Catharanthus Alkaloids XXIX: Isolation and Structure Elucidation of Vincoline

G. H. AYNILIAN*, S. G. WEISS*, G. A. CORDELL*, D. J. ABRAHAM‡, F. A. CRANE*, and N. R. FARNSWORTH* ˣ

Abstract □ Vincoline, an alkaloid of undetermined structure, previously isolated from *Catharanthus roseus, Catharanthus lanceus,* and *Vinca libanotica,* has been reisolated and its structure elucidated.

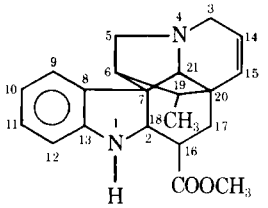
Keyphrases □ *Catharanthus* alkaloids—isolation, structure identification of vincoline □ *Vinca* alkaloids—isolation, structure identification of vincoline □ Vincoline—isolation, structure identification from *Catharanthus* and *Vinca* alkaloids

In the continuing search for novel alkaloids from Apocynaceae, the alkaloid vincoline was isolated from *Catharanthus roseus* and *Vinca libanotica.* This alkaloid was previously reported from *C. roseus* (1), from *C. lanceus* (2), and from *V. libanotica* (3); however, the structure of this base had not been deduced. In 1966, when this alkaloid was first isolated from *C. lanceus* (2), insufficient material was obtained to determine the structure. More recently, vincoline was isolated from the previncaleukoblastine fraction of *C. roseus* leaves in the same manner as described by Svoboda *et al.* (4) and simultaneously from *V. libanotica* (3). The structure elucidation studies reported at this time were made possible by the increased quantity of base available.

EXPERIMENTAL

Source and Properties of Vincoline—The vincoline used in this study was obtained from the crude previncaleukoblastine alkaloid fraction (4) of *C. roseus*[1]. It was found to have a melting point of 228–232°; UV[2] λ_{max}: 245 (log ϵ 3.91) and 299 (3.56) nm; $[\alpha]_D^{29}$ −242° (concentration 0.5% in chloroform); and important bands in the IR spectrum[3] at ν_{max} 3480 (OH), 3350 (NH), 1715 (ester), 1600 (indoline), and 740 (1,2-disubstituted benzene) cm^{-1}.

Acetylation of Vincoline—A solution of 5 mg of vincoline, 0.15 ml pyridine, and 0.2 ml acetic anhydride was heated at 45° for 4 hr. The solution was basified with 10% NH_4OH and extracted with three 5-ml portions of ether. After drying the combined ether solutions over anhydrous sodium sulfate, the solution was filtered and concentrated to afford a pure (TLC) but oily product. Mass spectral evidence (M^+ 410) indicated that monoacetylation had occurred.



I: vindolinine

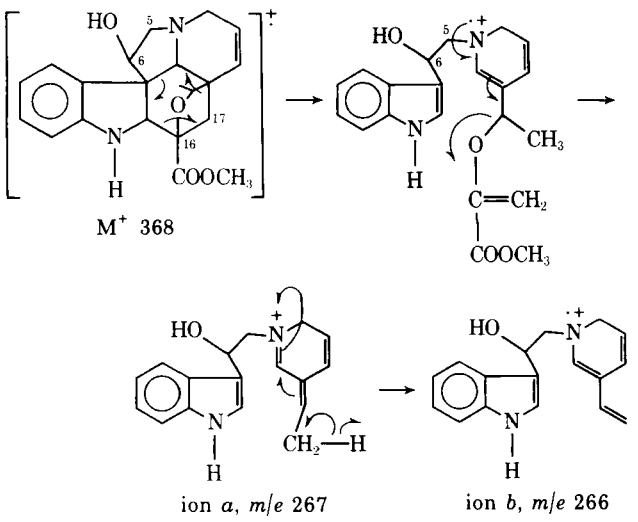
Attempted Sodium Borohydride Reduction of Vincoline—To a methanolic solution of 5 mg of vincoline, 5 mg of sodium borohydride was added. The solution was stirred for several hours at room temperature, neutralized, and extracted for alkaloid material with ethyl acetate. TLC and mass spectral data revealed that no reaction had occurred. The procedure was repeated several times under a variety of conditions, but no reaction was observed.

DISCUSSION

According to Svoboda *et al.* (1), the IR and NMR spectra of vincoline showed it to be related to vindolinine[4] (I); furthermore, the NMR spectrum showed the presence of a methoxyl group and indicated a dihydroindole moiety unsubstituted at *N*-1.

An examination of the NMR spectrum of vincoline[5] (Table I) revealed a multiplet between 7.11 and 6.44 ppm, integrating for four protons, together with a broad singlet at 5.56 ppm, indicating the presence of an indole moiety with the *N*-1 unsubstituted. Exchange of this proton with deuterium oxide confirmed this assignment. A 12-line pattern was assigned to two olefinic protons having two nonequivalent proton neighbors (C-3), while their chemical shift indicated the presence of a nearby deshielding group (*N*-4) (5). Therefore, the double bond was located at the expected position (5, 6). A singlet at 3.82 ppm indicated the presence of a methoxyl group. The presence of a methyl group on a carbon bearing one proton was indicated by a doublet (J = 6.6 Hz) at 0.60 ppm. This hinted that probably C-19 was attached to C-6, as in vindolinine (I).

The mass spectrum[6] of vincoline gave major peaks at M^+ 368 (55%), *m/e* 350 (25), 281 (25), 267 (100), 266 (62), 223 (16), 170 (12), 169 (10), 160 (68), 146 (10), 136 (14), 122 (23), 121 (44), 108 (62), 107 (10), and 93 (20). The molecular formula of vincoline,



M^+ 368

ion *a*, *m/e* 267 ion *b*, *m/e* 266

Scheme I

[1] The plants were cultivated at the Department of Pharmacognosy and Pharmacology, Drug and Horticultural Experiment Station, Downers Grove, Ill. The crude alkaloid fraction was prepared from about 1000 kg of air-dried leaves by Eli Lilly and Co., Indianapolis, Ind. The plant material was authenticated by F. A. Crane as *C. roseus* (L.) G. Don (Apocynaceae), and voucher specimens representing the collection were deposited in the Herbarium of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Ill.

[2] Recorded using a Beckman model DB-G grating UV spectrophotometer.

[3] Recorded using a Beckman model IR-18A IR spectrophotometer.

[4] The number system shown in the structure is that of J. LeMen and W. I. Taylor, *Experientia,* **21**, 508(1965).

[5] Recorded using a Brüker 90-MHz NMR spectrometer.

[6] Recorded using a Hitachi Perkin-Elmer model RMU-6D mass spectrometer.

Table I—NMR Spectrum of Vincoline[a]

Chemical Shifts, ppm	Integration for Protons	Pattern of Splitting	Coupling Constants
7.11–6.44 (aromatic protons)	4	Multiplet	—
5.37–5.89 (olefins)	2	Multiplet	—
5.56 (NH)	1	Broad singlet	—
3.89 (C*H*—CH_3)	1	Quartet	J = 6.6 Hz
3.82 ($COOCH_3$)	3	Singlet	—
3.81 (C—2H)	1	Singlet	—
0.60 (CH—C*H*$_3$)	3	Doublet	J = 6.6 Hz
0.6–3.7	9	Complex system which could not be interpreted	—

[a] The spectrum was recorded at 90 MHz.

Table II—High-Resolution Mass Spectral Measurements for Vincoline

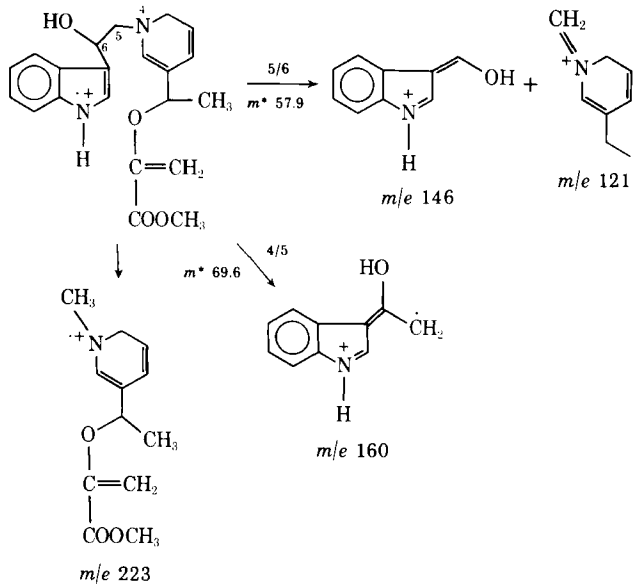
Formula	Observed m/e	Calculated m/e
C_6H_7N	93.05785	93.05820
$C_7H_{10}N$	108.08132	108.08301
$C_8H_{10}N$	120.08132	120.08265
$C_8H_{11}N$	121.08915	121.09022
$C_8H_{12}N$	122.09697	122.09737
C_9H_8NO	146.06059	146.06080
$C_{10}H_{10}NO$	160.07624	160.07852
$C_{12}H_{17}NO_3$	223.12084	223.12353
$C_{17}H_5N_2$	247.12352	246.12352
$C_{17}H_{18}N_2O$	266.14191	266.14377
$C_{17}H_{19}N_2O$	267.14526	267.14481
$C_{18}H_{21}N_2O$	281.1653	281.1654
$C_{19}H_{19}N_2O$	291.14974	291.15071
$C_{19}H_{21}N_2O_2$	309.16030	309.15999
$C_{21}H_{22}N_2O_3$	350.16304	350.16009
$C_{21}H_{24}N_2O_4$	368.17361	368.17094

$C_{21}H_{24}N_2O_4$, was established by high-resolution mass spectral measurements[7] (Table II). The mass spectral fragmentation pattern indicated the compound to have an aspidospermine-type skeleton (7). In the mass spectrum of many aspidospermine-type alkaloids, a retro-Diels–Alder reaction occurs, expelling C-16 and C-17 with any substituents (6, 8). In the case of vincoline, this process afforded the base peak in the spectrum, ion *a* at m/e 267, and a close relative, ion *b* at m/e 266 (Scheme I). High-resolution mass measurements of these two species indicated molecular formulas of $C_{17}H_{19}N_2O$ and $C_{17}H_{18}N_2O$, respectively. Since these two species contain only one oxygen atom each, the carbomethoxy group could be at either C_{16} or C_{17} and expelled by the retro-Diels–Alder reaction. In view of the overwhelming biogenetic precedence for the existence of such a carbomethoxy grouping at C_{16} in all related indole alkaloids (6), it was placed at C_{16} rather than C_{17} in vincoline.

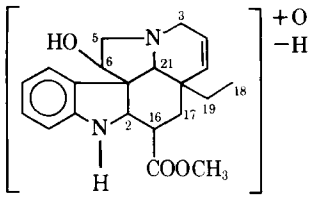
Acetylation (acetic anhydride–pyridine) afforded only a monoacetyl derivative (mol. wt. 410), suggesting that only one oxygen was in the form of an alcohol. The mass spectrum of the monoacetyl derivative showed a base peak at m/e 309, 42 mass units higher than the base peak of vincoline. It followed that the oxygen atom in the ions at m/e 267 and 266 was in the form of an alcohol. The possibility of a carbinolamine at C-2, C-3, C-5, or C-21 was ruled out when vincoline remained unchanged after treatment with sodium borohydride, suggesting that the alcohol function was at C-6. Additional evidence for this assignment was the occurrence of a fragment at m/e 160 (68%) ($C_{10}H_{10}NO$), consisting of an indole moiety with two carbon atoms, placing the hydroxyl group at C-5 or C-6. The base peak underwent 5–6 cleavage to give fragments at m/e 146 and 121 (Scheme II and Table II). Analysis of these ions showed that only the former contained oxygen. The rearranged parent ion also underwent 5–6 cleavage to give ions at m/e 146 and 223, the latter containing three of the four oxygens (Scheme II). The hydroxyl group was thus placed at C-6. This eliminated the vindolinine skeleton for vincoline; at this stage, Structure II could be written for this base.

The nature of the fourth oxygen could not be determined directly. The presence of the two peaks in the mass spectrum at m/e 267 and 266 indicated attachment of this oxygen at either C-16 or C-17. However, if it were a hydroxyl group, acetylation would have yielded a diacetyl derivative instead of a monoacetate.

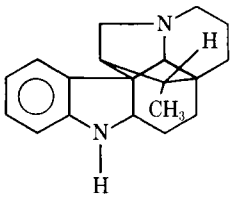
The nature of this oxygen atom was determined from NMR studies. The NMR spectrum, as mentioned earlier, showed a doublet integrating for three protons and having a chemical shift of 0.60 ppm (J = 6.6 Hz). This clearly indicated the presence of a methyl group (C-18) attached to a carbon atom (C-19) with a sin-



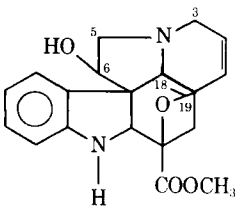
Scheme II



II

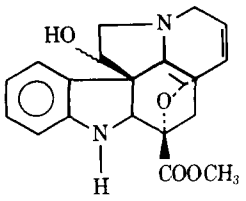


III: tuboxenine

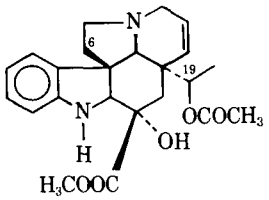


IV

[7] Recorded using a double-focusing mass spectrometer, model 110 (Consolidated Electro Dynamics Co.), operating at 70 ev.



V: vincoline



VI

gle proton. Double-resonance studies of tuboxenine (III), a vindolinine-type alkaloid, showed that this methine proton (C-19) gave a chemical shift of 1.7 ppm (5, 9), with a J value of about 7 Hz.

A double-resonance study of vincoline showed the methine proton to be a quartet (J = 6.6 Hz) centered at 3.89 ppm. However, only three lines were apparent, the fourth line being covered by the carbomethoxy signal. It was clear that C-19 was attached (in addition to C-20, C-18, and a proton) to an electronegative atom, in this case, oxygen. Therefore, the fourth oxygen moiety was in the form of an ether and, by points presented previously, was attached at C-16 or C-17. If a C-19—O—C-17 linkage existed, then the methine proton at C-17 should have been downfield at about 4.5 ppm (10). However, the region between 4.0 and 5.3 ppm was devoid of absorption. The only other possible linkage was one involving C-19—O—C-16. Structure IV was thus proposed for vincoline at this point.

Vincoline showed a negative rotation, so that the tryptamine bridge was beta (11). Molecular models indicated that stable structures could be derived if C-19, C-18 was either *cis* or *trans* to the tryptamine bridge. The chemical shift (0.60) of the methyl group at C-18 was best accounted for by the strong shielding effect of the aromatic ring current. In the structure where the tryptamine bridge and C-18, C-19 are *cis*, this possibility does not arise. However, in one of the C-19 epimers, when the tryptamine bridge and C-18, C-19 are *trans*, a close proximity of the dihydroindoline nucleus and C-18 is apparent. It is therefore suggested that vincoline has Structure V, omitting the stereochemistry of the hydroxyl group. The occurrence of a *cis*-relationship between C-18, C-19, and C-5, C-6 is rare, examples thus far only being observed in the vindolinine series. Biogenetically, vincoline may be produced from a molecule such as VI *via* nucleophilic attack at C-19 with subsequent hydroxylation at C-6. This again suggests a *trans*-relationship of these groups.

REFERENCES

(1) G. H. Svoboda, M. Gorman, and R. H. Tust, *Lloydia*, **27**, 203(1964).

(2) N. R. Farnsworth, H. H. S. Fong, and R. N. Blomster, *ibid.*, **29**, 343(1966).

(3) G. H. Aynilian, J. Trojanek, and N. R. Farnsworth, *ibid.*, in press.

(4) G. H. Svoboda, N. Neuss, and M. Gorman, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 659(1959).

(5) C. Djerassi, S. E. Flores, H. Budzikiewicz, J. M. Wilson, L. F. Durham, J. LeMen, M.-M. Janot, M. Plat, M. Gorman, and N. Neuss, *Proc. Nat. Acad. Sci. USA*, **48**, 113(1962).

(6) M. Hesse, "Indolalkaloide in Tabellen," Springer-Verlag, Berlin, Germany, 1968.

(7) C. Djerassi, M. Cereghetti, H. Budzikiewicz, M.-M. Janot, M. Plat, and J. LeMen, *Helv. Chim. Acta*, **47**, 827(1964).

(8) G. H. Aynilian, M. Tin-Wa, N. R. Farnsworth, and M. Gorman, *Tetrahedron Lett.*, **1972**, 89.

(9) C. Knoup, J. Seible, and H. Schmid, *Helv. Chim. Acta*, **47**, 358(1964).

(10) L. M. Jackman and S. Sternhell, "International Series of Monographs in Organic Chemistry," vol. 5, 2nd ed., Pergamon, New York, N.Y., 1969, p. 199.

(11) I. Kompis, M. Hesse, and H. Schmid, *Lloydia*, **34**, 269(1971).

(12) G. H. Aynilian, B. Robinson, and N. R. Farnsworth, *Tetrahedron Lett.*, **1972**, 391.

(13) G. H. Aynilian, C. L. Bell, and N. R. Farnsworth, *Lloydia*, in press.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 13, 1973, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612 and the ‡Department of Medicinal Chemistry, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213

Accepted for publication October 12, 1973.

Supported in part by Research Grant CA-12230, National Institutes of Health, U.S. Department of Health, Education, and Welfare, Bethesda, MD 20014

This paper constitutes Part VII of the series "Alkaloids of *Vinca* Species."

The authors are grateful to Dr. M. Gorman, Lilly Research Laboratories, Indianapolis, Ind., for providing high-resolution mass spectra for vincoline, and to Mr. Richard Dvorak, for providing all other mass spectral data. For the previous paper in the *Catharanthus* series, see Ref. 12; for the previous paper in the *Vinca* series, see Ref. 13.

ˣ To whom inquiries should be directed.