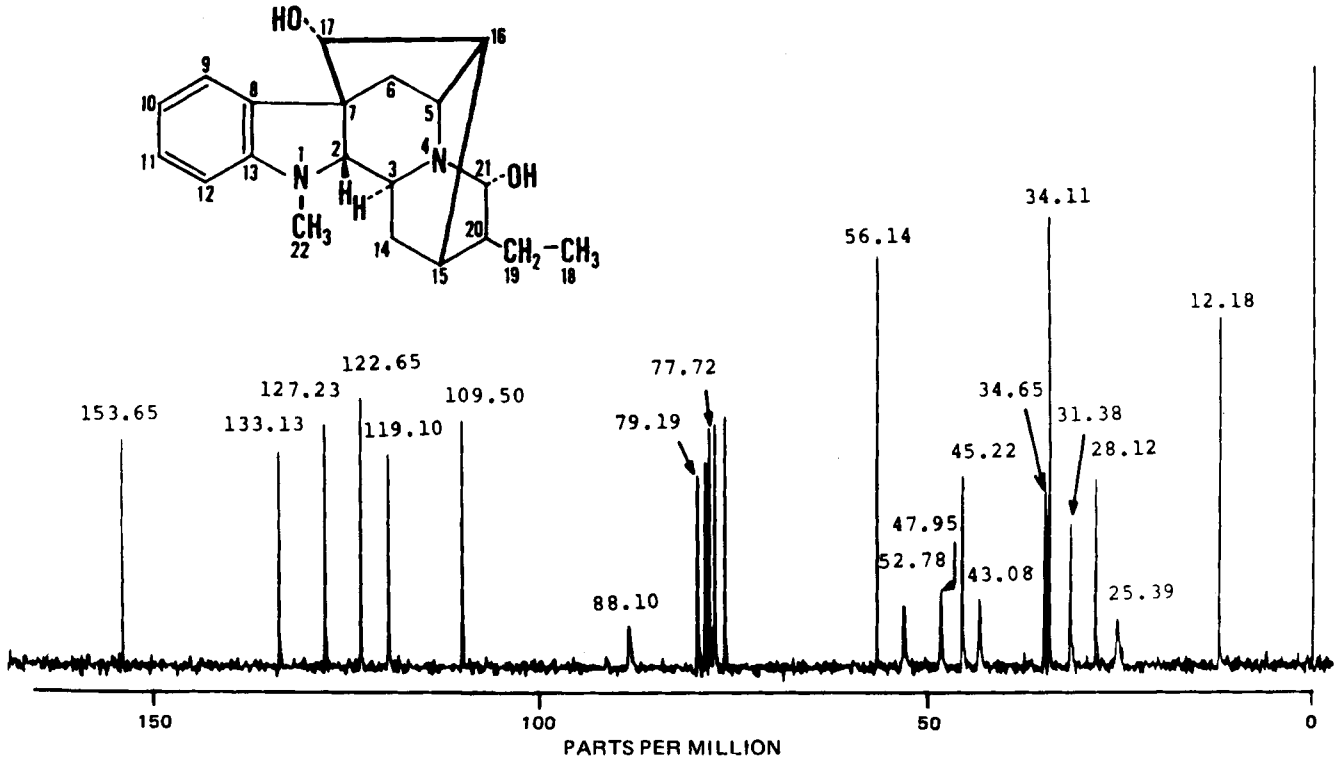


Figure 1—*Structure and complete proton-decoupled ^{13}C-NMR spectrum of ajmaline.*

vinylpyrrolidone was investigated (Fig. 2). Although the chemical shift of the C-17 signal was not distinctly observed by addition of 0.015 and 0.06 *M* polyvinylpyrrolidone due to overlapping with the signals derived from deuterochloroform, the signals of C-21 and C-17 tended to be shielded by an increase in the polyvinylpyrrolidone concentration. However, no significant changes of linewidth in the ajmaline signals induced by polyvinylpyrrolidone could be detected.

By using UV and ^1H-NMR spectra, the hydrogen bond formed between the C-21 hydroxyl group in ajmaline and the carbonyl groups of the pyrrolidone rings in polyvinylpyrrolidone was recognized previously (5). The upfield shifts of C-17 and C-21 were observed by the addition of polyvinylpyrrolidone in the present study. In ^{13}C-NMR spectra, the chemical shift variation is dominated by the electron density (11). The enhancements of electron density in C-21 and C-17 of ajmaline provide sufficient grounds to expect hydrogen bond formation with polyvinylpyrrolidone.

To ascertain this point, the effect of acetone on 2-butanol was examined. The C-2 in 2-butanol was shifted upfield by the addition of 1 drop of acetone (not shown). This upfield shift of C-2 in butanol obviously was attributable to the hydrogen bond formed between 2-butanol and acetone. Accordingly, it is almost certain that the C-17 and C-21 hydroxyl groups in ajmaline formed hydrogen bonds with the carbonyl groups of the pyrrolidone rings in polyvinylpyrrolidone. The fact that the upfield shift of C-21 was smaller than that of C-17 by hydrogen bond formation seemed to result from the smaller influence of C-21 than of C-17 by diacetylation. In addition, from consideration of the changed C-7 signal by diacetylation, the upfield shift of C-7 by the addition of polyvinylpyr-

Table I—Chemical Shift Assignments in ^{13}C-NMR Spectra of Ajmaline and 17,21-Diacetylajmaline [a]

Carbon	Multiplicity	Chemical Shift: Ajmaline (δ_a)	Chemical Shift: Diacetylajmaline (δ_b)	$\delta_b - \delta_a$
13	s	153.65	153.83	+0.18
8	s	133.13	132.09	−1.04
11	d	127.23	127.65	+0.42
10	d	122.65	122.35	−0.30
9	d	119.10	119.24	+0.14
12	d	109.50	109.72	+0.22
21	d	88.10	88.73	+0.63
2	d	79.19	80.03*	+0.84
17	d	77.72	79.22*	+1.50
7	s	56.14	54.37	−1.77
5	d	52.78**	53.56**	+0.78
3	d	47.95**	48.37**	+0.42
16	d	45.22	43.65***	−1.57
15	d	43.08	43.01***	−0.07
6	t	34.65****	36.15****	+1.50
22	q	34.11	34.65	+0.54
14	t	31.38****	31.83****	+0.45
20	d	28.12	27.27	−0.85
19	t	25.39	25.02	−0.37
18	q	12.18	12.17	−0.01
$COCH_3$	s		170.37	
	s		169.11	
$COCH_3$ (X2)	q		21.27	

[a] *, **, ***, and **** may be interchanged in each column. The numbering of the carbons is illustrated in Fig. 1. The designations for signal multiplicity are s = singlet, d = doublet, t = triplet, and q = quartet. Chemical shifts are expressed in parts per million relative to tetramethylsilane.

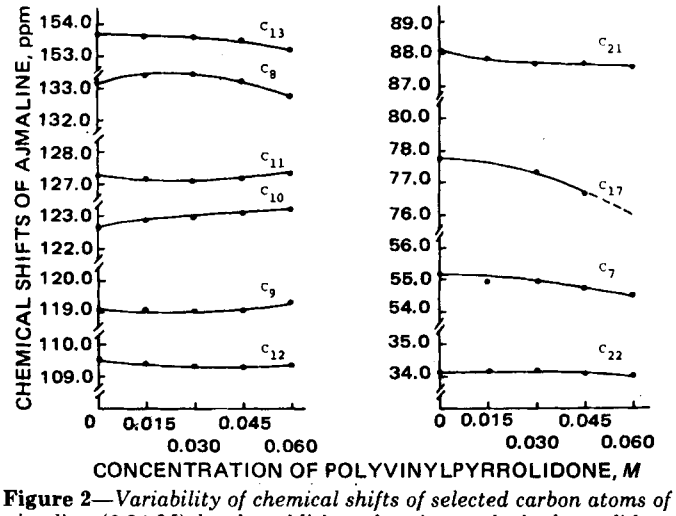
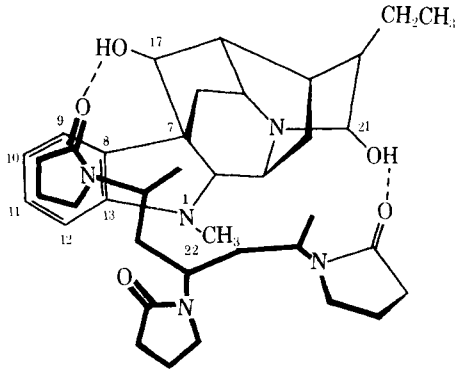


Figure 2—*Variability of chemical shifts of selected carbon atoms of ajmaline (0.34 M) by the addition of various polyvinylpyrrolidone concentrations.*



I

rolidone seemed to be influenced by hydrogen bond formation (Table I).

In addition to the influence in the signals of C-7, C-17, and C-21, there was a relatively large influence in the signals of aromatic carbons in ajmaline by polyvinylpyrrolidone; that is, the signals of C-8 and C-13 tended to shift upfield and those of C-9 and C-10 shifted downfield. The electron densities of C-8 and C-13 became higher due to the presence of polyvinylpyrrolidone, and those of C-9 and C-13 became lower.

Considering the relatively small changes of the aromatic carbons by diacetylation, it does not seem that these electron density changes were brought about indirectly through hydrogen bond formation. Furthermore, the upfield shift of C-13 and the downfield shift of C-10 do not seem to support the interaction with the nitrogen atom at position 1 in ajmaline. Accordingly, it is reasonable to consider that a complex was formed between the amide groups of the pyrrolidone ring in polyvinylpyrrolidone and the aromatic ring in ajmaline, as suggested previously (5). It is expected that the electron densities of C-8 and C-13 of the aromatic ring in ajmaline are affected and become higher because of the approach of the positively charged nitrogen atom of the amide group of the pyrrolidone rings of polyvinylpyrrolidone. Considering such mechanisms of complex formation, Laszlo (12) described it as a dipole–induced dipole complex in view of the nomenclature of these complexes.

From these results, an interaction model between polyvinylpyrrolidone and ajmaline is proposed (I). In the coprecipitate, ajmaline apparently was molecularly dispersed in solid polyvinylpyrrolidone through these interactions. As a result, the dissolution rate of ajmaline from the coprecipitate was enhanced markedly.

REFERENCES

(1) T. Tachibana and A. Nakamura, *Kolloid-Z. Z. Polym.*, **203**, 130 (1965).

(2) H. Matsumaru, S. Tsuchiya, and T. Hosono, *Chem. Pharm. Bull.*, **25**, 2504 (1977).

(3) T. R. Bates, *J. Pharm. Pharmacol.*, **21**, 710 (1969).

(4) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **55**, 1323 (1966).

(5) T. Hosono, S. Tsuchiya, and H. Matsumaru, *Chem. Pharm. Bull.*, **27**, 58 (1979).

(6) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic, New York, N.Y., 1972, pp. 55–207.

(7) G. W. Gribble, R. B. Nelson, J. L. Johnson, and G. C. Levy, *J. Org. Chem.*, **40**, 3720 (1975).

(8) E. Wenkert, C. J. Chang, H. P. S. Chawla, D. W. Cochran, E. W. Hagaman, J. C. King, and K. Orito, *J. Am. Chem. Soc.*, **98**, 3645 (1976).

(9) M. Christl, H. J. Reich, and J. D. Roberts, *ibid.*, **93**, 3463 (1971).

(10) P. G. Lukacs, M. De Bellefon, L. Le Men-Oliver, J. Levy, and J. Le Men, *Tetrahedron Lett.*, **1974**, 487.

(11) R. Hagen and J. D. Roberts, *J. Am. Chem. Soc.*, **91**, 4504 (1969).

(12) P. Laszlo, in "Progress in Nuclear Magnetic Resonance Spectroscopy," vol. 3, J. W. Emsley, J. Feeney, and L. H. Sutcliffe, Eds., Pergamon, London, England, 1967, pp. 383–386.

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Polarographic Determination of Edetate Disodium in Eyewash and Ophthalmic Decongestant Solutions

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Abstract □ The quantitative analysis of edetate disodium in nonprescription eyewash and ophthalmic solutions is described. The method involves differential pulse polarography using a dropping mercury electrode. A known concentration of cadmium or zinc is added to a buffer in a polarographic cell. The sample solution is incremented into the cell with a micropipet. The peak current decreases because the resulting chelate is not reducible at the potentials used. The quantity of edetate disodium in the sample then is determined graphically. Some contact lens cleaning and wetting solutions containing polymeric compounds are amenable to assay for edetate disodium if extraction, precipitation, centrifugation, or dilution steps minimize the maximum suppressor effect of the additives. These steps are very effective with cellulose ether compounds but are ineffective with polyvinyl alcohol.

Keyphrases □ Edetate disodium—polarographic determination in eyewash and ophthalmic decongestant solutions □ Polarography, differential pulse—analysis, edetate disodium in eyewash and ophthalmic decongestant solutions □ Ophthalmic preparations—polarographic determination of edetate disodium in eyewash and ophthalmic decongestant solutions

Edetate disodium (I) is added to eyewash and ophthalmic solutions containing bactericides such as benzalkonium chloride, chlorobutanol, and thimerosal to increase their bactericidal properties (1). Compendial methods (2–4) employ classical titrimetric procedures for the quantitative determination of I, but these methods are not suitable for the levels encountered in the drug preparations (0.01–0.25%).

A literature search revealed two methods used to determine I in pharmaceutical preparations. One employed colorimetric detection for ophthalmic solutions (5); the other used atomic absorption for an antibiotic preparation (6).

This paper proposes a sensitive, rapid, and quantitative polarographic method for determining I concentrations by stepwise addition of sample to the cell. The resulting

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