Tetrahedron. Vol. 27, pp. 4857 to 4862. Pergamon Press 1971. Printed in Great Britain

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

^{13}C SPECTRA OF THE VERATRUM ALKALOIDS, JERVINE AND VERATRAMINE*

PETER W. SPRAGUE,† DAVID DODDRELL and JOHN D. ROBERTS
Gates and Crellin Laboratories of Chemistry[1]
California Institute of Technology, Pasadena, California 91109

(*Received in USA* 24 *February* 1971; *Received in the UK for publication* 18 *May* 1971)

Abstract—The natural-abundance ^{13}C magnetic resonance spectra of jervine and veratramine have been determined at 15·08 MHz. Chemical-shift correlations between these molecules and degradation products of jervine together with proton-decoupling techniques have made unambiguous assignments of most of the resonances possible.

THE STEROIDS and steroidal alkaloids have historically provided chemists with difficult and frustrating structural problems. The extent of the literature dealing with instrumental methods, such as mass spectrometry, tailored specifically to help solve these problems is enormous. No truly omnipotent method has yet been discovered and the best results usually are obtained by application of a combination of analytical techniques. Carbon-13 magnetic resonance spectrometry (CMR) has shown great promise as an aid to structural elucidation of organic substances of this degree of complexity,[2,3] and is here applied to the Veratrum alkaloids.

The proton magnetic resonance (PMR) spectra of the Veratrum alkaloids are limited in usefulness because of small differences in chemical shift and complex spin–spin couplings. The CMR spectra, augmented by auxiliary techniques such as noise-modulated total proton decoupling[4,5] and specific and off-resonance single-frequency proton decoupling[2,6] have allowed assignment of nearly all of the skeletal atoms of two members of the Veratrum family of alkaloids, jervine and veratramine.[7]

The methods used here to assign the chemical shifts have been fully discussed elsewhere.[2,3,5] Because of the complex nature of the proton spectra of the steroidal alkaloids, specific proton decoupling techniques were not very useful. To make matters worse, the CMR spectra were themselves sufficiently complicated (*cf.* Fig. 1) to render single frequency off-resonance (SFOR) decoupling of little help, except to identify quaternary carbon atoms. The most fruitful techniques involved modifications of the jervine ring structure which changed some chemical shifts and not others. Model compounds, including simple steroids, which duplicated portions of the alkaloid system also proved useful in the chemical-shift assignments. The assignments for jervine (**1**), $^{5,12(17),16(20)}$ethyletiojervatriene-3-ol-11-one and its acetate (**2a** and **b**),[8] $^{5,12(17)}$ethyletiojervadiene-3-ol-11,16-dione acetate (**3**),[8] veratramine (**4a**) and

* Supported by the Public Health Service, Grant No. GM-11072, from the Division of General Medical Sciences, and by the National Science Foundation.

† California State College, San Bernardino.

its diformate (**4b**), and a piperidine model compound (**5**) are listed in Table 1 and correlated in Fig. 2.

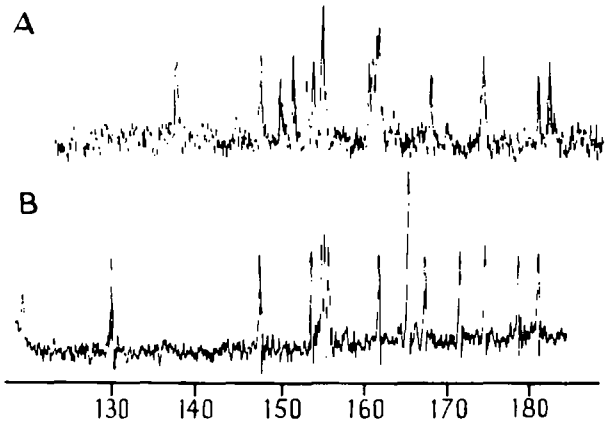


FIG 1. (a) Noise-decoupled ^{13}C spectrum at 15·08 MHz of 0·4 M jervine (**1**) in pyridine; 688 scans at a sweep width of 1000 Hz (scale in ppm from CS_2) and 50 sec/scan. (b) Noise-decoupled ^{13}C spectrum at 15·08 MHz of 0·26 M ethyletiojervatriene-3-ol-11-one acetate (**2b**) in pyridine; 149 scans at a sweep width of 1000 Hz (scale in ppm from CS_2) and 50 sec/scan

The unsaturated (sp^2) carbon atoms

Ketone carbonyl carbons have been shown to come into resonance at quite low fields[9] in CMR spectra. Assignment of C-11 in jervine is therefore obvious. Because of the similarities between the steroids and steroidal alkaloids, many assignments are possible by direct comparison of the CMR spectra of these substances. Carbons C-5 and C-6 were assigned in this way, which leaves the remaining unsaturated resonances in the jervine spectra to be assigned to C-12 and C-13. Carbon C-13 was assigned to the lower-field resonance because it is β to the carbonyl group.[5]

In the veratramine spectra, assignments of the sp^2 carbon atoms were more difficult. Three of the resonances are so close that differentiation is, at best, speculative. Carbons C-6, C-15 and C-16, being methines, are discernible as higher-field resonances. Carbon-16 was assigned to the lowest methine unsaturated resonance because of steric interactions with the heterocyclic ring which should affect this atom more than C-15 or C-7. Carbon C-13 was assigned to the highest-field quaternary unsaturated atom resonance because, of all the quaternary sp^2 atoms, this atom is least substituted.

Steroid ring atoms

Removal of the heterocyclic ring from the jervine system, as in **2**, simplifies the spectrum by eliminating six resonances. Of the remaining sixteen unassigned atoms in the jervine spectrum, C-3, C-17 and C-10 are readily assignable. Carbons C-3 and C-17 are hydroxylated in jervine and come into resonance at lower fields than the others. In addition, C-17 is quaternary and is discernible from C-3 by SFOR-decoupling experiments. Carbon C-10 is also quaternary and therefore easily identified.

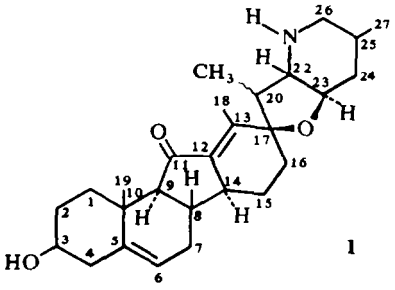
TABLE 1. THE CHEMICAL SHIFTS AND ^{13}C RESONANCE ASSIGNMENTS FOR THE VERATRUM ALKALOIDS. JERVINE (1), VERATRAMINE (4), AND RELATED COMPOUNDS[a,b]

Carbon	1	2a	2b	3	4a	4b	5[c]
1	154·4	156·3	156·8	157·1	155·2	154·8	
2	162·0	161·9	166·1	165·9	163·0	166·0	
3	121·9	122·8	120·1	120·4	122·7*	118·9	
4	152·1	151·3	155·9	155·8	150·9	154·8	
5	47·3	50·7	52·3	53·0	50·9**		
6	72·6	73·1	72·6	71·9	72·5		
7	153·6	155·0	155·9	156·4	152·3	151·3	
8	148·2	149·1	148·9	149·6	148·5	150·7	
9	130·3	130·9	131·1	131·0	136·2	135·7	
10	155·6	156·3	156·3	156·4	156·6	155·6	
11	−12·6	−12·1	−12·5	−12·4	163·0	162·2	
12	56·6	56·1	56·2	e	52·4		
13	50·5	52·5	52·6	51·8	60·9		
14	162·2	163·0	162·8	163·4	49·9**		
15	168·3	166·5	166·3	166·4	74·2		
16	155·6	168·3	168·1	156·4	67·4		
17	107·7	66·4	66·2	−5·9	49·9**		
18	182·0	181·7	181·4	183·8	177·7	176·8	
19	174·5	175·3	175·3	175·6	174·5	173·4	
20	161·0*	e	e		161·3***	162·2*	164·4*
21	180·7	179·5	179·3		174·5	174·3**	172·8
22	125·7				125·6	126·9	126·5**
23	116·2				123·0*	124·8	125·9**
24	150·5				157·9	161·8	155·1
25	161·4*				161·7***	164·9*	165·9*
26	137·8				139·2	141·6	140·4
27	174·5				172·7	172·8**	173·5
CH_3[d]			172·5	172·8			
C=O[d]			23·4	24·2			

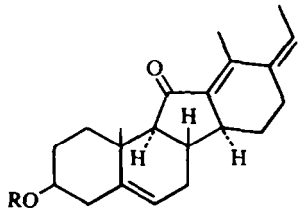
[a] Measured at 15·08 MHz in ppm from CS_2. Resonances marked with asterisks (single, double or triple) represent uncertain assignments which may be reversed. [b] The solvent was pyridine, except for **4b** which was in $CHCl_3$. [c] Using a numbering system analogous to the steroidal alkaloids. [d] Methyl and carbonyl of acetyl group. [e] These resonances under solvent peaks.

In the veratramine spectrum, the two hydroxylated atoms, C-3 and C-23, have shifts too close together to be individually assigned. The remaining atoms in ring A are assignable by comparison of the spectra of **2a**, **4a**, **2b**, and **4b** with the spectra of cholesterol and cholesteryl acetate.[2] Carbons C-1, C-2, and C-4 in the alkaloids have nearly the same chemical shifts as their counterparts in the cholesterol series. In addition, these resonances exhibit similar upfield shifts when the oxygen at C-3 is acetylated or formylated.[2]

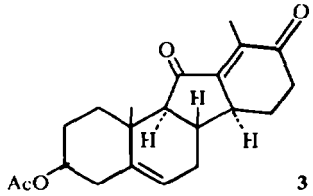
Of the remaining unassigned atoms in ring B, C-9 is assignable by comparison of the spectra of **1** with 9-deuteriojervine. The spectrum of the deuterated alkaloid clearly shows the loss of a peak at 130·3 ppm.[2] Carbons C-7 and C-8 are difficult to assign with certainty. There are only two resonances common to the spectra of **1**, **2a** and **b**, and **4a** and **b**, that could not be assigned to other atoms. The lower-field resonance was assigned to C-8 since this atom is a methine while C-7 is a methylene.



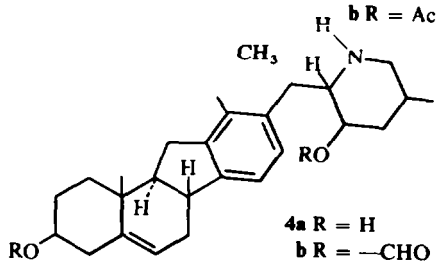
1



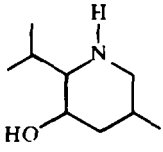
2a R = H
b R = Ac



3



4a R = H
b R = —CHO



5

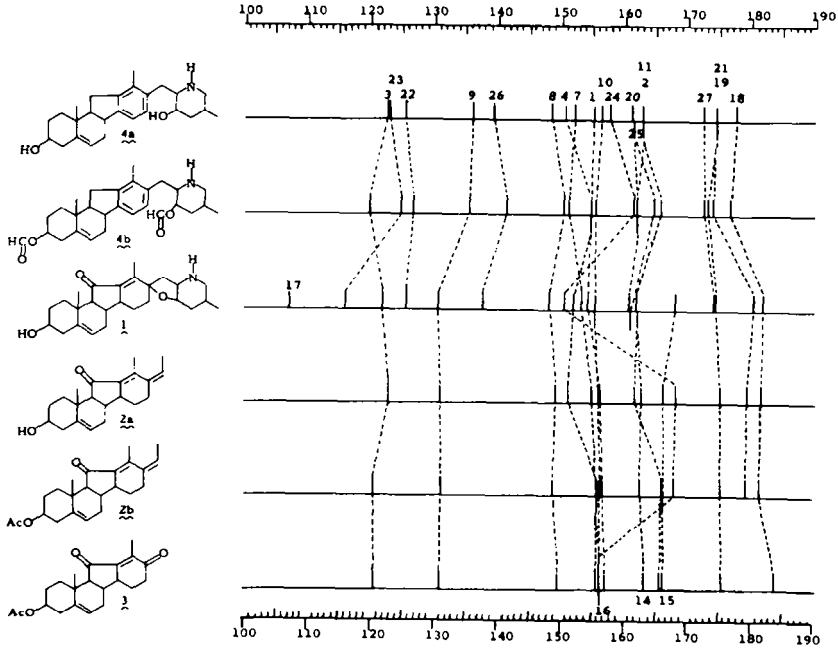


FIG 2. Chemical-shift correlations for jervine, veratramine and related compounds

Carbon-11 is the only unassigned atom in the C ring of veratramine. This atom is a methylene and not likely to be affected by modifications at the electronegative atoms in the molecule. The assignment was therefore made to a high-field resonance which was not shifted by formylation of veratramine.

Of the three unassigned atoms in ring D of jervine, C-14, C-15 and C-16, C-16 was assigned to the resonance that exhibited a + 12·7 ppm shift on deoxygenation of C-17, (**1** converted to **2b**), and a − 11·7 ppm shift on ketonization of C-17 (**2b** converted to **3**). These shifts are in line with those observed in similar cases in the steroid work.[2] The two resonances which did not shift on deoxygenation in the same region were assigned to C-14 and C-15 according to their degree of substitution.

The remaining two atoms in the steroid part of the alkaloids, C-18 and C-19, give rise to high-field resonances distinguishable by comparison of the spectra of **2b** with **3**. Loss of the side chain from C-17 in **2b** would be expected to influence the chemical shift of C-18 more than C-19. This argument leads to assignment of the higher-field resonance to C-18.

Heterocyclic carbons

Assignment of the eight atoms connected with this part of the molecule was facilitated by the use of the piperidine model compound (**5**). Its spectrum was simple enough to allow SFOR-decoupling data showing the degree of substitution of each atom in the molecule. The spectrum of **5** revealed three resonances at low field corresponding to atoms substituted by electronegative atoms and therefore attributable to carbon atoms C-22, C-23 and C-26 in the alkaloids. In addition, carbons C-22 and C-26 were distinguishable according to their degree of substitution. These three resonances appeared in nearly the same positions in the spectra of jervine and veratramine. There were two resonances in the spectrum of **5** occurring at 155 ppm and near 165 ppm, which were shown by SFOR-decoupling to correspond to a methylene and a methine carbon atom, respectively. These two resonances also appeared in the alkaloid spectra in nearly the same positions and were assigned to C-24 and C-25 in the heterocyclic ring. Only C-20, C-21 and C-27 remain unassigned and, as the last two atoms are Me groups, they are assigned to high-field resonances. It is expected that C-27 should resonate at essentially the same field in both alkaloids, whereas C-21 may not, as it is in a dissimilar environment in each compound. Carbon 20, being a methine, was assigned to the remaining medium-field resonance (near 160 ppm), thus completing the round of assignments in the spectra of jervine and veratramine.

EXPERIMENTAL

The ^{13}C spectra were obtained on the DFS CMR spectrometer which has been described elsewhere.[4] The compounds were run at concentrations of between 0·2 and 1·5 molar, depending on individual solubilities. Pyridine was used as solvent, except for compound **4b** which was run in $CHCl_3$. Chemical shifts were calculated from the β carbon of the solvent py., using the following relationship: $\delta_C^{CS_2} = \delta_C^{Py^{\beta C}} + 70{\cdot}0$ ppm. The chemical shifts for **4b** were calculated from $CHCl_3$ as an internal reference with the relationship: $\delta_C^{CS_2} = \delta_C^{CHCl_3} + 115{\cdot}3$ ppm.

The degradation products of jervine were synthesized according to the procedures of Fried and Klingsberg.[8] The piperidine model compound (**5**) was synthesized by standard procedures for the synthesis of 3-hydroxypyridines,[10] followed by reduction under conditions similar to those for Marion's synthesis of pseudoconhydrine.[11] The physical properties of the product indicated that it was a diastereomeric

mixture which, after sublimation, had mp 54–60°. No attempt was made to separate the diastereomers because the model compound was used only to allow estimates of the chemical shifts for the corresponding atoms in the alkaloid systems. In fact, the ^{13}C spectra of compound **5** showed only nine major peaks, indicating one diastereomer predominated. (Calcd. for $C_9H_{19}ON$: C, 68·74; H, 12·18; N, 8·90. (Found: C, 68·33; H, 12·26; N, 8·67%).

Acknowledgement—We are much indebted to Dr M. W. Klohs of the Riker Laboratories of 3M (Northridge, Calif.) for providing the alkaloid samples used in this work.

REFERENCES

[1] Contribution No. 4205
[2] H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert and J. D. Roberts, *J. Am. Chem. Soc.* **91**, 7445 (1969)
[3] W. O. Crain, Jr., W. C. Wildman and J. D. Roberts, *Ibid.* **93**, 990 (1971)
[4] F. J. Weigert, M. Jautelat and J. D. Roberts, *Proc. Nat. Acad. Sci. (U.S.)* **60**, 1152 (1968)
[5] [a] L. F. Johnson and M. E. Tate, *Can. J. Chem.* **47**, 63 (1969);
[b] R. R. Ernst, *J. Chem. Phys.* **45**, 3845 (1966)
[6] M. Jautelat, J. B. Grutzner and J. D. Roberts, *Proc. Nat. Acad. Sci. (U.S.)* **65**, 288 (1970)
[7] S. M. Kupchan and A. W. By, *The Alkaloids*, Vol. X, p. 193. R. H. F. Manske, Ed., Academic Press, New York, N.Y. (1968)
[8] J. Fried and A. Klingsberg, *J. Am. Chem. Soc.* **75**, 4929 (1953)
[9] J. W. Emsley, J. Feeney and L. H. Sutcliffe, *High Resolution Nuclear Magnetic Resonance Spectroscopy*, p. 1009. Vol. 2, Pergamon Press, New York, N.Y. (1966)
[10] H. Meislich, *Pyridine and its Derivatives*, (Edited by E. Klingsberg), Interscience, New York, N.Y. (1962)
[11] L. Marion and W. F. Cockburn, *J. Am. Chem. Soc.* **71**, 3402 (1949)