

The Botanical, Chemical, Biosynthetic and Pharmacologic Aspects of *Catharanthus roseus* (L.) G. Don (Apocynaceae)

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Since 1958, *Catharanthus roseus* (L.) G. Don in the family Apocynaceae has been the most actively studied alkaloid containing plant, and work on these alkaloids has been at the forefront of several aspects of alkaloid chemistry for many years. This very brief review will attempt to illustrate some of the crucial advances that have occurred as a result of work on these alkaloids.¹⁾

Botany: The isolation of alkaloids from *Catharanthus roseus* and from plants in the genus *Vinca* led to a need to clarify the relationship between the genera *Vinca* and *Catharanthus* since *C. roseus* had been referred to as *Vinca rosea* L.

The genus *Vinca* was established by Linnaeus in 1753 and *Catharanthus* by G. Don in 1835. *Vinca rosea* was first described by Linnaeus in 1759, but the flower did not fit his generic description, particularly as regards the stamens. Reichenbach in 1828 proposed the name *Lochnera* from *Vinca*, and according to Stearn²⁾ is therefore a *nomen nudum*. The correct name is therefore *Catharanthus roseus* (L.) G. Don. The genus consists of seven species endemic to Madagascar and one *C. pusillus*, endemic to India (Table 1). *Catharanthus roseus* is the most important member of the genus and has become a pantropical ornamental. *Vinca* species which total six, on the other hand are native

Table 1. The genus *Catharanthus*

<i>Catharanthus lanceus</i>	(Boj. ex A.DC.) Pich.
<i>Catharanthus longifolius</i>	(Pich.) Pich.
<i>Catharanthus trichophyllus</i>	(Bak.) Pich.
<i>Catharanthus roseus</i>	(L.) G. Don
<i>Catharanthus coriaceus</i>	Mgf.
<i>Catharanthus ovalis</i>	Mgf.
<i>Catharanthus pusillus</i>	(Murr.) G. Don
<i>Catharanthus scitulus</i>	(Pich.) Pich.

Table 2. Folklore activity of *Catharanthus roseus* leaves*

Reported Use	Locality
Antidiabetic	S. Africa, India, Australia, Phillipines
Antigalactogoue	France
Menorrhagia	S. Africa
Topical Antiseptic	S. Vietnam
Purgative	Malagasy Republic
Anticancer	Kenya

* Partial listing

to Southern Europe, particularly the Mediterranean, and the Near East.

Folklore: -Some of the more important folklore activity reported for *C. roseus* is summarized in Table 2, but the one which led to the initial, independent studies by the groups of Noble, Beer and Cutts and at Eli Lilly was the possibility of an oral insulin substitute.

Isolation:- The Canadian group found overwhelming *Pseudomonas* infections in the rats receiving *C. roseus* extracts intraperitoneally due to the development of severe leukopenia as the result of a lack of immune protection.³⁾ Bioactivity-directed fractionation led to the isolation of vincalukoblastine (vinblastine, VLB). Neither this group nor the Eli Lilly group could substantiate the hypoglycemic activity of crude extracts, but the latter group did find anti-leukemic activity in the P-1534 leukemia system and an intensive search for the active principles led to the isolation of vincalukoblastine and leurosine,⁴⁾ through a quite different extraction and purification scheme than had been used previously.

However, it was observed that certain fractions not containing these two alkaloids produced a high percentage of "indefinite" survivors and these fractions could be separated through a new technique of gradient pH extraction^{5,6)} This technique utilizes the differing basicities of the alkaloids in the complex mixture, in which the least basic alkaloids are separated first and the more basic alkaloids are obtained as the pH is raised. In this way the extremely potent alkaloid leurocristine (VCR) was isolated in approximately $2 \times 10^{-4}\%$ yield. Subsequent work, principally by the Lilly group, has led to the isolation of over ninety alkaloids, several of which display anticancer activity, but to date only two of these VLB (VELBAN) and VCR (ONCOVIN) are commercially available.

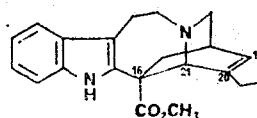
Clinical activity:- VLB became a prescription product in 1961 and VCR in 1963, and these highly profitable products are becoming of increasingly wider use. Vincalukoblastine has been used successfully in the treatment of Hodgkin's disease,⁷⁾ epidermoid carcinoma of the head and neck⁸⁾ and ovarian and breast

cancer.⁹⁾ In combination, VLB and chiorambucil are more effective in the treatment of Hodgkin's disease than either agent alone,¹⁰⁾ and the combination of VLB and bleomycin has proved quite effective for the treatment of testicular cancer.

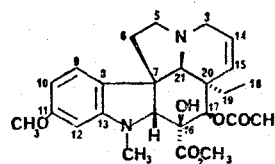
VCR has a quite different and wider spectrum of activity. In combination with prednisone it is used in acute childhood lymphocytic leukemia¹¹⁾ and with other agents it is effective for the treatment of Wilms' tumor, breast cancer, Hodgkin's disease and large bowel cancer.¹²⁾

Structure determination:- The spectral data, initially only IR and UV and subsequently low field (40 MHz) NMR, indicated¹³⁾ a close structural relationship between the dimers and two of the major monomeric alkaloids¹⁴⁾ of the leaves of *C. roseus* catharanthine (1)¹⁵⁾ and vindoline (2).¹⁶⁾ Indeed one of the key observations was the reductive cleavage of either VLB or VCR to afford cleavamine (3) and desacetylvindoline (4). Cleavamine (3) is also a degradation product of catharanthine (1).¹⁷⁾

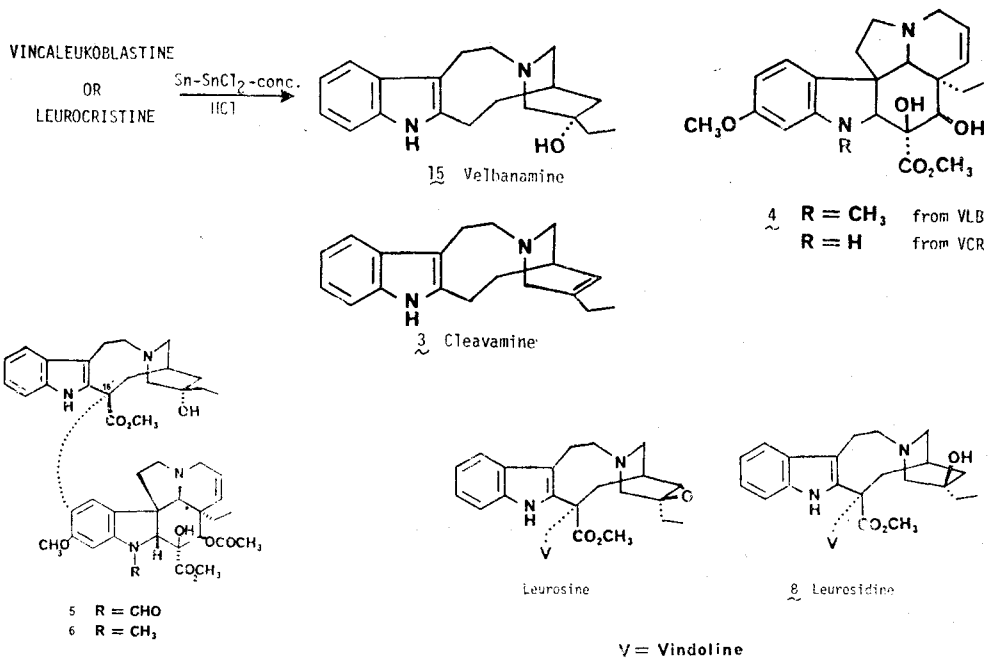
The complete molecular structure of VCR was established through X-ray analysis¹⁸⁾ of leurocristine methiodide dihydrate to be 5, differing from VLB (6) only in the oxidation level of the Na substituent. Two of the other important bisindole alkaloids leurosine (7) and



1 Catharanthine



2 Vindoline



leurosidine (8) were assigned tentative structures by the Lilly group,^{19,20} but these were subsequently revised through mass spectral interpretation (leurosine) and evaluation of the ¹³C NMR spectral data (leurosine and leurosidine).^{22,23}

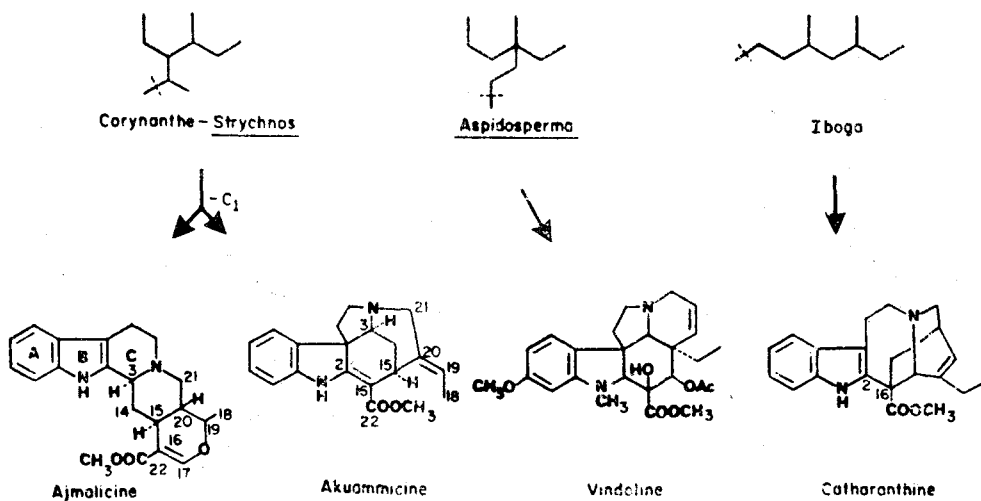
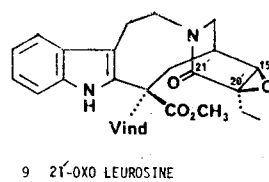


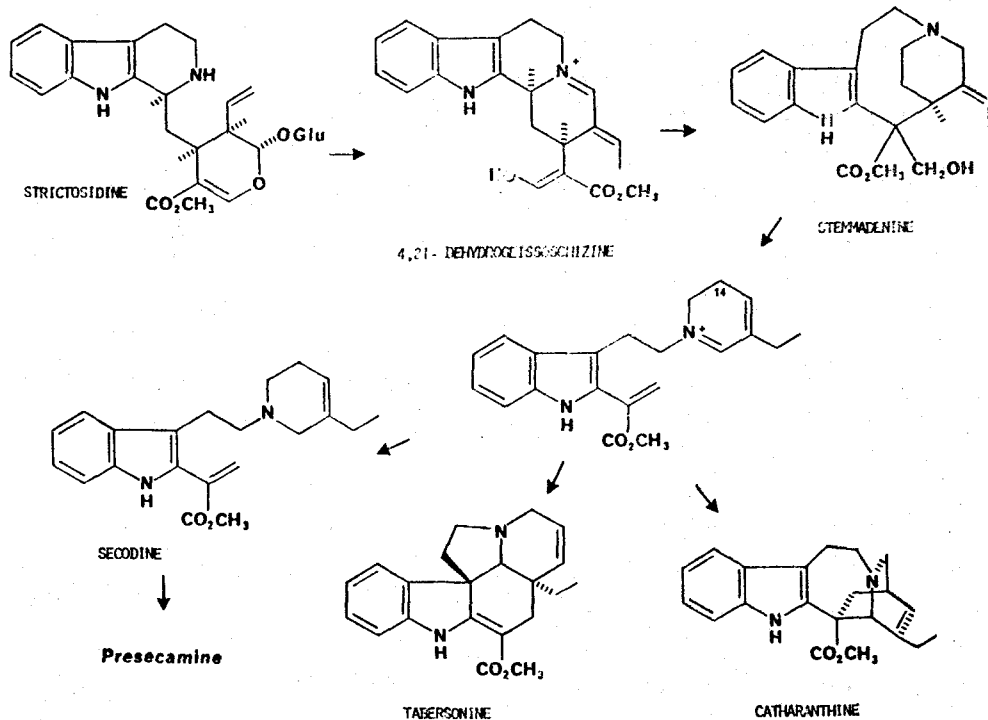
Fig. 1. Fundamental indole alkaloid structure types

The establishment of the ^{13}C NMR data for bisindole alkaloids, in particular, has permitted the structure determination of several new alkaloids. Thus the structure of 21'-oxoleurosine (9) was readily deduced because the resonance of C-21' of leurosine (53.9ppm) was absent, replaced by a new amide carbonyl resonance at 167.9 ppm.²⁴⁾ In conformation of this, a deshielded proton at 4.66 ppm was assigned to a proton on C-6 lying in the plane of the amide carbonyl.

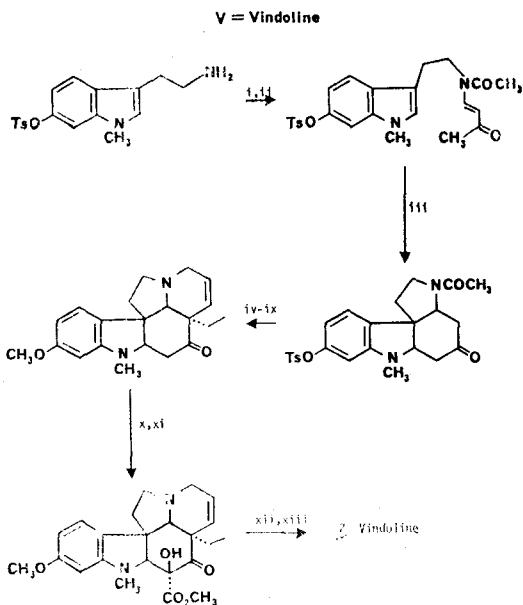
Biosynthesis:- The 1200 or so indole alkaloids show a wide variety of skeletal types, but three of the most important of these are those in the corynanthe, *Aspidosperma* and Iboga series (Fig. 1). Very few plants in the Apocynaceae contain all these alkaloids types, but *C. roseus* does and it has therefore been subjected to numerous studies²⁵⁾ in order to establish the overall scheme, which is summarized in Scheme 1. Over the years this has

been an area of considerable controversy, particularly with regard to the C-3 stereochemistry of the nitrogenous glycoside precursor. More recently²⁶⁾ Zenk and co-workers have established that C-3 α H isomer is the precursor of indole alkaloids and have also obtained²⁷⁾ the enzyme system, strictosidine synthase which produces this intermediate. Some progress has been made on evaluating the formation of VLB from the monomeric units.²⁸⁾

Synthesis:- The low yield of the important bisindole alkaloids from the plant led to vast synthetic efforts aimed at not only producing the iboga and *Aspidosperma* skeleta, but also the clinical entities themselves. However, since the yield of VLB may be 1000 times that of VCR, considerable effort was also made at carrying out this conversion. As a result this can now be achieved either chemically, through oxidation with CrO_3 at low temperature, followed by formylation,²⁹⁾ by Pd/oxygen



Scheme 1. Biosynthesis of monoterpenoid indole alkaloids



Reagents: i) $\text{ClCH=CHCOMe-Et}_3\text{N-EtOH}$,
 ii) $\text{Ac}_2\text{O-Et}_3\text{N-C}_6\text{H}_6$, Δ , iii) $\text{BF}_3\text{-Et}_2\text{O}$,
 iv) 20% KOH-MeOH , Δ , v) $(\text{CH}_3)_2\text{SO}_4\text{-K}_2$
 $\text{CO}_3\text{-(CH}_3)_2\text{CO}$, Δ , vi) $(\text{C}_2\text{H}_5)_3\text{OBF}_4\text{-NaHCO}_3$,
 RT, vii) acrolein- NaOMe , viii) MesCl ,
 ix) $\text{Et-tBuOH-KO}^t\text{Bu-DME}$, xii) $\text{AlCl}_3\text{-THF-NaAlH}_2$
 $(\text{OCH}_2\text{CH}_2\text{OCH}_3)_2$, -25° ,
 xiii) $\text{Ac}_2\text{O-NaOAc}$.

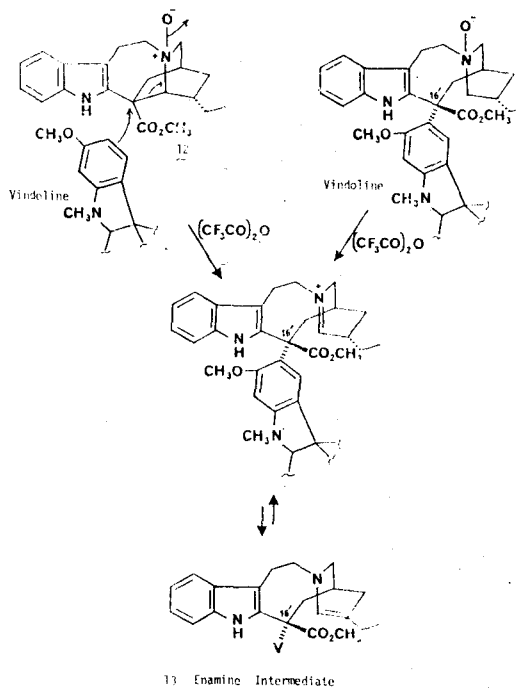
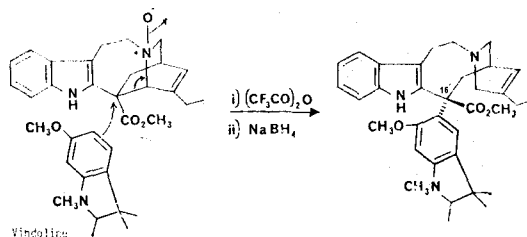
Scheme 3. Büchi synthesis of vindoline

catalyzed oxidation in the presence of formic acid,³⁰⁾ or microbologically with *Streptomyces albobrisesolus* followed by formylation.³¹⁾

Catharanthine (1) has been synthesized by the groups of Kutney^{32,33)} and Büchi³⁴⁾ and the route developed by Kutney is shown in Scheme 2. Vindoline (2) has also been synthesized by these two groups,^{35,36)} although others³⁷⁾ have improved certain of the early steps. The synthetic procedure of Büchi and co-workers is summarized in Scheme 3.

Prior to these successful synthetic efforts an attempt was made to join the two monomeric units.³⁸⁾ Although this did produce a bisindole species the stereochemistry at the crucial C-16' center was the unnatural one. In this respect

it is worth noting that when this stereochemistry is inverted, antileukemic activity is lost. Crucial to further work was the development of circular dichroism^{39,40)} as a technique to determine the C-16' stereochemistry in synthetic products. The key to the successful joining of the two monomer units was the modified Polonovskii reaction,⁴⁰⁾ which when carried out with catharanthine N-oxide (10) and vindoline (2) at low temperature afforded anhydrovinblas-



13 Enamine Intermediate

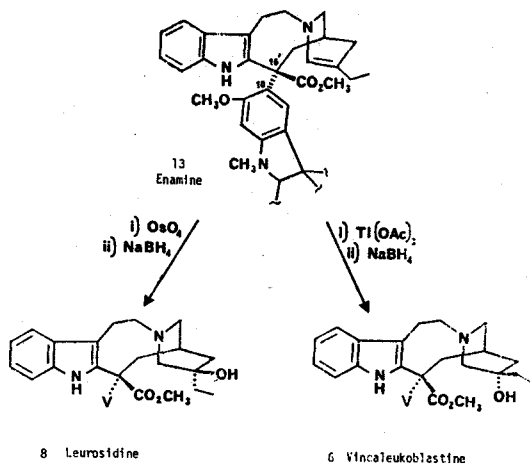
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Scheme 4. Synthesis of anhydrovinblastine

tine (11) (Scheme 4) with the natural C-16 stereochemistry.^{41,43)} In the formation of VLB and leurosidine from dihydrocatharanthine-N-oxide (12) and vindoline (2), the enamine 13, produced from the intermediate imminium species 14, is crucial (Scheme 5). For reaction with osmium tetroxide followed by NaBH₄ reduction afforded leurosidine (8) stereospecifically,⁴⁴⁾ and oxidation with thallium triacetate followed by NaBH₄ reduction yielded vincal leukoblastine (6).^{45,46)} A similar strategy had been used by Kutney in an earlier synthesis of velbanamine (15).^{32,33)}

Summary: Obviously this brief description of twenty years of effort with the alkaloids *Catharanthus roseus*, from the isolation of VLB to its successful synthesis, can hardly do justice to the many hundreds of articles published in the area, but Table 3 places some of the key developments into a historical perspective. Almost as important though, the study of the monomeric and dimeric alkaloids of *Catharanthus* has led to substantial scientific progress in a number of areas (Table 4).

But what of the future? Table 5 indicates



Scheme 5. Synthesis of leurosidine and vincal leukoblastine

Table 3. Historical perspective for the alkaloids of *C. roseus*.

1959	Isolation of vincal leukoblastine (VLB)
1961	VLB first marketed as VELBAN
1962	Degradation of VLB to deacetyl vindoline and velbanamine
1963	Isolation of leurocristine (VCR)
1963	VCR first marketed as ONCOVIN
1965	Acid rearrangement of catharanthine
1965	X-Ray structure of leurocristine methiodide
1967	Synthesis of a dimeric species
1968	Synthesis of velbanamine and catharanthine
1968	Biosynthesis of vindoline/catharanthine from loganin
1974	Biosynthesis of VLB from vindoline/catharanthine
1975	Synthesis of vindoline
1975	Synthesis of anhydrovinblastine
1975	Carbon-13 NMR spectrum of VLB
1977	Biosynthesis of vindoline/catharanthine from strictosidine
1979	Synthesis of vincal leukoblastine

Table 4. Study of the *Catharanthus* alkaloids has led to advances in the areas of:

- Alkaloid isolation techniques
- Structure elucidation of indole alkaloids
- Chemistry of iboga alkaloids
- Synthesis of *Aspidosperma* alkaloids
- Synthesis of iboga alkaloids
- Biosynthesis of indole alkaloids
- Spectroscopy of bisindole alkaloids
- Microbial oxidation of indole alkaloids
- Treatment of human neoplastic disease

Table 5. *Catharanthus roseus*-future prospects

- Isolation of alkaloids having activity in other tumor systems
- Synthesis of new compounds which are more active and/or less toxic
- Synthesis of the bisindoles from the monomers on a solid-phase enzyme system
- New combination regimens to develop an even wider spectrum of activity

what some of the future aims are and possibly the most stunning of these would be the solid phase enzyme synthesis from the monomers.

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