Alkaloids of *Vinca* Species V: Structure Elucidation of Herbadine, an Alkaloid Isolated from *Vinca libanotica*

G. H. AYNILIAN, C. L. BELL, and N. R. FARNSWORTH *

Abstract □ Herbadine, a novel dihydroindole alkaloid, was isolated from the aerial parts of *Vinca libanotica* Zucc. (Apocynaceae). The physical and spectral data (UV, IR, NMR, and mass spectroscopy) indicated the alkaloid to be a derivative of ajmaline. Comparative high-resolution NMR studies with quebrachidine and high-resolution mass spectral studies established the structure of the alkaloid to be 2-epi-3-hydroxyquebrachidine. Herbadine was previously isolated from another species by Russian workers and a structure was postulated. The current paper gives evidence for a corrected structure of herbadine.

Keyphrases □ Herbadine—structure elucidation, *V. libanotica* alkaloid □ *Vinca libanotica* alkaloids—structure elucidation of herbadine □ Alkaloids—*Vinca* species, structure elucidation of herbadine

In a previous article (1) the structures and NMR spectra of vincamajine (I) and herbamine (III), both isolated from *Vinca libanotica* Zucc. (Apocynaceae) (2), were reported. The structure of another dihydroindole alkaloid is now elucidated. This alkaloid, also isolated from *V. libanotica* (2), has a molecular formula of $C_{21}H_{24}N_2O_4$ and possesses physical and spectral properties very similar to herbadine (V), recently reported to be isolated from *V. herbacea* (3). However, in accordance with the revised structure for herbamine (1) and based on high-resolution mass spectral studies of herbadine together with high resolution NMR studies at 220 MHz of both herbadine and the structurally related quebrachidine (II), a new structure (IV) is postulated for herbadine.

EXPERIMENTAL[1]

Previously, the isolation and characterization of several alkaloids including herbadine from *V. libanotica* were reported (2). Herbadine exhibited a melting point of 202–205° dec. and a UV spectrum in methanol having maxima at λ 292 (log ε 3.48), 242 (3.98), and 212 (4.57) nm, which are typical of alkaloids having the dihydroindole moiety. An IR spectrum (KBr) gave absorption bands at ν_{max} 3450 (s) (NH and/or OH), 2950 (s), 2875 (w), 2400 (w), 1725 (s) ($COOCH_3$), 1615 (s) (indoline), 1560 (w), 1540 (w), 1460 (w), 1380 (w), 1310 (w), 1290 (w), 1240 (s) ($COOCH_3$), 1200 (w), 1140 (s), 1100 (w), 1060 (s), 1040 (w), 980 (w), 950 (w), 900 (m), 860 (m), 810 (w), 780 (w), 760 (w), and 735 (1,2-disubstituted benzene) cm^{-1}.

The mass spectrum gave a molecular ion at M^+ 368 (32%), followed by ions at *m/e* 337 (2), 252 (2), 251 (2), 178 (3), 168 (1), 167 (1), 166 (1), 158 (1), 157 (1), 150 (3), 147 (3), 144 (5), 143 (20), 135 (5), 130 (10), 121 (2), and 117 (100).

The high-resolution mass spectrum revealed that the fragments at *m/e* 130 and 143 corresponded to the N_a-indole moiety with one and two additional carbon atoms. The peak at *m/e* 144 was a doublet consisting not only of $C_{10}H_{10}N$ but also of C_9H_6NO; the latter requires retention of the oxygen at C_{17} and is found in all ajmaline derivatives bearing a 17-hydroxy group (4). This is not the case in the structure (V) proposed for herbadine by the Russian workers, who did not determine the elemental composition of the mass spectral fragments. The base peak at *m/e* 117 (C_8H_7N) could be best represented by the N_a-indole skeleton.

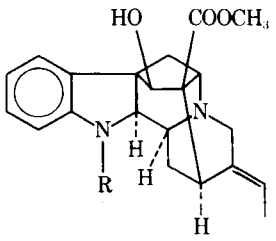
The NMR spectrum of the alkaloid, determined in CD_3OD at 90 MHz, exhibited some similarities with the spectrum of quebrachidine. Thus, it was assumed initially that herbadine (M^+ 368) was an hydroxyl derivative of quebrachidine (M^+ 352), the structure of which was established based on mass spectral studies (5).

Since NMR data can be used as a simple means of differentiating between alkaloids of the ajmaline and 2-epiajmaline series (1) and since no comprehensive NMR assignments have been reported for quebrachidine, the high-resolution NMR spectra of both herbadine and quebrachidine were determined in pyridine-d_5 at 220 MHz (Table I).

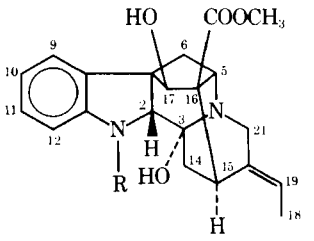
DISCUSSION

The basic differences observed between the NMR spectra of herbadine and quebrachidine were the same as the ones observed between herbamine and vincamajine (1), thus proving that herbadine belongs to the ajmaline series. In addition, in the high-resolution mass spectrum, the elemental composition of the prominent ions (M^+ 368, *m/e* 144, 143, 130, and 117) was 14 units ($—CH_2—$) lower than the ions of herbamine, suggesting that herbadine was the N_a-demethyl derivative of herbamine.

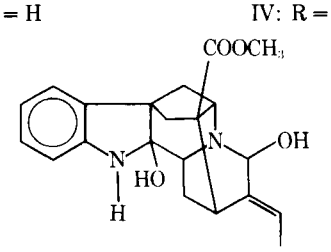
In the NMR spectrum of herbadine, two doublets at δ 4.50 and 3.40 (J = 17 Hz) were attributed to the two C_{21} protons; in quebra-



I: R = CH_3
II: R = H



III: R = CH_3
IV: R = H



V

[1] The UV spectrum was determined using a Beckman model DB-G spectrophotometer, and the IR spectrum was determined using a Beckman model IR 18A spectrophotometer in a KBr pellet. NMR spectra of herbadine and quebrachidine were recorded in pyridine-d_5 solutions on a Varian HR-220 instrument operating at 220 MHz, with tetramethylsilane as an internal standard and with chemical shifts reported in δ (parts per million) units. The high-resolution mass spectrum (70 ev) of herbadine was recorded using a C.E.C. model 110 double-focusing spectrometer at Eli Lilly and Co.

The plant material was authenticated as *V. libanotica* Zucc. (Apocynaceae) by Dr. W. T. Stearn, British Museum of Natural History, London, England. A voucher specimen (SP-2315) representing the collection has been placed in the Herbarium of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center.

Table I—NMR Spectral Data of Herbadine and Quebrachidine Determined at 220 MHz in Pyridine-d_5

Assignments	Herbadine		Quebrachidine	
	Chemical Shift, δ[a]	Coupling Constants, Hz	Chemical Shift, δ[a]	Coupling Constants, Hz
C_{9}—H	7.75–7.30 (m)		7.55–6.95 (m)	
C_{11}—H	7.75–7.30 (m)		7.55–6.95 (m)	
C_3—OH	7.75–7.30 (m)		—	
C_{17}—OH	7.75–7.30 (m)		7.55–6.95 (m)	
C_{12}—H	7.17 (d)	$J_{11,12} = 6.5$	7.55–6.95 (m)	
C_{10}—H	7.00 (t)	$J_{9,10} = J_{10,11} = 6.5$	7.55–6.95 (m)	
$-N_bH$	6.65 (d)	$J_{2,NH} = 3$	6.10 (d)	$J_{2,NH} = 4$
C_{19}—H	5.32 (q)	$J_{18,19} = 6$	5.32 (q)	$J_{18,19} = 7$
C_{17}—H	4.85 (d)	$J_{17,OH} = 5$	4.84 (d)	$J_{17,OH} = 6$
$C_{21\beta}$—H	4.50 (d)	$J_{21\alpha,21\beta} = 17$	3.62 (m)	
C_2—H	4.24 (d)	$J_{2,NH} = 3$	4.17 (d × d)	$J_{2,3} = 3.5$, $J_{2,NH} = 4$
C_5—H	4.16 (d)	$J_{5,6\beta} = 4.5$	3.99 (d)	$J_{5,6\beta} = 6$
$-COOCH_3$	3.90 (s)		3.88 (s)	
C_{15}—H	3.86 (d)	$J_{14\beta,15} = 4$	3.80 (d)	$J_{14\beta,15} = 5$
$C_{21\alpha}$—H	3.42 (d)	$J_{21\alpha,21\beta} = 17$	3.62 (m)	
$C_{14\beta}$—H	3.40 (d × d)	$J_{14\beta,15} = 4$	2.91 (d × d)	$J_{14\beta,15} = 5$
		$J_{14\alpha,14\beta} = 15$	3.62 (m)	$J_{14\alpha,14\beta} = 14$
$C_{6\beta}$—H	3.23 (d × d)	$J_{6\alpha,6\beta} = 12$	3.25 (d × d)	$J_{5,6\beta} = 6$
		$J_{5,6\beta} = 4.5$		$J_{6\alpha,6\beta} = 12$
$C_{6\alpha}$—H	2.12 (d)	$J_{6\alpha,6\beta} = 12$	2.02 (d)	$J_{6\alpha,6\beta} = 12$
$C_{14\alpha}$—H	1.84 (d)	$J_{14\alpha,14\beta} = 15$	1.50 (d × d)	$J_{3,14\alpha} = 10$
				$J_{14\alpha,14\beta} = 14$
C_{18}—CH_3	1.71 (d)	$J_{18,19} = 6$	1.72 (d × t)	$J_{18,19} = 7$
				$J_{18,21} = 2$

[a] Chemical shifts measured in parts per million downfield from internal tetramethylsilane reference.

chidine, the signals of both C_{21} protons were in the form of a multiplet at δ 3.62. This finding was in accordance with the fact that the C_{21} protons in the ajmaline series are in nonequivalent environments due to their orientation with respect to the highly anisotropic ethylidine side chain and the unshared pair of electrons of N_b, while in the 2-epiajmaline series, the C_{21} protons are symmetrically disposed with respect to these two groups. Thus, in herbadine the C_{21} does not bear an alcohol group and this makes Structure V not feasible.

In the spectrum of quebrachidine, the signal of C_2—H is made up of a doublet of doublets at δ 4.17 due to the coupling to both C_3—H and NH protons. By contrast, in herbadine, the C_2—H signal is only a doublet at δ 4.24, showing only coupling to the NH proton. A Dreiding model of herbadine revealed that the signal of C_2—H should not show appreciable coupling to the C_3 proton, regardless of the presence of a C_3 proton, since the dihedral angle between these two protons is about 85° ($J_{2,3} \cong 0$). A similar phenomenon was observed in sandwicine and its derivatives (6).

A doublet at δ 4.85 and 4.84 in the spectra of herbadine and quebrachidine, respectively, represents the C_{17} protons of the two alkaloids. The presence of this signal in herbadine is an additional proof that an OH group should be present at C_{17} and not as shown in V.

A doublet at δ 1.84 ($J_{14\alpha,14\beta} = 15$ Hz) represents $C_{14\alpha}$—H, showing that only geminal coupling exists. However, in quebrachidine, the signal of $C_{14\alpha}$—H is made up of a doublet of doublets at δ 1.50 ($J_{3,14\alpha} = 10$ Hz, $J_{14\alpha,14\beta} = 14$ Hz), showing that, in addition to the geminal coupling, this proton is coupled to C_3—H. Since in both the ajmaline and 2-epiajmaline series the dihedral angle between $C_{14\alpha}$—H and C_3—H is about 10° and a large coupling constant is expected, then in herbadine the C_3—H must be substituted with an OH group since the signal of $C_{14\alpha}$—H is a simple doublet.

The remaining protons in both compounds give signals that are well separated from signals of adjacent protons, and the resultant spectrum is easily interpreted on the basis of first-order analysis. Furthermore, the spectra of herbadine and quebrachidine agree closely with those of herbamine and vincamajine, respectively, as reported previously (1), and assignments of signals were based on this prior analysis.

The NMR data, together with the other physical constants, establishes herbadine to be 2-epi-3-hydroxyquebrachidine.

REFERENCES

(1) G. H. Aynilian, C. L. Bell, N. R. Farnsworth, and D. J. Abraham, *Lloydia*, in press.

(2) G. H. Aynilian, N. R. Farnsworth, and J. Trojánek, *ibid.*, **37**, 299(1974).

(3) V. Y. Vachnadze, V. M. Malikov, K. S. Mudzhirl, and S. Y. Yunusov, *Khim. Prir. Soedin.*, **1972**, 341.

(4) K. Biemann, P. Bommer, A. L. Burlingame, and W. J. McMurray, *J. Amer. Chem. Soc.*, **86**, 4624(1964).

(5) M. Gorman, A. L. Burlingame, and K. Biemann, *Tetrahedron Lett.*, **1963**, 39.

(6) F. Ronchetti, G. Russo, E. Bombardelli, and A. Bonati, *Phytochemistry*, **10**, 1385.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 22, 1974, from the *Department of Pharmacognosy and Pharmacology and the Department of Medicinal Chemistry, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication September 11, 1974.

The authors thank Dr. S. S. Danyluk, Division of Biology and Medicine, Argonne National Laboratory, for allowing them to obtain the 220-MHz spectra, and Dr. M. Gorman, Eli Lilly and Co., for recording the high-resolution mass spectrum of herbadine.

[x] To whom inquiries should be directed.