# Alkaloids of *Vinca rosea* L. (*Catharanthus roseus* G. Don) XXXVI: Isolation and Characterization of New Dimeric Alkaloids

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**Abstract** □ In a continuing effort to study thoroughly the alkaloids of *Catharanthus roseus*, new dimeric alkaloids were isolated and characterized. Structures are proposed for leurocolombine and vinamidine based on UV, IR, PMR, high-resolution mass spectrometry, and CMR. Pseudovincaleukoblastine diol was identified by PMR and mass spectrometry. Leurocolombine exhibited antimitotic activity and marginal antitumor activity against the Ridgeway osteogenic sarcoma.

**Keyphrases** □ Alkaloids—isolation of three from *Catharanthus roseus*, characterization by UV, IR, NMR, and mass spectra □ *Vinca rosea*—isolation of three dimeric alkaloids, characterization by UV, IR, NMR, and mass spectra □ *Catharanthus roseus*—isolation of three dimeric alkaloids, characterization by UV, IR, NMR, and mass spectra □ Leurocolombine—isolation from *Catharanthus roseus*, UV, IR, NMR, and mass spectral structure identification □ Vinamidine—isolation from *Catharanthus roseus*, UV, IR, NMR, and mass spectral structure identification □ Pseudovincaleukoblastine diol—isolation from *Catharanthus roseus*, UV, IR, NMR, and mass spectral structure identification

In continuing efforts to study thoroughly the alkaloids of *Catharanthus roseus* G. Don[1], three new dimeric alkaloids were isolated: leurocolombine (I), vinamidine (II), and pseudovincaleukoblastine[2] diol (III). Postvincaleukoblastine–preleurocristine (1) production-scale chromatographic column cuts were subjected to preparative high-pressure liquid chromatography (HPLC), gradient pH purification (2), or gradient pH followed by preparative HPLC or preparative TLC. Structure elucidation studies of the three new dimeric alkaloids are reported in this paper.

Leurocolombine demonstrated antimitotic activity[3] at $2 \times 10^{-2}$ μg/ml and produced 27% inhibition against the Ridgeway osteogenic sarcoma at 15 mg/kg. Vinamidine was not toxic to Swiss white mice at 30 mg/kg and showed no antimitotic activity, so *in vivo* testing was not attempted. Pseudovincaleukoblastine diol did not show antimitotic activity, and insufficient material was available for further testing.

## EXPERIMENTAL

**Isolation of Leurocolombine (I)**—Twenty grams of a production-scale postvincaleukoblastine–preleurocristine chromatographic column cut was subjected to gradient pH purification. Leurocolombine cocrystallized from methanol with vincaleukoblastine, leurosine, and other trace impurities from pH 3.90 and 4.40 as the free base. Each pH fraction was subjected to sulfate formation in the usual manner[4]. Sulfates from pH 3.90, 4.40, and 4.90 were shown by TLC on silica gel[5] in ethyl acetate–ethanol (3:1) to contain vincaleukoblastine, leurosine, desacetylvincaleukoblastine, leurocolombine, and trace quantities of unidentified compounds.

The mixed sulfates were combined and dissolved in 2 liters of hot ethanol for recrystallization. Upon cooling to room temperature, a white amorphous precipitate deposited and was harvested to give 2.2184 g of first crop material. Three subsequent crops of amorphous sulfates, totaling 0.7023 g, were harvested from the chilled concentrated mother liquor. All crops in the attempted recrystallization of the mixed sulfates were amorphous mixtures, and none was appreciably enriched in I. Consequently, the second, third, and fourth crops of mixed sulfates were converted to their free bases for preparative TLC.

Between 80 and 100 mg of bases was applied to 20 × 20-cm silica gel preparative plates[6], which were developed in ether–diethylamine–toluene–methanol (100:5:5:5). Bands were distinguished by their UV-quenching effect and removed and eluted with chloroform and methanol. This method yielded 105 mg of I. The first crop sulfate (2.2184 g) was converted to its free base (1.98 g) and subjected to gradient pH purification to enrich fractions prior to preparative TLC. Leurocolombine was again found in pH 3.90, 4.40, and 4.90 (total weight 1.07 g) and cocrystallized with vincaleukoblastine and other trace impurities as both the free base and sulfate. Preparative TLC of all mixtures yielded an additional 150.6 mg of I.

Leurocolombine had a typical indole–dihydroindole UV spectrum[7]: $\lambda_{max}$ 217 ($a_m$ = 51,091) and 265 ($a_m$ = 15,666) nm with shoulders at 290 and 298 nm. The IR spectrum[8] had a strong band at 3570 $cm^{-1}$ (OH) in addition to the other typical bands found in vincaleukoblastine and leurosine (3). Leurocolombine has pKa values of 5.05 and 6.3. The mass spectrum[9] indicated a molecular ion at *m/e* 826.

**Acetylation of Leurocolombine (I)**—To a solution of 21.9 mg of I in 0.5 ml of dry pyridine, excess acetic anhydride was added. The reaction mixture was allowed to stand at room temperature overnight. After evaporating to dryness *in vacuo*, the mixture was shown by TLC on silica gel in ethyl acetate–ethanol (3:1) to contain unreacted I and one reaction product. Preparative separation afforded leurocolombine acetate ($M^+$ 868).

**Isolation of Vinamidine (II)**—Ten grams of a production-scale chromatographic preleurocristine fraction was fractionated in a series of preparative HPLC runs. Chromatography was carried out in stainless steel columns, using neutral alumina packing[10] and ethyl acetate–methylene chloride (25:75). The initial run was made on a 2.5-cm × 7-m column at 900 psi with a consequent flow rate of 66 ml/min. Eluent was subdivided based on the profile generated by a 280-nm UV flow monitor.

TLC of fractions on silica gel in ether–diethylamine–toluene–methanol (100:5:5:5) revealed a fraction containing a new alkaloid. This fraction was further purified by three sequential runs on an 8-mm × 7-m column at 320 psi with a 7-ml/min flow rate. Final purification was completed in an 8-mm × 2-m column using meth-

[1] Plant material obtained from Meer Corp. and Madis Labs.

[2] Pseudovincaleukoblastine is desacetoxyvincaleukoblastine. See previous paper in series, XXXV: N. Neuss, A. J. Barnes, and L. L. Huckstep, *Experientia*, in press.

[3] Metaphase arrest was observed using Chinese hamster ovary cells. Vincaleukoblastine was active at $2 \times 10^{-3}$ μg/ml.

[4] See U.S. pat. 3,225,030 (Dec. 21, 1965).

[5] Precoated silica gel 60 F-254, 0.25-mm plates, E. Merck, Darmstadt, Germany.

[6] Quantum Industries, Fairfield, NJ 07006

[7] Recorded on a Cary 15 UV spectrophotometer in ethanol.

[8] Recorded on a Perkin-Elmer 457A IR spectrophotometer in chloroform.

[9] Recorded on a Varian MAT model 731 double-focusing spectrometer. Samples were inserted directly into the ion source by direct probe.

[10] Woelm W-200 (60–180 μm).

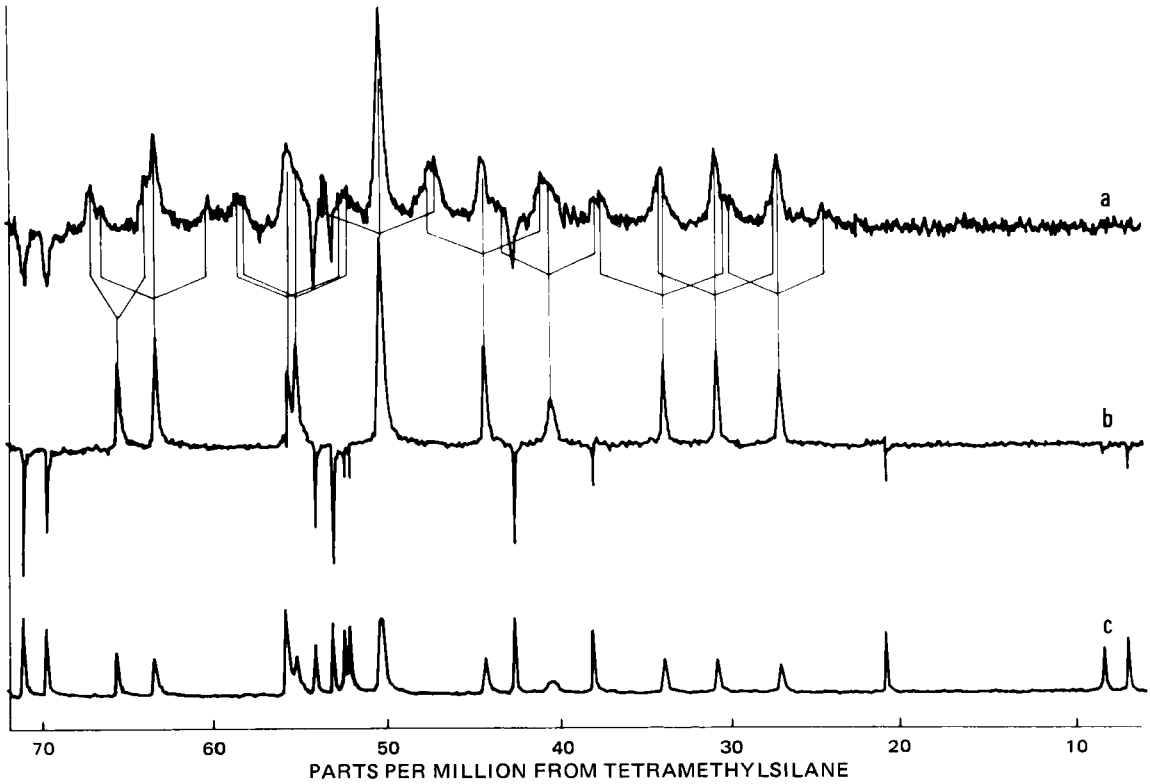



**Figure 1**—*Upfield portion of $^{13}C$ spectrum of leurocolombine. Key:* a, *off-resonance decoupled spectrum at $\tau$ = 0.4 sec;* b, *proton decoupled spectrum at $\tau$ = 0.4 sec; and* c, *proton decoupled spectrum.*

yl acetate–methylene chloride (60:39) at 1 atm of pressure and a flow rate of 1.5 ml/min. A total of 152 mg of II was isolated, being equivalent to 19 ppb of *C. roseus* leaf.

Vinamidine exhibited $\lambda_{max}$ 216 ($a_m$ = 57,278) and 268 ($a_m$ = 16,815) nm with shoulders at 285, 295, and 311 nm. The IR spectrum exhibited an intense band at 1660 $cm^{-1}$, which is typical of an amide. The mass spectrum indicated a molecular ion at *m/e* 824.

**Acetylation of Vinamidine (II)**—Vinamidine acetate ($M^+$ 866) was prepared in the manner described for I.

**Sodium Borohydride Reduction of Vinamidine (II)**—Thirty milligrams of vinamidine base was dissolved in anhydrous methanol. An excess of sodium borohydride was added slowly with stirring. After stirring at room temperature for 0.5 hr, 10 ml of water was added and the methanol was removed *in vacuo*. The aqueous suspension was then extracted with chloroform; the chloroform extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* to yield a one spot reaction product ($M^+$ 826).

**Acetylation of Vinamidine Reduction Product**—The vinamidine reduction product was acetylated in the manner described for I to yield two reaction products, subsequently identified as a monoacetate ($M^+$ 868) and a diacetate ($M^+$ 910) of the vinamidine reduction product.

**Isolation of Pseudovincaleukoblastine Diol (III)**—To isolate III, 11.75 g of a postvincaleukoblastine–preleurocristine production-scale chromatographic column cut was subjected to gradient pH purification. After sulfates were made in the usual manner, mixed sulfates containing vincaleukoblastine, leurocristine, and a third component were obtained from pH 5.40 and 5.90. A 100-mg sample of sulfate from pH 5.40 was fractionated in a preparative HPLC run on a stainless steel 0.793-cm × 6-m column packed with neutral alumina[11] using a linear gradient of 0–5% ethanol in methylene chloride.

The weighed sample was dissolved in 5 ml of water, and ammonium acetate was added to pH 9.0. The precipitate was centrifuged, the supernate was decanted, and the precipitate was dissolved in 3 ml of methylene chloride for application to the column. The run was made at 1100 psi (uncorrected) with a consequent flow rate of 180 ml/hr[12]. Fractions were collected every 3 min once peaks were observed on a UV profile. Compound III ($M^+$ 768) was located in fractions 30–32 by TLC on silica gel in ether–diethylamine–toluene–methanol (100:5:5:5). The remainder of the mixed sulfates containing the compound was subjected to preparative TLC in the same system since better yields were obtained than with the preparative HPLC run. After two additional preparative TLC runs, a total of 25.2 mg of III was isolated.

**Acetylation of Pseudovincaleukoblastine Diol (III)**—Compound III was acetylated in the manner described previously to yield its diacetate ($M^+$ 852) as the major reaction product with traces of a monoacetate ($M^+$ 810).

## DISCUSSION

In the structure analyses of the new dimeric alkaloids, it is helpful to relate the unknown structures to the known structure of vincaleukoblastine (4). The vindoline portion of the dimers can readily be identified by characteristic chemical shifts in the PMR spectrum (5), by characteristic fragments in the mass spectrum (6), and by characteristic carbon resonances in the CMR spectrum (7).

**Leurocolombine (I)**—The mass spectrum of I indicated a molecular weight of 826, and high-resolution measurements verified a molecular formula of $C_{46}H_{58}N_4O_{10}$ (Table I). Deuterium exchange in combination with mass spectrometry disclosed that I had one more exchangeable proton than vincaleukoblastine, indicating that the additional oxygen was most likely a hydroxy group. The vindoline half of the dimer appeared intact, as evidenced by peaks at *m/e* 121, 122, 135, 282, 469, and $M^+ - C_6H_7O_5$ (6). Since typical indole fragments at *m/e* 143 and 144 were present, the extra hydroxy was assumed to be in the alicyclic portion of the indole half of the molecule.

In vincaleukoblastine, *m/e* 154 is characteristic for the alicyclic portion of the velbanamine portion of the molecule (8). If the extra hydroxy was in this portion, a peak at *m/e* 170 would be expected; indeed, a weakly intense peak did appear, but high resolution indicated it was a mass triplet. One component did correspond to the

[11] Woelm N-18 (18–30 μm).

[12] Lapp LS-30 Pulsafeeder pump.

Table I—High-Resolution Mass Spectral Measurements for Leurocolombine

| Formula | Observed *m/e* | Calculated *m/e* |
|---|---|---|
| $C_{46}H_{58}N_4O_{10}$ | 826.41613 | 826.41530 |
| $C_{44}H_{55}N_4O_8$ | 767.40267 | 767.40199 |
| $C_{40}H_{51}N_4O_5$ | 667.38395 | 667.38595 |
| $C_{26}H_{33}N_2O_6$ | 469.22936 | 469.2338 |
| $C_{14}H_{20}NO_5$ | 282.13126 | 282.13415 |
| $C_{12}H_{12}N$ | 170.09755 | 170.09697 |
| $C_9H_{16}NO_2$ | 170.11731 | 170.11810 |
| $C_{11}H_{10}N$ | 156.08161 | 156.08132 |
| $C_8H_{14}NO_2$ | 156.10182 | 156.10245 |
| $C_9H_{14}NO$ | 152.10906 | 152.10754 |
| $C_{10}H_{10}N$ | 144.08187 | 144.08132 |
| $C_{10}H_9N$ | 143.07408 | 143.07350 |

desired molecular formula for the additional hydroxy while the other components were determined to be the indole portion of the molecule together with attached carbon and nitrogen atoms.

A peak at *m/e* 152 had an empirical formula corresponding to loss of water from the $C_9H_{16}NO_2$ component at *m/e* 170. This region of the mass spectrum was further complicated by a mass doublet at *m/e* 156, the more intense component together with its associated $^{13}C$ isotope peak corresponding to the alicyclic portion of the molecule and containing two oxygens. Deuterium exchange in this region showed an increase in intensity at *m/e* 157 with no other discernible significant changes.

Inspection of the PMR spectrum of I showed few differences from that of vincaleukoblastine. The resonances that are usually identified as vindoline protons were present with few changes. The aromatic resonances, H-9 and $OCH_3$-11 closest to the bond linking the two monomers, were broadened, suggesting possible restriction of rotation around that bond. In addition, a doublet at $\delta$ 4.16 was resolved, with a coupling constant of 15 Hz characteristic of geminal coupling of a methylene proton attached to a heteroatom.

The key to placement of the extra hydroxy in I came from CMR studies[13]. The initial experiment involved running the proton-decoupled CMR spectrum to get chemical shift values for as many of the carbons as possible. Overlap made it difficult to distinguish all 46 carbon resonances, and additional experiments were performed to resolve the resonances and to gain data useful for making assignments.

From relaxation time ($T_1$) measurements on vincaleukoblastine and other dimeric alkaloids, the nonprotonated carbons with long $T_1$ values were easily distinguished from protonated carbons that have shorter $T_1$ values (9). Average $T_1$ values were 1–3 sec for nonprotonated carbons; 0.5 sec for methyl carbons attached to carbon, oxygen, or nitrogen; 0.1 sec for methine carbons; and 0.05 sec for methylene carbons. The $T_1$ values of methines and methylenes were not always clearly differentiated, so a procedure using the inversion-recovery (180–$\tau$–90) method (10) in conjunction with off-resonance decoupling (11) was used to distinguish these two types of carbons.

In the inversion-recovery method, resonances are inverted by a 180 $R_f$ pulse and allowed to relax only partially before being measured by a 90 pulse. A resonance having a relaxation time, $T_1$, is nulled if an interval ($\tau$) of ln $2T_1$ (0.69 $T_1$) is used. By using an interval of 0.35 sec (0.69 × 0.5), the methyl resonances were nulled in vincaleukoblastine. Leurocolombine required a $\tau$ value of 0.4 sec. The resulting spectrum showed nonprotonated carbon resonances as inverted peaks and the methines and methylenes as upright peaks (Fig. 1*b*). At this point, nonprotonated carbons and methyl carbons were clearly identified, but methine and methylene carbon resonances were not yet differentiated. The inversion-recovery spectrum was subsequently recorded with off-resonance decoupling to produce methines as "doublets" and methylenes as "triplets" (Fig. 1*a*).

The $^{13}C$ resonances of the vindoline half of vincaleukoblastine have been reported (7). These resonances were subjected to the inversion-recovery/off-resonance decoupling procedure to test both the assignments and the procedure. Excellent agreement was obtained. Resonances in I with chemical shifts similar to the assigned vindoline resonances in vincaleukoblastine were checked by the procedure to confirm their assignments in I (Table II).

[13] Recorded on a JEOL PS-100 spectrometer in Fourier transform mode operating at 25 MHz with repetition times of 2–3 sec. Chemical shifts are reported relative to internal tetramethylsilane, using concentrations of 50–200 mg/2 ml deuterated chloroform, depending on availability of sample.

Table II—$^{13}C$ Chemical Shifts of Vindoline Half of Vincaleukoblastine and Leurocolombine

| | Vincaleukoblastine | Leurocolombine |
|---|---|---|
| Acetate C=O | 171.6 | 171.5 |
| Ester C=O | 170.8 | 170.8 |
| C-11 | 158.0 | 158.6 |
| C-13 | 152.5 | 153.6 |
| C-15 | 129.9 | 129.8 |
| C-14 | 124.4 | 124.4 |
| C-9 | 123.5 | 123.6 |
| C-8 | 122.6 | 122.3 |
| C-10 | 121.1 | 120.0 |
| C-12 | 94.2 | 94.6 |
| C-2 | 83.3 | 83.2 |
| C-16 | 79.7 | 79.4 |
| C-17 | 76.4 | 76.2 |
| C-21 | 65.5 | 65.7 |
| $OCH_3$ | 55.7 | 55.9 |
| C-7 | 53.2 | 53.2 |
| $COOCH_3$ | 52.1 | 52.2 |
| C-3 | 50.2 | 50.4 |
| C-5 | 50.2 | 50.4 |
| C-6 | 44.6 | 44.3 |
| C-20 | 42.7 | 42.6 |
| $N\text{-}CH_3$ | 38.3 | 38.1 |
| C-19 | 30.8 | 30.8 |
| $OCOCH_3$ | 21.1 | 21.0 |
| C-18 | 8.3 | 8.3 |

By schematically representing the I spectrum (Fig. 2*a*) and subtracting the vindoline resonances (Fig. 2*b*), only those resonances corresponding to the velbanamine-like half of the dimer remained (Fig. 2*c*). This procedure reduced the complexity of the total spectrum.

The aromatic carbons of the indole half and the ester carbonyl and methoxy were readily assigned by comparison of chemical shifts to vincaleukoblastine (7), and these assignments were confirmed by the inversion-recovery/off-resonance decoupling procedure (Table III). This procedure confirmed the deduction based on mass and PMR spectra that the indole portion of the molecule was unsubstituted. From the remaining resonances, it was observed that I contained three "singlets," *i.e.*, nonprotonated carbons, rather than two as in vincaleukoblastine. One singlet corresponded well to C-20′ of vincaleukoblastine, and one was in the range of C-16′. The third singlet resonated at 71.2 ppm in the range of carbons singly bonded to oxygen (12) and thus had the extra hydroxy. Since the methine doublet corresponding to C-14′ in vincaleukoblastine is absent in I, the hydroxy must be placed at C-14′. These data are accommodated by Structure I.

Since vincaleukoblastine and I have identical C-18′ chemical shifts, it may be concluded that the configuration at C-20′ is the same in I as in vincaleukoblastine. At present, no configuration has been determined for C-14′.

A hydroxy at C-14′ could possibly sterically hinder rotation around the bond linking the two monomers, and this would ex-

Table III—$^{13}C$ Chemical Shifts of Selected Carbons of Indole Half of Vincaleukoblastine and Leurocolombine

| | Vincaleukoblastine | Leurocolombine |
|---|---|---|
| Ester C=O | 174.9 | 174.3 |
| C-13′ | 134.9 | 135.1 |
| C-2′ | 131.3 | 130.4 |
| C-8′ | 129.4 | 129.2 |
| C-10′ | 122.1 | 122.3 |
| C-11′ | 118.7 | 118.9 |
| C-9′ | 118.4 | 118.4 |
| C-7′ | 117.0 | 117.1 |
| C-12′ | 110.4 | 110.4 |
| C-20′ | 69.4 | 69.9 |
| C-16′ | 55.7 | 54.2 |
| $COOCH_3$ | 52.1 | 52.5 |
| C-18′ | 6.9 | 6.9 |

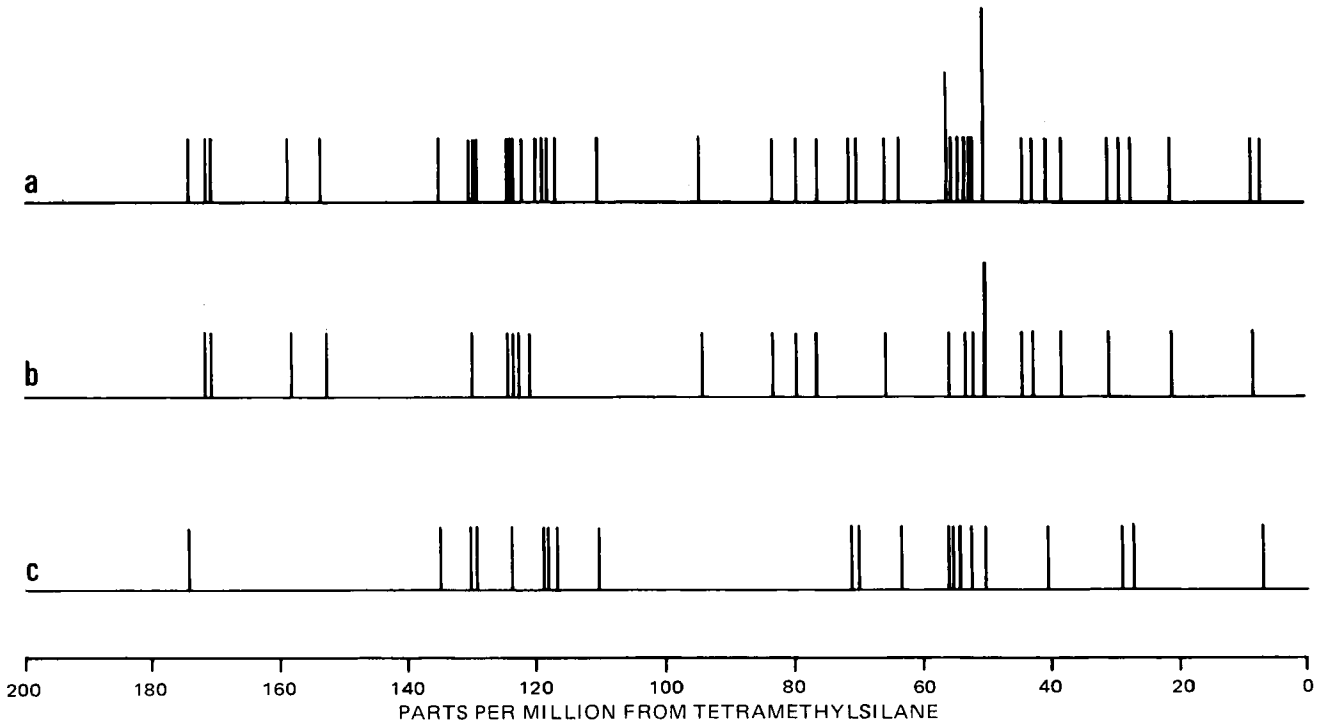


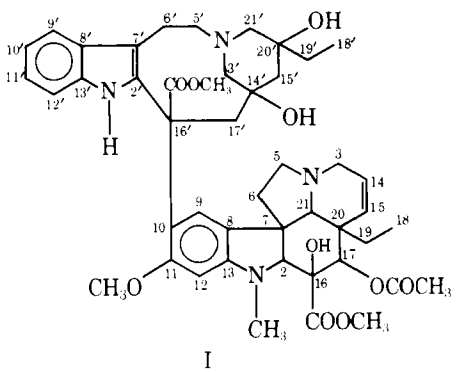

**Figure 2**—*Schematic diagrams of* $^{13}C$ *spectra. Key:* a, *total leurocolombine spectrum;* b, *vindoline spectrum; and* c, *indole half of leurocolombine.*

plain the broadened signals observed in the PMR spectrum. Both C-16′ and C-10 showed 1-ppm shifts in the CMR spectrum, which could reflect an effect on that bond.

**Vinamidine (II)**—The mass spectrum of II indicated a molecular weight of 824, and high resolution established a molecular formula of $C_{46}H_{56}N_4O_{10}$, which is isomeric with leurocristine (13). The IR spectrum had an intense band at 1660 $cm^{-1}$, characteristic of an amide.

In the PMR spectrum, the characteristic chemical shifts for the vindoline half of the molecule were observed. Likewise, the carbon resonances for the vindoline half of vinamidine agreed well with the assigned chemical shift values for the vindoline carbons in vincaleukoblastine and were checked by the inversion-recovery/off-resonance decoupling procedure. Consequently, an *N*-formyl group in the vindoline half was eliminated as the source of the intense amide band in the IR spectrum.

The CMR studies indicated the presence of a ketone at 210.4 ppm (12) in the indole half of the molecule. Sodium borohydride reduction resulted in the disappearance of the ketone resonance, with a new signal appearing at 68.4 ppm, indicative of an alcohol (12). The vinamidine reduction product formed a diacetate, while the parent compound formed a monoacetate. The CMR spectrum also had a resonance at 163.4 ppm, which appeared as an upright doublet in the inversion-recovery/off-resonance decoupling procedure. This downfield chemical shift, coupled with the fact that the carbon bears one proton, suggested a formamide. An $N_b$-formyl group would be consistent with the observed CMR resonance as well as the IR band. The reduction reaction affected neither this carbon resonance nor the IR band.

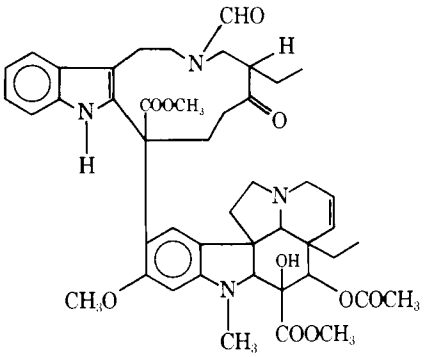


I

The ketone was placed at C-15′, although C-14′ is the biogenetically preferred position. Despite C-20′ being nonoxygenated, the chemical shift of C-18′ in II is quite similar to C-18′ in vincaleukoblastine. A ketone at C-15′ rather than C-14′ would exert a shielding effect (14) on C-18′ to cause a slight upfield shift and effectively compensate for any downfield shift due to no oxygenation at C-20′. From these data, Structure II is proposed for vinamidine.

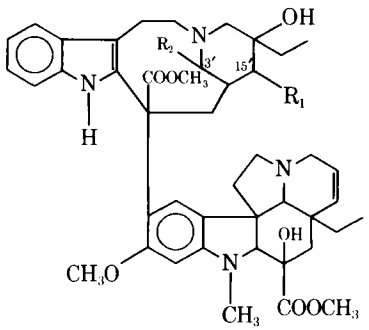
The structure of catharine, another *Catharanthus* indole–dihydroindole alkaloid, was reported recently[14]. It differs from II by having a double bond between C-20′ and C-21′ and a ketone at C-14′ rather than C-15′. The hydrogenation products of the two compounds were not identical, thus indicating that II was not merely dihydrocatharine. Sodium borohydride reduction of catharine produced a lactone between the carbomethoxy carbonyl and the reduced ketone, but II formed only the alcohol to substantiate again different placement of the ketone.

**Pseudovincaleukoblastine Diol (III)**—The mass spectrum of III indicated a molecular ion of $M^+$ 768, which corresponds to the molecular weight of desacetylvincaleukoblastine; however, the remainder of the spectrum showed that the compounds differed. The characteristic vindoline fragments of *m/e* 469 and 282 were shifted



II

[14] Presented by P. Potier at the IUPAC Natural Products Symposium in Ottawa, Canada, July 1974.



$R_1 = OH$ $R_2 = H$ or $R_1 = H$ $R_2 = OH$

III

58 mass units to *m/e* 411 and 224, respectively. Similarly, the usual $M^+ - 160$ (5) was shifted to $M^+ - 102$, which indicated a desacetoxy rather than desacetyl compound. Comparison to the mass spectrum of pseudovincaleukoblastine[2] showed identical fragments of *m/e* 411, 224, and $M^+ - 102$. The peaks at *m/e* 154 and 355, which are characteristic of the velbanamine half of vincaleukoblastine (8), were shifted to *m/e* 170 and 371 in III to indicate an extra hydroxy group in the alicyclic portion of the indole half of the dimer. These two peaks are also seen in the mass spectrum of vincadioline[15].

Inspection of the PMR spectrum of III showed the compound lacked an acetate peak. In the PMR of desacetyl compounds of the vindoline-containing dimeric alkaloids, H-17 is shifted to $\delta$ 4.075 (5) and the vinyl protons coincide in a multiplet around $\delta$ 5.85 (15). Compound III had no signal near $\delta$ 4.0 assignable to H-17 and had a broad multiplet at $\delta$ 5.46–5.78, which was clearly different from the multiplet observed in a simple desacetyl derivative.

Compound III formed a diacetate with one acetate group in the vindoline half and the other acetate in the indole half as evinced by the peak at *m/e* 371 shifting to *m/e* 413. More specifically, the peak at *m/e* 170 is shifted to *m/e* 212 to locate the acetate group in the alicyclic portion. The two acetates were observed in the PMR spectrum at $\delta$ 1.88 and 1.90. The peak at $\delta$ 1.90 was assigned to the acetate at C-16 in the vindoline half, which is identical to the chemical shift of the C-16 acetate in desacetoxyvincaleukoblastine acetate[2]. The acetate peak at $\delta$ 1.88 is quite similar to the one observed C-15′ acetate in vincadioline diacetate at $\delta$ 1.85[15]. The acetate derivatives of both III and vincadioline exhibited a new one-proton singlet at $\delta$ 4.47–4.48, assigned to the proton at the C-15′ acetate. In the parent compounds, this proton is hidden but, upon acetylation, shifts downfield to be clearly identified.

From these data, Structure III, where $R_1$ is OH, is proposed for pseudovincaleukoblastine diol. There is a possibility that the hydroxy could be placed at C-3′ or C-21′ since a carbinolamine could form an acetate under the conditions used. The most likely placement, however, of the hydroxy is at C-15′, based on comparison to vincadioline. The small amount of compound precluded further evidence of the structure.

[15] Vincadioline is 15′-hydroxyvincaleukoblastine, a minor alkaloid from *C. roseus;* unpublished results from Lilly Research Laboratories.

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