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Structure Elucidation and Chemistry of *Catharanthus* Alkaloids. XXX. Isolation and Structure Elucidation of Vincarodine[1]

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Continuing work on antileukemic alkaloids of *Catharanthus roseus* has afforded an alkaloid vincarodine 9, containing a novel C-15–C-16 ether linkage in an Eburna-type skeleton.

The Madagascan periwinkle *Catharanthus roseus* has been the subject of intensive study with respect to the isolation[3] and biosynthesis of its contained alkaloids,[4] since the observation that an alkaloid from this plant, vincaleukoblastine, exhibited marked leukopenia in rats.[5]

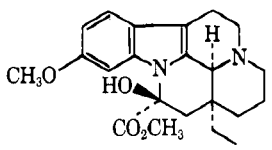
The extensive isolation work of Svoboda led to the introduction of vincaleukoblastine and vincristine as prescription products in the treatment of Hodgkin's disease and childhood leukemia, respectively.[6]

We have been reexamining some of the alkaloid fractions of *C. roseus*[7] active against 9KB. The first fraction we examined was the post-VLB–pre-VCR fraction previously described by Svoboda.[8] This fraction was active in both the 9KB and 3PS systems (3.5×10^{-1}; T/C 231, respectively).[9]

pH gradient fractionation[10] of the post-VLB–pre-VCR fraction afforded from pH fractions 4.5, 5.0, and 5.5, after crystallization from methanol, a crystalline alkaloid melting sharply at 246–247°. The product was homogeneous by tlc in solvent systems A, B, and C, where R_f values were 0.45, 0.30, and 0.65, respectively, and the chromogenic reaction with ceric ammonium sulfate (CAS) was blue, rapidly fading to colorless.[11]

The ir spectrum of this alkaloid indicated the presence of NH or OH functions and a saturated ester moiety. The nmr spectrum in $CDCl_3$ (Table I) confirmed the presence of a methyl ester moiety exhibiting a singlet at 3.82 ppm. A second methoxy group was also observed, and its position at 4.10 ppm, together with the integration for only three aromatic protons, suggested an aromatic methoxy group. A triplet at 1.07 ppm and a quartet (not further coupled) at 1.95 ppm indicated the presence of an ethyl group at a tertiary carbon atom. The complexity of the spectrum yielded little additional information at this stage.

The uv spectrum was quite unlike any we had previously encountered from *Catharanthus* species, exhibiting maxima at 272 and 295 nm (log ϵ 3.94 and 3.71, respectively) with no shift of maxima in acid or base. We could find only two compounds having a similar uv spectrum, namely, vincine 1[12] and vincinine 2,[13] compounds isolated from *Vinca minor*.



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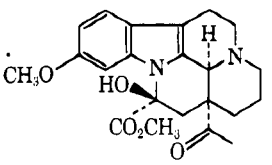

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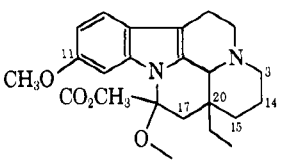
The physical data of our compound agreed well with those of vincarodine, an alkaloid isolated by Svoboda from a similar fraction of *C. roseus*.[14] Svoboda indicated that his compound had a probable molecular weight of 796 and a molecular formula of $C_{44}H_{52}N_4O_{10}$. Mass spectrometry of our compound indicated that the molecular weight was 398, there being no evidence for any dimeric material. The molecular formula should then be half that suggested by Svoboda and this was confirmed by high-resolution mass spectrometry (calcd for $C_{22}H_{26}N_2O_5$, 398.18477; found, 398.18417).

The similarity of the aromatic region of vincarodine with that of vindoline indicated that the methoxy group was at the 11 position.[15,16] At this point the partial structure 3 could be written.



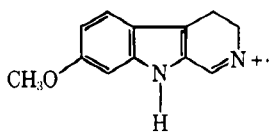
3

Comparison of the molecular formula of this partial structure with that obtained by high-resolution measurements indicated that a single further oxygen was contained. Acetylation of vincarodine afforded a monoacetate derivative (M^+ m/e 440) even under vigorous conditions. Reduction of vincarodine with $LiAlH_4$ or $NaBH_4$ gave a compound, mol wt 370, which upon acetylation afforded a diacetate derivative, mol wt 454, confirming the presence of a methyl ester function in the parent compound.

Table I
Nmr Spectrum of Vincarodine in $CDCl_3$

Functional group	Absorption, δ ppm	Multiplicity	Proton count
CH_3CH_2	1.07	Triplet	3
CH_3CH_2	1.92	Quartet	2
Aliphatics	2.17–3.38	Complex	8
$-CH_2CH_3$	3.82	Singlet	3
$ArOCH_3$	4.10	Singlet	3
-CHO-	3.79	Broadened doublet	1
-CHOH	4.00	Broadened doublet	1
ArCHN	4.10	Broadened singlet	1
Aromatic	6.56–7.50	Multiplets	3

An important observation was that in all of these compounds the base peak in the mass spectrum remained at *m/e* 200. High-resolution mass spectral analysis revealed a composition $C_{12}H_{12}N_2O$ for this ion, suggesting that it had the probable structure **4**.



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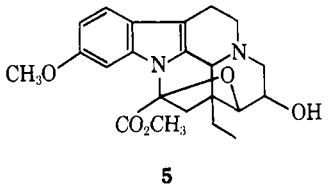
In the mass spectrum of vincarodine a number of metastable ions were observed, one of these being for the transition *m/e* 398 → 200, indicating a very interesting loss of nine of the ten iridoid-derived carbon atoms in one process. Evidence from the chemical reactions indicated that there must be four oxygen atoms in the iridoid-derived part of the molecule. Three of these were accounted for previously. There were only four positions where this one remaining oxygen atom could be placed, namely, positions 3, 14, 15, or 17.

Position 3 was eliminated as a possibility for oxygenation for two reasons: (1) the lack of borohydride reduction of any carbinolamine present, and (2) the persistent fragment at *m/e* 214 ($C_{13}H_{14}N_2O$) which contained C-3.

A common fragmentation pathway for the Eburna series of alkaloids is that of retro Diels–Alder reaction in the C ring followed by homolytic fission of the 15–20 bond.[17] This fragmentation in the case of vincarodine apparently leads to the ion *m/e* 297 of molecular formula $C_{18}H_{19}NO_3$. This ion contained only three oxygen atoms and did not lose hydroxyl radical, which indicated that there was no oxygenation at C-17. The ion *m/e* 101 (398 − 297) in the mass spectrum analyzed well for $C_4H_7NO_2$ and was shifted appropriately on acetylation of vincarodine, whereas *m/e* 297 was unaffected.

The two oxygen atoms were therefore limited to positions 16 and 14 or 15, and, from the molecular formula and absence of olefinic protons, an ether linkage must be involved. The possibility that a hydroxyl group existed at C-16 was eliminated by the ease of acetylation of vincarodine, by the lack of shift in the uv spectrum on addition of acid or base, and by the formation of ion *m/e* 101 described above.

Vincarodine was recoverable quantitatively from treatment with acid at room temperature, so that structures involving acetals or ketals could be eliminated. Two structures, **5** and **6**, were considered at this time for vincarodine, differing only in the attachment of the ether linkage from C-16 to either C-15 or C-14, and the subsequent placement of the hydroxyl group.



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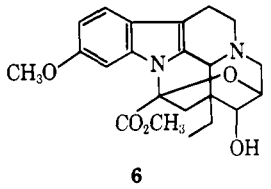

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Table II
High-Resolution Mass Spectrum of Vincarodine

Mass no.	Molecular formula	Calcd	Found
398	$C_{22}H_{26}N_2O_5$	398.18417	398.18477
380	$C_{22}H_{24}N_2O_4$	380.17361	380.17295
369	$C_{21}H_{25}N_2O_4$	369.17696	369.17493
368	$C_{21}H_{24}N_2O_4$	368.16913	368.16891
339	$C_{20}H_{23}N_2O_3$	339.17087	339.16935
321	$C_{20}H_{21}N_2O_2$	321.16030	321.16205
310	$C_{19}H_{20}NO_3$	310.14432	310.14201
309	$C_{18}H_{17}N_2O_3$	309.12392	309.12604
298	$C_{18}H_{20}NO_2$	298.13888	298.13984
297	$C_{18}H_{19}NO_2$	297.13649	297.13857
296	$C_{18}H_{18}NO_2$	296.12867	296.12697
283	$C_{17}H_{17}NO_3$	283.12084	283.12031
282	$C_{17}H_{16}NO_3$	282.11302	282.11368
281	$C_{17}H_{15}NO_3$	281.10519	281.10788
268	$C_{16}H_{14}NO_3$	268.09737	268.09747
267	$C_{17}H_{17}NO_2$	267.12593	267.12494
266	$C_{17}H_{16}NO_2$	266.11810	266.11829
254	$C_{16}H_{16}NO_2$	254.11810	254.11843
239	$C_{16}H_{17}NO$	239.13101	239.13098
238	$C_{16}H_{16}NO$	238.12310	238.12595
	$C_{15}H_{12}NO_2$	238.08680	238.08847
224	$C_{15}H_{14}NO$	224.10754	224.10931
223	$C_{15}H_{13}NO$	223.09971	223.10142
214	$C_{13}H_{14}N_2O$	214.11061	214.10838
211	$C_{14}H_{13}NO$	211.09971	211.09754
210	$C_{14}H_{12}NO$	210.09189	210.09184
208	$C_{14}H_{10}NO$	208.07624	208.07614
	$C_{15}H_{14}N$	208.11262	208.11202
200	$C_{12}H_{12}N_2O$	200.09049	200.08961
199	$C_{12}H_{11}N_2O$	199.08714	199.08507
198	$C_{10}H_{14}O_4$	198.08473	198.08468
197	$C_{13}H_{11}NO$	197.08406	197.08113
196	$C_{12}H_{12}NO$	186.09189	186.09408
184	$C_{12}H_{10}NO$	184.07624	184.07560
	$C_9H_{12}O_4$	184.07356	184.07247
180	$C_{13}H_{10}N$	180.08132	180.08388
173	$C_{11}H_{11}NO$	173.08406	173.08655
170	$C_{11}H_{10}N_2$	170.08440	170.08478
167	$C_{12}H_9N$	167.07350	167.07467
156	$C_8H_{12}O_3$	156.07867	156.07631
155	$C_8H_{11}O_3$	155.07082	155.07118
154	$C_{11}H_8N$	154.06567	154.06495
143	$C_{10}H_9N$	143.07350	143.07428
125	$C_7H_9O_2$	125.06025	125.06094
101	$C_4H_7NO_2$	101.04320	101.04046
97	C_6H_9O	97.06534	97.06718
95	C_6H_7O	95.04969	95.05046
72	C_3H_6NO	72.04494	72.04490

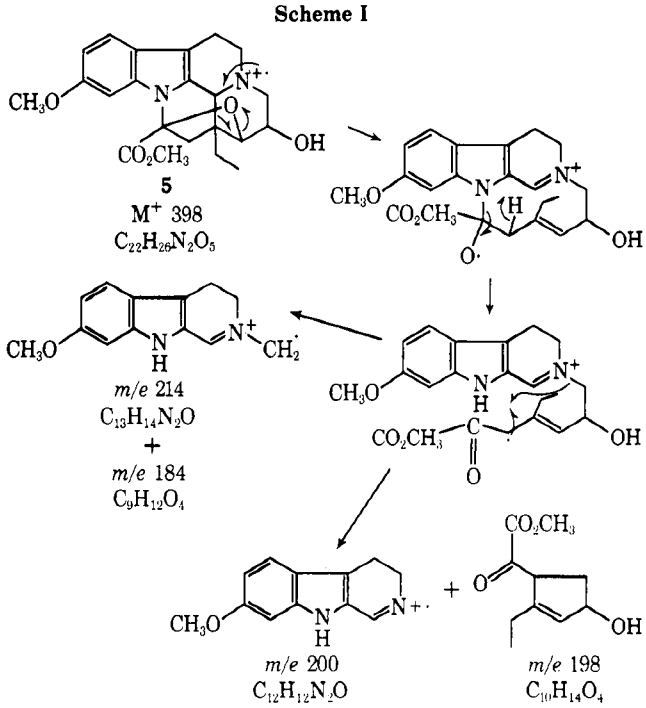
To distinguish between these two structures, careful analysis of the high-resolution nmr and mass spectral data was carried out. Table II summarizes the high-resolution mass spectral data for vincarodine, and the postulated schemes for two of the most intense fragments are summarized in Schemes I and II. Each of the ions tabulated can be placed on the fragmentation patterns of vincarodine and indeed a number of these pathways serve to distinguish between the two proposed structures.

Scheme I illustrates the formation of the base peak *m/e* 200, resulting in a loss (as one fragment) of *m/e* 198 from the parent ion. It is not possible to rationalize in reasonable terms the formation of this ion from the alternative structure **6**. A small fragment was observed at *m/e* 380 analyzing for $C_{22}H_{24}N_2O_4$. This represents a loss of water from vincarodine possible only for the skeleton represented by **5**. A fragment ion at *m/e* 368 ($C_{21}H_{24}N_2O_4$) is shifted by 42 mass units upon acetylation of vincarodine; *i.e.*, a loss of 30 mass units from the parent ion still occurs.

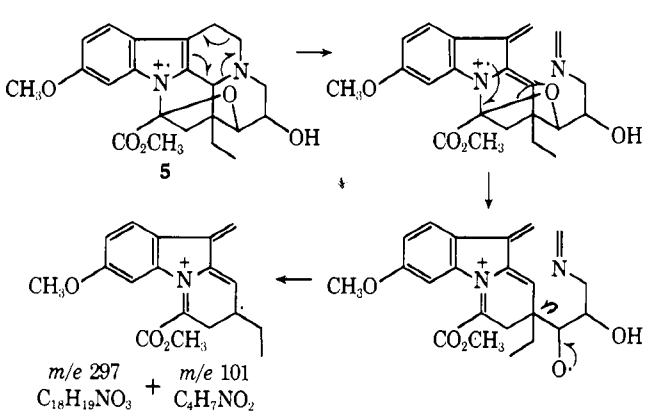
Table III
Nmr Spectra of Vincarodine and Acetylvincarodine

Functional group	Vincarodine (pyridine-*d*) Absorption, δ, ppm	Multiplicity	Proton count	Acetylvincarodine ($CDCl_3$) Absorption, δ, ppm	Multiplicity	Proton count
CH_3CH_2–	0.98	Triplet	3	1.04	Triplet	3
CH_3CH_2–	2.02	Multiplet	2	1.75	Quartet	2
Aliphatics	2.14–3.42			1.93–3.53	Complex	8
$-COCH_3$				2.05	Singlet	3
$-CO_2CH_3$	3.70	Singlet	3	3.80	Singlet	3
$ArOCH_3$	4.01	Singlet	3	4.05	Singlet	3
-CHO-	4.13	Multiplet	1	3.87	Broadened doublet	1
ArCHN–	4.13	Multiplet	1	4.10	Broadened singlet	1
-CHOR (R = H)	3.98	Multiplet	1	(R = $COCH_3$) 4.93	Multiplet	1
Aromatics	6.90–7.66	Multiplet	3	6.50–7.48	Multiplet	3

Scheme I

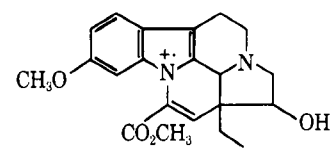


Scheme II



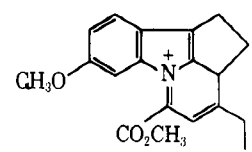
Cleavage of the tetrahydrofuran ring with loss of hydrogen radical at C-17 and homolytic fission of the C-15–C-20 bond gives rise to an allylically stabilized radical at C-20. Formation of a new D ring with loss of C-15 affords *m/e* 368 having structure **7**.

Standard retro Diels–Alder fragmentation of the C ring and aromatization of the E ring results in a measured loss of 72 mass units. This scheme is written with a strained imagination for the alternative structure **6**.



7, *m/e* 368
$C_{21}H_{24}NO_4$

A further important ion for structure elucidation purposes is that at *m/e* 310, formulated as having structure **8**. A mechanism for the formation of this ion can only be written from **5**. Scheme II illustrates the standard fragmentation pattern in this series of compounds as applied to vincarodine, resulting in the formation of *m/e* 297 and a measured loss of *m/e* 101.



8, *m/e* 310
$C_{19}H_{20}NO_3$

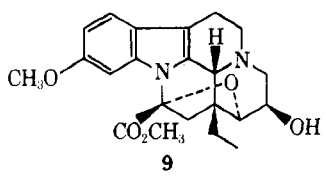
In summary, the formation of the ions *m/e* 200, 380, 368 (**8**), and 310 (**9**) do serve to distinguish between structure **5** and **6** and indicate structure **5** for vincarodine.

Additional evidence for structure **5** was obtained when further aspects of the nmr spectra were examined. In particular, the spectrum of vincarodine was obtained in pyridine-d_5 (see Table III). It was hoped that in this way a solvent shift of the hydrogen on the carbon bearing the hydroxyl group would be observed. In practice this technique failed to give a more informative spectrum for the purposes of a double-resonance study with one important exception. Close to the aromatic methoxyl group at 4.01 ppm, there was observed in the pyridine-d_5 spectrum a broadened singlet at 3.98 ppm. Irradiation of this signal collapsed multiplets in only two observable regions, 2.30 and 2.46 ppm. Triple irradiation at these two positions caused perturbation at 3.98 ppm to an apparent doublet. These observations are consistent with a single proton on a carbon attached to oxygen coupling with two protons on a carbon attached to a less electronegative atom and a second hydrogen in the region of 3.5–4.2 ppm. The small coupling (J = 3–4 Hz) of the proton at 3.98 ppm with the methylene protons indicates that this proton is almost equally disposed angularly between these two protons, *i.e.*, the proton at 3.98 ppm is equatorial.

To further examine this point and provide evidence on the precise structure of vincarodine, the nmr spectrum of acetylvincarodine was determined in $CDCl_3$ (Table III). As expected,[18] the proton on the carbon bearing the acetyl group was now shifted downfield by nearly 1 ppm to 4.93

ppm and appeared as a broad signal. Irradiation at this position produced collapse in only two regions, 3.87 and 2.30 ppm. In the former case the multiplet collapsed essentially to a singlet. Back-irradiation at 2.30 ppm caused collapse only at 4.93 ppm to a doublet that was slightly broadened. Triple resonance irradiation at 3.87 and 2.30 ppm collapsed the signal at 4.93 ppm to a singlet.

These data are only compatible with a hydroxy group at C-14 and an ether function at C-15. Furthermore, the evidence relating to the disposition of the proton on C-14 presented above indicates a trans (diaxial) relationship of the oxygen atoms on C-14 and C-15. At this point vincarodine is represented by structure 9 or its mirror image.



9

Vincarodine exhibited a negative rotation. By analogy with (−)-eburnamine, (+)-isoeburnamine, and (+)-vincine (1),[19] structure 9 represents the absolute stereochemistry of vincarodine. However, studies are now in progress by X-ray diffraction to confirm this assignment.

This compound is the first example of this indole alkaloid skeletal type from a *Catharanthus* species, although examples from closely related *Vinca* species are well known.[19]

Details of the biological activity of 9 will be reported elsewhere.

Experimental Section[20]

Vincarodine (9). A 100-g portion of a post-VLB-pre-VCR alkaloid fraction from the leaves of *Catharanthus roseus* (L.) G. Don was submitted to a pH gradient fractionation. The material was initially dissolved in 4 l. of 2% citric acid and extracted successively at half pH increments with benzene (3 × 1 l.). Adjustment of pH was made by addition of concentrated NH_4OH. From pH fractions 4.5, 5.0, and 5.5, crystallization from methanol afforded a total of 853 mg of white, crystalline material identified as vincarodine (9): mp 246–247° (lit. mp 253–256°); ir (KBr) 3600–3200 (m), 1755 (vs), 1620 (m), 1300 (s), 1065 (s), 945 (s), 810 (w), 785 cm^{-1} (w); uv λ_{max} 228, 272, 295 nm (log ϵ 4.46, 3.94, 3.71); nmr ($CDCl_3$) δ 1.05 (t, 3), 3.78 (s, 3), 4.05 (s, 3), 6.49–7.39 (m, 3); $[\alpha]^{27}D$ − 187° (1% pyridine); mass spectrum *m/e* (rel intensity) 398 (96, M^+), 368 (14), 339 (9), 310 (4.5), 297 (58), 296 (99), 282 (14), 268 (23), 266 (27), 238 (27), 223 (7), 214 (18), 200 (100), 199 (23), 170 (10).

Acetylvincarodine. A mixture of 100 mg of vincarodine (9), 5 ml of pyridine, and 5 ml of acetic anhydride was allowed to stand at room temperature for 2 hr. Work-up in the usual way and crystallization of the product from methanol afforded 106 mg of white, crystalline material (96%): mp 180–185°; ir (KBr) 2950 (m), 1750 (m), 1725 (s), 1220 (s), 1190 (s), 1005 cm^{-1} (s); uv λ_{max} 229, 272, 298 nm; mass spectrum *m/e* (rel intensity) 440 (91, M^+), 410 (10), 381 (9), 296 (41), 282 (13), 268 (19), 243 (14), 238 (21), 214 (14), 200 (100), 170 (13).

Hydride Reductions of Vincarodine. A. Borohydride Reduction. A mixture of 10 mg of vincarodine (9) and 100 mg of $NaBH_4$ in 5 ml of absolute ethanol was stirred at room temperature for 4 hr. Work-up in the usual way afforded 9 mg of a white, amorphous solid (97%): mp 157–160°; ir (KBr) 3400–3200 (s), 1600 (w), 1200 (s), 1000 cm^{-1} (s); uv λ_{max} 228, 272, 297 nm; mass spectrum *m/e* (rel intensity) 370 (56, M^+), 340 (5.0), 268 (20), 238 (10.5), 214 (50), 200 (100), 170 (12).

B. Lithium Aluminum Hydride Reduction. A mixture of 10 mg of vincarodine (9) and 50 mg of $LiAlH_4$ in 5 ml of dry THF was stirred at room temperature for 2 hr. Work-up in the usual way afforded a product identical with that obtained from borohydride reduction.

Acetylation of Hydride Reduction Product. Acetylation of the hydride reduction product with acetic anhydride and pyridine followed by work-up in the usual way afforded a single white, amorphous product in essentially quantitative yield: ir (KBr) 1725 (s), 1225 (vs), 1020 cm^{-1} (s); uv λ_{max} 228, 272, 298 nm; mass spectrum *m/e* (rel intensity) 454 (45, M^+), 424 (36), 395 (45), 364 (18), 334 (17), 214 (55), 200 (100), 170 (22).

Registry No.—7, 49849-77-6; 7 acetyl derivative, 49849-78-7; 7 hydride reduction product, 49849-79-8; 7 hydride reduction product diacetate, 49849-80-1.

References and Notes

(1) This research was supported, in part, by research grant CA-12230 from the National Institutes of Health, U. S. Department of Health, Education, and Welfare, Bethesda, Md. These data were first presented at the Mid-West Natural Products Regional Meeting held at the University of Illinois at the Medical Center, Chicago, Ill., April 28, 1973. It was subsequently presented at the American Society of Pharmacognosy Meeting, Jekyll Island, Ga., July 19, 1973.
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(20) All melting points were determined using a Kofler hot-stage instrument and are uncorrected. Infrared spectra were recorded using a Beckman Model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Absorption bands are reported in reciprocal centimeters and intensities as vs (very strong), s (strong), m (medium), and w (weak). Ultraviolet spectra were recorded using a Beckman Model DB-G spectrophotometer using methanol as solvent. Nmr spectra were recorded using a Varian Associates Model T60A instrument using tetramethylsilane (TMS) as the internal standard. The chemical shifts are reported in parts per million (δ). Mass spectra were recorded using a Hitachi Perkin-Elmer Model RMU-6D instrument. The specific rotation was determined using a Carl Zeiss optical polarimeter. Tetrahydrofuran was distilled from $LiAlH_4$ prior to use.