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Review Article

Current Status of Research on the Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don)

By GORDON H. SVOBODA, IRVING S. JOHNSON, MARVIN GORMAN, and NORBERT NEUSS

THE PANTROPICAL apocynaceous plant *Vinca rosea* Linn. is an erect, everblooming pubescent herb or subshrub, 1 to 2 ft. high. It is cosmopolitan in the tropics and is widely cultivated in gardens throughout the world as an ornamental. The pink and the white color varieties are found in the natural state, while three seed hybrids, *viz.*, blush pink with red eye, crimson, and white with red eye, are also commerically available.

Generally accepted synonyms for the genus *Vinca* are *Pervinca*, *Lochnera*, and *Catharanthus*. Pichon (2) has shown that the genera *Vinca* and *Lochnera* differ in 34 morphological characteristics and should not be used as synonyms. He furthermore found that *Catharanthus* G. Don predated *Lochnera* Reichb. by 7 months. While it would appear that the correct botanical name for this plant is *Catharanthus roseus* G. Don, we shall continue to use the more common *Vinca rosea* Linn. synonymously.

An early description of the medicinal properties of this plant mentions that an infusion of the leaves was used in Brazil in the treatment of hemorrhage and scurvy, as a mouthwash for toothache, and for healing and cleaning chronic wounds (3). Recently it has been reported that the total alkaloids possess a limited antibiotic activity against *V. cholera* and *M. pyogenes* var. *aureus*, as well as a significant and sustained hypotensive action (4). A recent review by Farnsworth (5) contains an exhaustive summary of early descriptions of medicinal properties of the plant, as well as those of other members of these genera.

The folklore reputation which this plant enjoyed as an oral hypoglycemic agent (6) prompted its phytochemical examination in two different laboratories, independently and unknown to each other. While neither group could substantiate this reported activity in either normal or experimentally-induced hyperglycemic rabbits, the Canadian group of Noble, Beer, and Cutts observed a peripheral granulocytopenia and bone marrow depression in rats associated with certain fractions (7, 8). Continued investigation led to their preparation of vincaleukoblastine (VLB) sulfate,[1] an alkaloid capable of producing severe leukopenia in rats (9–11). The Lilly group had demonstrated that certain alkaloidal fractions gave a reproducible effectiveness in DBA/2 mice infected with the P-1534 leukemia, a transplanted acute lymphocytic leukemia (12–15). An intensive phytochemical examination resulted in obtaining leurosine,[1] a new dimeric alkaloid closely chemically related to VLB, as well as VLB sulfate. The effectiveness of both of these alkaloids against the P-1534 leukemia in DBA/2 mice was first demonstrated in these laboratories.

Received from the Organic Chemical Development and Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.

Paper XIII (a review) in the series; the preceding paper appeared in THIS JOURNAL (1).

[1] The A.M.A. Council on Drugs has approved vinblastine, vinleurosine, vinrosidine, and vincristine as generic names for these alkaloids. VLB is supplied as Velban (vinblastine sulfate, Lilly).

Our early recognition of the fact that the "indefinite" survivors observed with certain amorphous fractions were due neither to VLB nor leurosine, nor to their combination, further stimulated the phytochemical search for the compound(s) responsible for this profound activity. These efforts culminated in the discovery of leurosidine[1] and leurocristine (16).[1]

PREPARATION OF ALKALOIDS

The earliest chemical investigation of this plant was conducted by Greshoff (17) who reported the presence of alkaloids, but who was unable to obtain pure crystalline compounds. Cowley and Bennett (18) prepared a crystalline tartrate and two sulfates from the total alkaloidal extract, but did not describe their physical or chemical properties. More recently, several groups of investigators have obtained the following alkaloids: ajmalicine (19), akuammine (19), tetrahydroalstonine (20), serpentine (19, 20), lochnerine (21), and reserpine (22), as listed in Table I. Inasmuch as these alkaloids are well documented, they do not merit further discussion.

During the course of our investigation, Beer obtained VLB as the sulfate (9), the preparation of which we were able to duplicate. Kamat and co-workers (23) reported on two amorphous and two crystalline alkaloids, one of which they described in detail and named vindoline, apparently being identical to our vindoline (24). An alkaloid, independently named lochnericine, has been reported by Nair and Pillay (25), and appears to be identical with our lochnericine (24). The occurrence of vindolinine was established simultaneously by Janot and co-workers (26) and our group, and the name was selected by mutual agreement.

The alkaloids reported to date solely from these laboratories include: leurosine, virosine, perivine, catharanthine, isoleurosine, lochneridine, sitsirikine, vincamicine, catharine, vindolicine (27), leurosidine, leurocristine (16), carosidine, carosine, pleurosine, neoleurosidine, vincarodine, catharicine, vindolidine, and neoleurocristine (1). These alkaloids, along with VLB and the three co-discovered ones, vindoline, lochnericine and vindolinine, are listed in Table II, along with their empirical formulas, m.p.'s, pK'a's, specific rotations, and ultraviolet absorption maxima.

TABLE I.—ALKALOIDS FROM *Vinca rosea* LINN.

Name	Formula
Ajmalicine	$C_{21}H_{24}N_2O_3$
Tetrahydroalstonine	$C_{21}H_{24}N_2O_3$
Serpentine	$C_{21}H_{22}N_2O_3$
Lochnerine	$C_{20}H_{24}N_2O_2$
Akuammine[a]	$C_{22}H_{26}N_2O_4$
Reserpine[a]	$C_{33}H_{40}N_2O_9$

[a] Akuammine (vincamajoridine) and reserpine have not been encountered in our investigation, while the presence of the other four has been confirmed.

Figure 1 represents the scheme used in the extraction of the drug. The process consists essentially of separating the plant alkaloids into those whose tartrates are soluble in organic solvents and those which are not, under the existing conditions. The basis for this method, in which the tartaric acid serves to bind the stronger bases in the crude drug, affording an initial separation of the weak and strong bases during extraction, was first described in 1957 (28) in a paper dealing with the root bark alkaloids of *Alstonia constricta* F. Muell.

The final purification of most of the alkaloids listed in Table II was achieved by elution chromatography on alumina deactivated by treatment with 10% acetic acid. The eluting solvents used were benzene, benzene-chloroform mixtures, chloroform, and chloroform-methanol mixtures. In order to obtain a number of the alkaloids it was necessary to subject certain selected post-VLB column fractions to a gradient pH technique, which is described as follows: A 10-Gm. sample of the crude amorphous chromatographic fraction was dissolved in 500 ml. of benzene. Any benzene-insoluble material which would result was removed by filtration and discarded. The alkaloids were extracted into 500 ml. of 0.1 *M* citric acid by a reduced pressure steam distillation. Insoluble material was removed by filtration and discarded. The filtrate volume was then adjusted to 500 ml. with water. The resulting clear acidic solution was extracted at pH levels of 2.80, 3.40, 3.90, 4.40, 4.90, 5.40, 5.90, 6.40, and 7.50 (± 0.02), with 500-ml. portions of benzene. The pH of the aqueous phase was raised by the addition of ammonia. The benzene extracts were dried over sodium sulfate and were concentrated *in vacuo* to dryness. The alkaloids were crystallized therefrom by the addition of either methanol or acetone.

It must be stated that no one column operation yielded every alkaloid that is listed as being obtained from a particular starting fraction. The following schemes, as presented in Figs. 2 and 3, will serve to illustrate the manner in which all of these alkaloids were obtained.

BIOLOGICAL PROPERTIES

Four of the recently isolated alkaloids from *Vinca rosea* Linn. have been of particular biological interest in these laboratories because of

TABLE II.—NEW ALKALOIDS FROM *Vinca rosea* LINN.

Name	Formula	M.p., °C.[a]	$[\alpha]_D^{26}$ $CHCl_3$	pK'a in DMF	U.V. $\lambda_{max.}^{EtOH}$, mμ[a]
Leurosine	$C_{46}H_{58}N_4O_9$[b]	202–205 (decompn.)	+72	5.5, 7.5 (H_2O)	214, 259
Vincaleukoblastine	$C_{46}H_{56}N_4O_9$	211–216 (decompn.)	+42[c]	5.4, 7.4 (H_2O)	214, 259
Virosine	$C_{22}H_{26}N_2O_4$[b]	258–264 (decompn.)	−160.5	5.85 (66%)	226, 270
Perivine	$C_{20}H_{24}N_2O_3$	180–181	−121.4	7.5 (66%)	226, 314
Catharanthine	$C_{21}H_{24}N_2O_2$	126–128	+29.8	6.8 (66%)	226, 284, 292
Lochnericine	$C_{21}H_{24}N_2O_2$	190–193 (decompn.)	−432	4.2 (66%)	226, 297, 327
Vindolinine·2HCl	$C_{21}H_{24}N_2O_2 \cdot 2HCl$	210–212 (decompn.)	−8 (H_2O)	3.3, 7.1 (66%)	245, 300
Vindoline	$C_{25}H_{32}N_2O_6$	154–155	−18	5.5 (66%)	212, 250, 304
Isoleurosine	$C_{46}H_{60}N_4O_9$[b]	202–206 (decompn.)	+61.2	4.8, 7.3 (66%)	214, 261, 287
Lochneridine	$C_{20}H_{24}N_2O_3$[d]	211–214 (decompn.)	+607.5	5.5 (66%)	230, 293, 328
Sitsirikine·1/2 H_2SO_4	$C_{21}H_{26}N_2O_3 \cdot 1/2$ H_2SO_4	239–241 (decompn.)	+23 (Base)	7.6 (66%)	224, 282, 288
Vincamicine	(Dimeric)	224–228 (decompn.)	+418	4.80, 5.85 (66%)	214, 264, 315, 341
Catharine	$C_{46}H_{52}N_4O_9 \cdot CH_3OH$	271–275 (decompn.)	−54.2	5.34 (66%)	222, 265, 292
Vindolicine	$(C_{25}H_{32}N_2O_{62})$	248–251 (melts, recryst.) 265–267 (decompn.)	−48.4	5.4 (66%)	212, 257, 308
Leurosidine	(Dimeric)	208–211 (decompn.)	+55.8	5.0, 8.8 (33%)	214, 265
Leurocristine	$C_{46}H_{54}N_4O_{10}$	218–220 (decompn.)	+17.0 ($EtCl_2$)	5.0, 7.4 (33%)	220, 255, 296
Carosidine	(Dimeric)	263–278, 283 (decompn.)	−89.8	...	212, 254, 303
Carosine	$C_{46}H_{56}N_4O_{10}$[b]	214–218	+6.0	4.4, 5.5 (33%)	255, 294
Pleurosine	$C_{46}H_{56}N_4O_{10}$[b]	191–194 (decompn.)	+61.0	4.4, 5.55 (33%)	267, 308
Neoleurosidine	$C_{48}H_{62}N_4O_{11}$[b]	219–225 (decompn.)	+41.6	5.1 (33%)	214, 268
Vincarodine	$C_{44}H_{52}N_4O_{10}$[b]	253–256 (decompn.)	−197.4	5.8 (66%)	230, 272, 298
Catharicine	$C_{46}H_{52}N_4O_{10}$[b]	231–234 (decompn.)	+34.8	5.3, 6.3 (33%)	214, 268, 293, 315
Vindolidine	$C_{48}H_{64}N_4O_{10}$[b]	244–250 (decompn.)	−113.2	5.3 (33%)	261, 311
Neoleurocristine	$C_{46}H_{56}N_4O_{12}$[b]	188–196 (decompn.)	−57.87	4.68 (33%)	220, 257, 298

[a] The melting points were determined on a Kofler microstage. The ultraviolet absorption spectra were obtained using a Cary model 14 spectrophotometer. [b] While these molecular formulas agree well with the analytical results for each particular alkaloid, it should be noted that they are to be considered as proximate at this time, in light of our experience with the other dimeric alkaloids (58). [c] Determined on VLB etherate. [d] On the basis of mass spectrometric evidence obtained by H. Budzikiewicz and J. M. Wilson of Stanford, we now prefer the C_{20} formulation rather than the C_{19} first reported (27).

their effects as chemotherapeutic agents in experimental neoplasias. These are: vincaleukoblastine, leurosine, leurocristine, and leurosidine. The transplantable tumor used as a bioassay for these alkaloids is the P-1534 leukemia. This is an acute lymphocytic leukemia which, in our laboratory, is transplanted only in DBA/2 mice. It has been used to detect and predict clinical activity of a number of antineoplastic compounds (13, 15, 29–34).

The techniques by which drugs are assayed with this and other tumors in our laboratory have been previously described (15).

The activity observed with the initial total extract of the plant and with the first crude fractions is shown in Table III. This led to the production of leurosine and VLB. Their activity against the P-1534 leukemia and other murine tumors has been described in detail (15, 34). Briefly, animals implanted with the P-1534 leukemia and treated with VLB survived 40–150% longer than saline-treated controls, when VLB was administered at levels ranging from 0.05–0.6 mg./Kg. Similar results were obtained with leurosine by therapy with levels ranging from 3.0–7.5 mg./Kg. (Table IV). A number of other transplantable tumors were also affected, including Freund, Ehrlich, and S-180 ascites tumors, B82A leukemia, and the Walker carcinosarcoma 256.

Extensive experimental evaluation indicated the "indefinite" survivors, obtained by administering certain of the crude extracts to animals implanted with the P-1534 leukemia, were not due to VLB or leurosine.

Continuing chemical investigation of those fractions (Table V), which gave high prolongation or indefinite survival of P-1534 animals, with such activity as a bioassay, led to the production of leurocristine and leurosidine (16). The activity of these alkaloids will be described in detail elsewhere, but prolongations ranging from

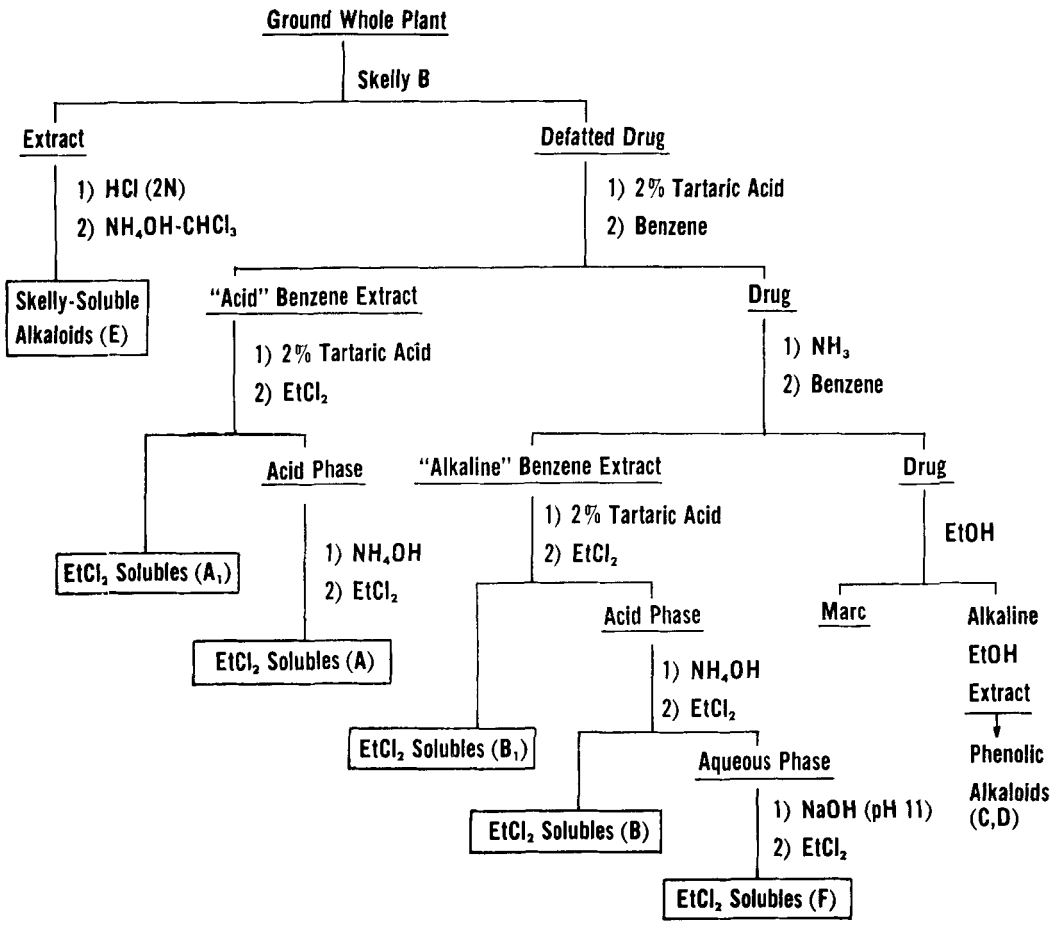


Fig. 1.—Extraction scheme.

30–220% over controls were obtained with leurocristine at levels from 0.05–0.35 mg./Kg., and 20–130% at levels from 2.0–10 mg./Kg. with leurosidine (Table VI) (35). At the higher levels of these ranges, 60–100% of the animals may be "cured" and survive indefinitely. The experimental tumor spectra of leurocristine and leurosidine are slightly broader than VLB and leurosine (Table VII).

With the exception of leurosine, all of these alkaloids may significantly prolong life of P-1534 animals, even when treatment is delayed until the animals are in near terminal states. In addition, in the case of leurocristine and leurosidine, very preliminary studies suggest that if such animals survive, they may become resistant to additional challenges with the same tumor. "Curing" of strain specific tumors in highly inbred strains of laboratory animals is in itself rare, but resistance to subsequent challenges of the same tumor is almost unique. It is unknown whether such reactions can be extrapolated usefully to any clinical situation, either concerning neoplasia or transplantation phenomena.

Pharmacological and toxicological studies with these alkaloids have not been published in detail. Vincaleukoblastine and leurocristine have been more extensively studied (36). Acute i.v. LD_{50}'s for VLB and leurocristine in mice are approximately 17.0 and 2.0 mg./Kg., respectively. Multiple doses of VLB caused marked leucopenia in rats and dogs at 0.50 and 0.1–0.2 mg./Kg., respectively, causing death in some instances. Leurocristine, leurosidine, and leurosine do not exert leucopenic effects in rats and dogs to this same degree. Transient rises in blood pressure of dogs receiving VLB or leurocristine have been observed. Leurocristine has also been observed to cause a slight paralysis of hind limbs in mice with a single i.v. administration of 2.0 mg./Kg. Other studies of possible neurological effects of these alkaloids are in progress.

Clinically, VLB has been more extensively studied than any of the other alkaloids. The

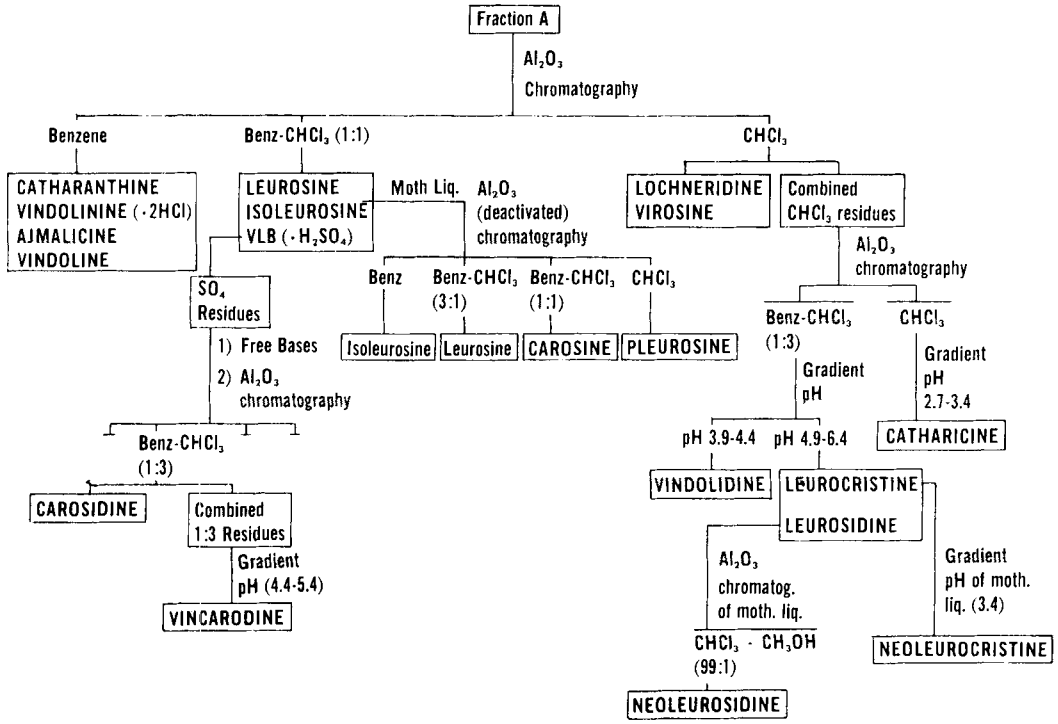


Fig. 2.—Flow scheme for alkaloids from fraction A.

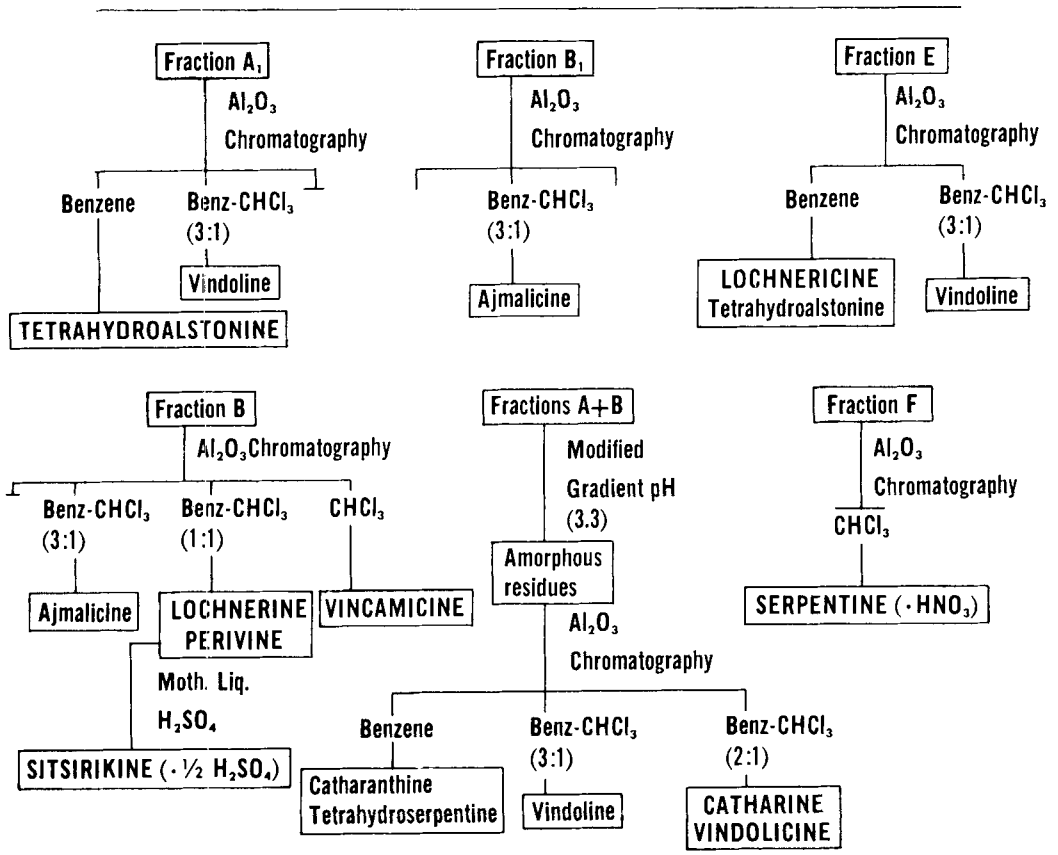


Fig. 3.—Flow schemes for alkaloids from fractions A_1, B_1, B, (A + B), E, and F.

TABLE III.—ACTIVITY OF ORIGINAL EXTRACT OF WHOLE PLANT AND CRUDE FRACTIONS AGAINST P-1534 LEUKEMIA

Material	Dosage, mg./Kg./day	Av. Wt. Change, Gm., T/C	Av. Survival Time, days, T/C	% Increase in Survival Time	% Indefinite Survival
Defatted whole plant	120.0	+0.1/+2.7	25.6/14.8	73	0
Fraction A	0.5	−0.6/+1.2	26.5/19.2	38	20
Fraction B	30.0	−1.2/+1.2	24.0/19.0	25	40
Total alkaloids	6.0	+0.8/+2.9	27.7/17.2	61	20
	7.5	+0.2/+0.6	29.8/13.4	122	0
	15.0	−2.3/+0.6	20.3/13.4	51	60
	15.0	+0.3/+0.5	30.0/13.0	130	0
	75 (Oral)	−1.5/+0.6	20.6/13.4	53	0

TABLE IV.—RESULTS OBTAINED WITH LEUROSINE AND VINCALEUKOBLASTINE TREATMENT OF THE P-1534 LEUKEMIA

Material	Dosage, mg./Kg./day	Av. Wt. Change, Gm., T/C	Av. Survival Time, days, T/C	% Increase in Survival Time
Leurosine	7.5	+0.2/+1.9	22.0/14.6	50
	3.0	+1.4/0.0	27.3/13.0	110
	6.0	+1.4/+1.3	26.4/17.2	53
	150.0 (Oral)	−1.2/+0.6	19.6/13.4	46
Vincaleukoblastine	0.05	+2.7/+0.7	23.0/16.2	41
	0.1	+1.7/+0.7	24.8/16.2	53
	0.3	+0.6/+0.9	22.8/13.4	70
	0.45	+0.3/+0.3	27.0/13.6	98
	0.45	+0.8/+0.6	30.6/13.2	131
	0.6	−1.7/−1.6	28.6/11.4	150
	0.6	−2.0/−1.6	26.0/11.4	128
	0.6	+0.3/−1.6	24.6/11.4	115
	1.5 (Oral)	−1.2/+0.7	27.6/16.2	70

most striking results of VLB therapy have been obtained in the treatment of Hodgkin's disease (37–41). Patients have been maintained in clinical remission for periods of over a year. Choriocarcinoma and carcinoma of the breast have also been treated effectively with this compound (42, 43). In all of these studies, beneficial results have been obtained even in tumors resistant to other commonly used chemotherapeutic agents.

A number of other types of tumors have also been observed to respond to VLB therapy. These include carcinomas of the bronchus and colon. Isolated instances of responses in seminoma, teratoblastoma, and melanoma have been reported.

Some investigators have obtained temporary remissions in various types of leukemia, including acute lymphocytic, acute monocytic, and myeloblastic. The remissions have not generally included complete bone marrow remissions. The results obtained with VLB in leukemias and tumors of the gastrointestinal and genitourinary tract do suggest the need for further evaluation in numbers of patients treated and types of treatment regimens.

Only the most preliminary clinical studies have been made with leurocristine. These reports suggest a striking percentage of complete hematological remissions in acute lymphocytic leukemia

TABLE V.—ANTI-P-1534 ACTIVITY OF AMORPHOUS FRACTIONS FREE OF VINCALEUKOBLASTINE AND LEUROSINE

mg./Kg.[a] XIX10	Toxic Deaths	Prolongation, %	Indefinite Survivors
3.0	0/5	?	5/5
3.0	0/5	?	5/5
3.0	2/5	?	3/5
0.6	2/5	181	0/5
18.0	2/5	147	0/5
4.5	0/5	151	1/5
9.0	1/5	147	2/5
9.0	0/5	174	1/5
6.0	0/5	123	4/5
20.0	0/5	148	1/5
30.0	0/5	238	4/5

[a] Intraperitoneally.

and responses in a spectrum of solid tumors (44–51). Proper placement of leurocristine in the clinical armamentarium against the oncological process must await further clinical study. At the present time there is no clinical information available concerning leurosine or leurosidine.

The mechanisms by which these agents exert antitumor effects are unknown. Tissue culture studies with VLB suggested an interference in metabolic pathways leading from glutamic acid to the citric acid cycle and to urea. Extension of these studies to experimental *in vivo* systems tend to confirm the *in vitro* results (15, 34, 52). Similar findings have also been made clinically (41, 53). Various abnormal mitotic figures including

TABLE VI.—ANTI-P-1534 ACTIVITY OF LEUROCRISTINE AND LEUROSIDINE

Material	mg./Kg.[a] XIX10	Toxic Deaths	Prolongation, %	Indefinite Survivors
Leurocristine	0.35	2/5	30	1/5
	0.30	2/5	?	3/5
	0.25	0/5	226	3/5
	0.20	1/5	110	2/5
	0.15	0/5	55	2/5
	0.12	0/5	49	0/5
	0.09	0/5	32	0/5
	0.06	0/5	24	0/5
Leurosidine	10.0	1/5	?	4/5
	7.5	0/5	?	5/5
	5.0	0/5	127	0/5
	4.0	0/5	75	0/5
	3.0	0/5	30	0/5
	2.0	0/5	21	0/5

[a] Intraperitoneally.

TABLE VII.—COMPARISON OF ANTITUMOR ACTIVITY OF FOUR "ACTIVE" ALKALOIDS

Tumor	Host	Alkaloids[a] A	B	C	D
Lilly mammary	DBA/1	+	...	...	+
Sarcoma 180	CAF[1]	...	...	...	...
Adenocarcinoma 755	C57B1/6	...	...	...	+
C-1498 Leukemia	C57B1/6	...	...	...	...
P-1534 Leukemia	DBA/2	+++	++	+++	+++
L-1210 Leukemia	DBA/2	...	...	...	...
Ridgeway osteogenic sarcoma	AKR	...	...	+++	+++
Mecca-lymphosarcoma	AKR	...	...	+	+
AKR Leukemia	AKR	...	+	...	++
Ehrlich ascites	Cox std.	+++	+++	...	...
Freund ascites	CAF[1]	+++	+++	++	++
S-180 Ascites	CAF[1]	+++	...	+	+++
B-82B Leukemia	C58	++	+	++	+++
Walker carcinosarcoma 256	Rat	+	+	+	...
Lilly mammary	C3H	...	...	...	...
Gardner lymphosarcoma	C3H	...	...	+	...
S-91 Melanoma	DBA/1	...	...	...	...
X-5563 Myeloma	C3H	N.D.	N.D.	++	+
High malignancy clone	C3H	N.D.	N.D.	...	...
Lilly rhabdomyosarcoma	Rat	+	...	...	..

[a] A, Vincaleukoblastine; B, leurosine; C, leurosidine; D, leurocristine. +, 30–50% Inhibition of solid tumors or prolongation of survival time in leukemias; ++, 50–100% inhibition of solid tumors or prolongation of leukemias; +++, 100% inhibition of solid tumors or >100% prolongation of leukemias; N.D., not done.

C-mitosis, ball metaphase, etc., have been obtained both *in vitro* and *in vivo* (15, 34, 52, 54). It seems unlikely that the therapeutic responses obtained are due to these cytological effects, since these are observed clinically and experimentally in tumors in the absence of any oncolytic effects.

Experimental studies in combination with other known cancer drugs, and the lack of cross resistance to them clinically, suggest mechanisms different than those of any such known compounds. In spite of the probable close structural relationships among these four alkaloids, there appears to be a lack of cross resistance and probable different mechanisms of action among them. These agents, regardless of mechanism, represent new and hitherto unknown leads towards effective chemotherapeutic treatment of a variety of human neoplasms.

CHEMISTRY

Prior to the discovery of the oncolytic properties of certain alkaloidal extracts prepared from the plant *Vinca rosea* Linn., a number of miscellaneous plant extracts had been reported to have some degree of antitumor activity (55). In several instances the pure chemical substance (s) had been isolated, *viz.*, podophyllin (56) and colchicine (57), and had been subjected to clinical testing.

Preliminary studies showed that the *Vinca rosea* Linn. alkaloids were not related to other naturally occurring oncolytic agents (12, 15) and that, by and large, they were indole and dihydroindole in character (58). Table I lists a number of alkaloids from this plant which had been described by earlier workers (14). They are all found in other genera and species of the *Apocynaceae*, and their structures are well

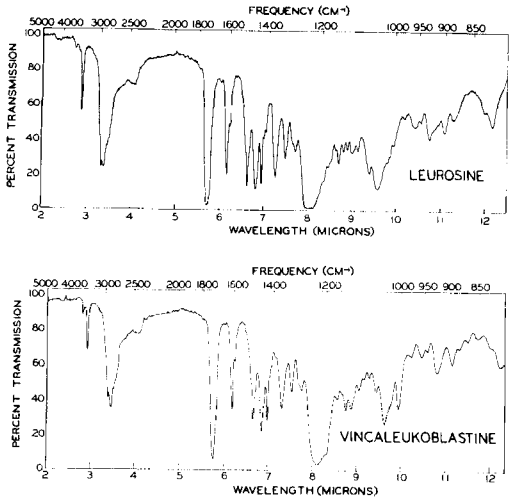


Fig. 4.—Infrared spectra of leurosine and vincaleukoblastine.

documented. The remaining alkaloids (Table II) were of unknown structure and it seemed pertinent, on the basis of the unique biological properties of certain of these alkaloids, to undertake a detailed study of their chemistry, with particular emphasis on leurocristine (16), VLB (59), leurosine (12), and leurosidine (16).

Investigation of the physical properties of leurosine and VLB (58) indicated that they were nonsymmetrical dimeric alkaloids. Titration showed the presence of two basic nitrogens, pK'a 5.5 and 7.0, one of which would quaternize when treated with methyl iodide or similar reagents. Analyses of the free bases, sulfates, dihydrochlorides, and quaternary salts indicated that they were $C_{46}H_{56-58}N_4O_9$ compounds (59). A study of the infrared spectra of the bases further supported this contention (thus the intensity of the indole N—H was approximately one-half that expected for a typical indolic C_{21} alkaloid) and indicated that VLB contained a hydroxyl group ($\lambda_{max.}^{CHCl_3}$ 2.8, 10.0 μ) that was not present in leurosine. Aside from this difference, the infrared spectra were very similar (Fig. 4) (58).

Comparison of these spectra with those of several other *Vinca rosea* alkaloids, notably catharanthine and vindoline (Table II), clearly indicated a close interrelationship between these compounds. When the infrared spectrum of a solution containing equimolar quantities of catharanthine and vindoline was compared to those of leurosine and VLB, it was virtually superimposable from 2–8 μ and quite similar up to 16 μ (Fig. 5). It was therefore assumed that the double molecules leurosine and VLB were composed of catharanthine and vindoline moieties, with minor molecular modification, bonded together in some unique manner (58). With this thought in mind, we turned our attention to the study of these two smaller molecules.

Catharanthine (I) was found to be a $C_{21}H_{24}N_2O_2$ compound and to form a methiodide readily. The physical characteristics of the quaternary salt showed that the basic nitrogen (pK'a 7.0) was analogous to the one forming the salt in the dimeric compounds. The ultraviolet spectrum was typical of an unsubstituted indole, while the infrared spectrum showed indole N—H (2.9 μ), carbomethoxyl moiety (5.78, 8.05 μ), and a triplet at 6.8 μ, characteristic of the Iboga-type alkaloids. The presence of a single double bond was seen from hydrogenation studies which yielded dihydrocatharanthine (II), thereby indicating that the parent base was pentacyclic (24).

Treatment of dihydrocatharanthine (II) under reflux with hydrazine in absolute ethanol, or saponification followed by brief acid treatment, resulted in decarboxylation in a manner analogous to that reported for voacangine (III) and related alkaloids (60, 61).

The new descarbomethoxy base, epi-ibogamine (IV), $C_{19}H_{24}N_2$, was further characterized as the hydrochloride $C_{19}H_{24}N_2 \cdot HCl$ (62). The analogous reaction on catharanthine (I) was unsuccessful; however, by the use of vigorous conditions (refluxing in concentrated hydrochloric acid) a poor yield (*ca.* 1%) of descarbomethoxycatharanthine (V) could be obtained. This compound was hydrogenated (platinum dioxide in alcohol) and afforded epi-ibogamine (IV). The failure of catharanthine to decarboxylate readily may be explained if the position of the double bond is as shown in (I). The corresponding intermediate for this type of reaction (60, 62) would require the highly strained structure shown in (VI).

The position of the carbomethoxyl at C-18 was also shown by the formation of the tetrahydro-1, 3-oxazine (VII) of catharanthinol (VIII), (the lithium aluminum hydride reduction product of I). This derivative was obtained by the treatment of catharanthinol with dry hydrogen chloride in acetone (63). As expected, the compound does not have any indole N—H or OH absorption in the infrared spectrum; $C_{23}H_{28}N_2O_2$ (62).

The relationship of catharanthine (I) and its derivatives to the corresponding Iboga alkaloids [*e.g.*, ibogamine (IX) and coronaridine (carbomethoxyibogamine) (X)] was indicated from the similarities of their infrared spectra and

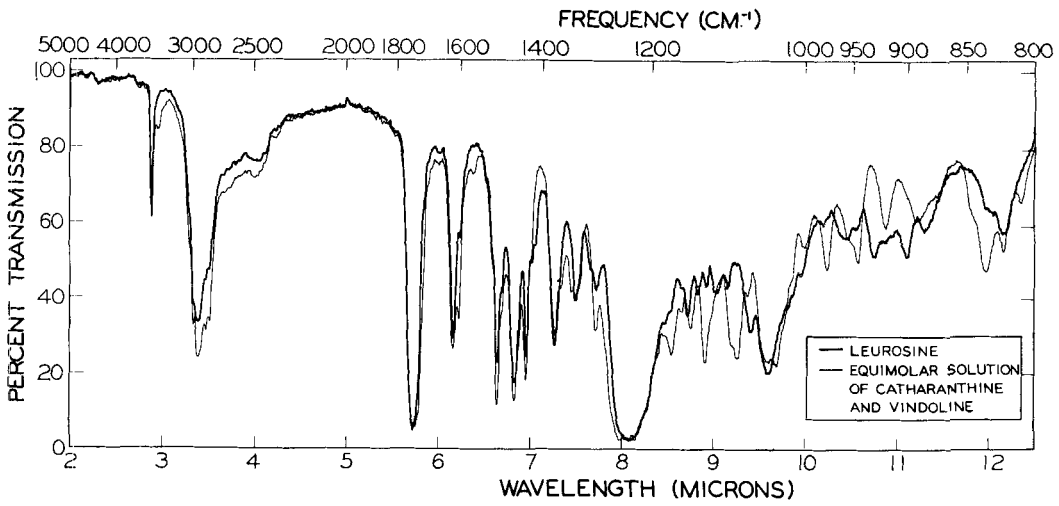
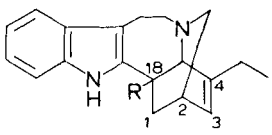
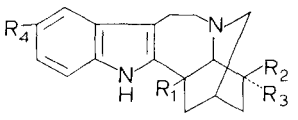


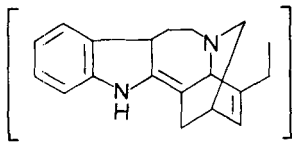
Fig. 5.—Infrared spectra of leurosine *vs.* an equimolar solution of catharanthine and vindoline.



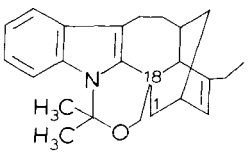


I R = $COOCH_3$
VIII R = CH_2OH
V R = H

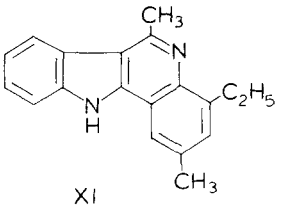
II R_1=$COOCH_3$; R_2=H; R_3=C_2H_5; R_4=H; Dihydrocatharanthine
III R_1=$COOCH_3$; R_2=C_2H_5; R_3=H; R_4=OCH_3; Voacangine
IV R_1=H; R_2=H; R_3=C_2H_5; R_4=H; Epi-ibogamine
IX R_1=H; R_2=C_2H_5; R_3=H; R_4=H; Ibogamine
X R_1=$COOCH_3$; R_2=C_2H_5; R_3=H; R_4=H; Coronaridine
XIII R_1=H; R_2=C_2H_5; R_3=H; R_4=OCH_3; Ibogaine



VI



VII



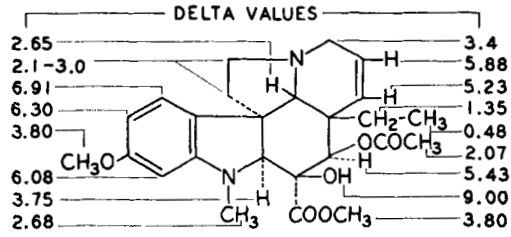
XI

finally proved by the isolation of the indoloquinoline (XI) (64) from the selenium dehydrogenation of epi-ibogamine (IV). This product (XI) was found to be identical in every respect (X-ray powder pattern, infrared, ultraviolet, and mass spectra) with an authentic sample obtained by dehydrogenation of ibogamine (IX) (64).

The position of the endocyclic double bond was indicated by the NMR spectrum of catharanthine (I) which has shown the presence of an ethyl group (methyl triplet centered at 1.10 δ with peaks separated by 7 c.p.s.) and a single proton on a double bond (peak at 5.90 δ). The position of the double bond was corroborated by products resulting from dehydrogenation of catharanthine (I) and dihydrocatharanthine (II), using palladium on carbon (62).

The former gave a good yield of 3-ethyl pyridine (XII) at 150–160°, while the latter required a temperature of 230–250° to afford 3-methyl-5-ethyl pyridine in a manner similar to that previously described for voacangine (III) (65). The formation of (XII) is explained by the fission of the C-1–C-2 allylic bond, while in dihydrocatharanthine (II) the bond breaks as in voacangine (III) between C-1 and C-18.

The hydrogenation of catharanthine led to



XIV

only one isomer (*vide supra*). From the models of (I), it is apparent that the hydrogen must come in from the side nearest to N_b to give the axial ethyl group shown in (II). Therefore, dihydrocatharanthine (II) and epi-ibogamine (IV) are epimeric at C-4 with coronaridine (X) and ibogamine (IX), respectively, since the C-ethyl group in the Iboga alkaloids, *e.g.*, ibogaine (XIII) has been shown to be equatorial (66), and catharanthine must be Δ^3-dehydrocoronaridine (62).

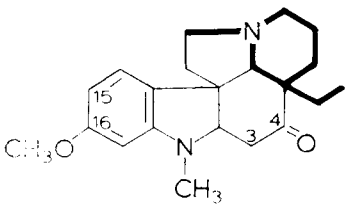
With this information in hand, attention was turned to the structure elucidation of vindoline. Vindoline (XIV), the most abundant alkaloid in the leaves, was found to be a $C_{25}H_{32}N_2O_6$ dihydroindole compound (24). Its basic nitrogen did not form a methiodide in analogy to the nitrogen pK'a 5.4 in the dimeric alkaloids. The nature of the six oxygens was determined by the formation of suitable derivatives. Thus, treatment of vindoline with acid (8 min. reflux—N_2) afforded desacetylvindoline (XV), $C_{23}H_{30}N_2O_5$, corresponding to the loss of an acetyl group. This was further corroborated in the infrared spectrum by the appearance of a hydroxyl band (2.7 μ) and corresponding reduction of intensity of the carbonyl band (24).

The presence of the methyl ester was shown by lithium aluminum hydride reduction of the base with simultaneous removal of the acetyl to yield vindolinol, $C_{22}H_{30}N_2O_4$ (24). The infrared spectrum of vindoline, in which a broad band at 3.5–4.0 μ was conspicuous, showed that the fifth oxygen was a hydrogen-bonded hydroxyl. Formation of the diacetate $C_{27}H_{34}N_2O_7$ (acetic anhydride in pyridine) was accompanied by the disappearance of this broad band in the infrared spectrum (24). From the study of the ultraviolet and infrared spectra, the sixth oxygen was shown to be present as an aromatic methoxyl on a dihydroindole chromophore. The confirmation of the assignment of the oxygen functional groups was obtained from the NMR spectra (XIV) (Fig. 7) (67). Vindoline consumed one mole of hydrogen at atmospheric pressure, showing it to be a pentacyclic compound and afforded dihydrovindoline (XVI) which could be converted to an amorphous hygroscopic hydrochloride. Pyrolysis of this salt at 195–200° in vacuum gave a distillate from which a $C_{21}H_{28}N_2O_2$ compound (XVII) was obtained in 15% overall yield by direct crystallization from hexane. The methoxydihydroindole portion of this compound was the same as in vindoline (XIV), and the second oxygen was found to be present as a ketonic carbonyl ($\lambda_{max.}^{CHCl_3}$ 5.85 μ) (67).

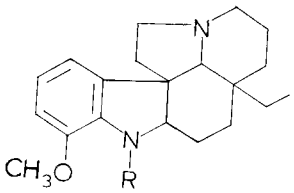
Careful comparison of the mass spectra of the pyrolysis ketone (XVII) and dihydrovindoline (XVI) with that of N-methyl desacetyl aspidospermine (XVIII) (68) indicated the presence of the latter ring system in vindoline and its derivatives, since in all three compounds identical intense peaks were found at m/e 124, 174, 188, and 298 (68).

The position of the carbonyl in (XVII) was assigned on the basis of the presence of the peak at m/e 298 (M-42) (XXII) which suggested that this group involved either C-3 or C-4. Position 4 was selected for two reasons: the typical ABX pattern at low field (3 to 3.5 δ) in the NMR spectrum (also consistent with structure XVII) indicated three protons between the carbonyl group and nitrogen; and equilibration with CH_3OD/methoxide resulted in the introduction of only two deuterium atoms per molecule (M = 342). The peak at m/e 124 (XIX) comprises the atoms outlined by heavy lines in structure (XVII) while the 174 (XX) and 188 (XXI) peaks contain the dihydroindole moiety (as indoles) with one or two carbons, respectively, of the "tryptophan" dimethylene bridge attached. The fragment of mass 298 (XXII) involves the loss of the two-carbon bridge (C-3 and C-4) containing the oxygen functions (except the aromatic methoxyl) of each derivative. The only other major peak in the ketone occurs at m/e 166 (XXIII) which shifts to m/e 168 in the deutero derivative. Structure (XXIII) is assigned to it since this peak corresponds to fragment (XXIV) of mass 152 (69) in the spectrum of aspidospermine (XXV). The fragmentation is more complex in dihydrovindoline, giving rise to a series of peaks at m/e 398, 311, 284, and 224 in addition to those already mentioned. The proposed structure for vindoline is consistent with the formation of these additional ions.

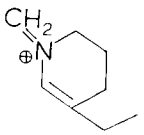
The position of the aromatic methoxyl was shown to be at either C-15 or C-16 by examination of the typical 1, 2, 4 aromatic proton pattern (*ortho* splitting J = 8 c.p.s., *meta* splitting J = 2.5 c.p.s.). The final selection of C-16 is based on the comparison of the infrared and ultraviolet spectra of vindoline with 6-methoxy-N-



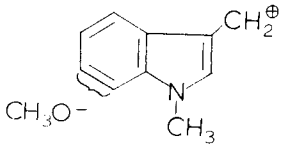
XVII



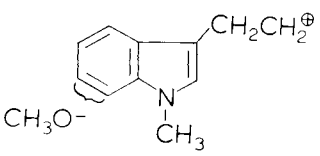
XVIII R = CH_3 O
XXV R = CH_3C–



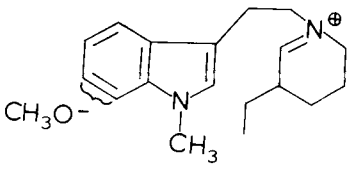
XIX



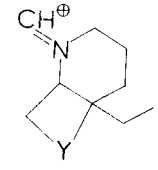
XX



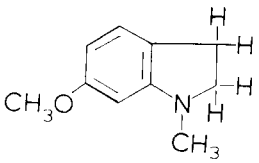
XXI



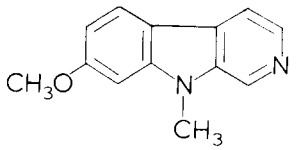
XXII



XXIII Y = C=O
XXIV Y = CH_2



XXVI

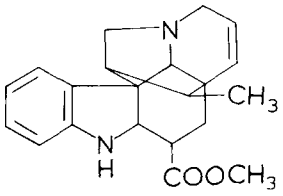


XXVII

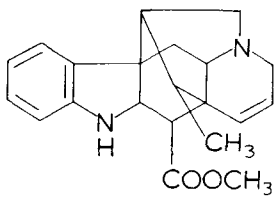
methyldihydroindole (XXVI) and by the isolation of *ind*-N-methylnorharmine (XXVII); m.p. 112–114°, hydrochloride m.p. 232–236°, molecular weight (M) 212 (by mass spectrometry), $\lambda_{max.}^{EtOH}$ 243 (37,500), 303 (15,100), 336 (4,300), from a soda lime distillation of vindoline at 325°. This derivative also indicates the position of the N-CH_3 as being on the anilino nitrogen. A second product (XVII) which was identical to the C_{21} ketone obtained above was also isolated from the soda lime reaction. Examination of the NMR spectrum of vindoline (XIV) (Fig. 7) allows the assignment of each proton as shown. The following assignments, however, deserve some comment. The unsplit peak of the proton on the carbon bearing the acetoxyl moiety shifts to 4.07 δ in desacetyl vindoline (XVIII). The protons of the methylene portion of the C-ethyl group are not equivalent and are found as a 12-band multiplet centered at 1.35 δ (J = 7.5 c.p.s.). The methyl triplet, however, is almost symmetrical in vindoline, being found at an exceptionally high field 0.48 δ (J = 7.5 c.p.s.) for a grouping of this type. This high field appears to be due to increased shielding by ring currents from the aromatic ring. The coupling constant of the two *cis* vinyl protons is J = 10 c.p.s., and one of these (5.88 δ) is further split by two non-equivalent adjacent protons (3.4 δ) with coupling constants J = 5 and 2 c.p.s. (67).

The mass spectral evidence, besides indicating the ring system of vindoline, shows that the oxygen functions other than the aromatic methoxyl can only be present at positions 3 and 4, and therefore must be arranged as shown in structure (XIV) to be consistent with NMR data. The formation of the ketone is also easily explained from this formulation (dehydration, hydrolysis, decarboxylation) (67). The stereochemistry of vindoline is as yet tentative and rests on evidence which will be presented elsewhere.

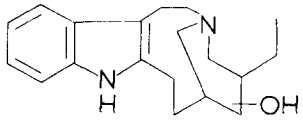
It is interesting to note that vindolinine (24, 70), another *Vinca* alkaloid with a slightly modified aspidospermine (XXV) skeleton, contains a double bond at the same position as vindoline (XIV). The structure of this compound is most probably best represented by (XXVIII) or (XXIX) and was arrived at by a joint effort of Djerassi's group at Stanford, Le Men and coworkers in Paris, and the writers at the Lilly Laboratories (71). The structural assignment of this compound is unique in that it is based largely on interpretation of mass spectrometric fragmentation patterns, combined with NMR measurements, without utilization of classical chemical degradations. In all, over 30 derivatives were studied, and these were obtained by the suitable use of acetylation, reduction, and acylation reactions (71).



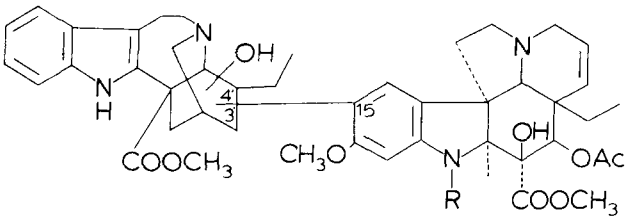
XXVIII



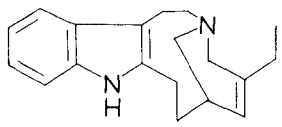
XXIX



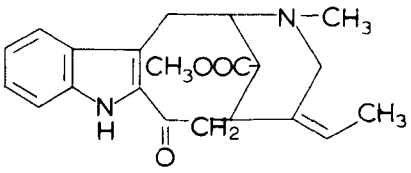
XXXIV



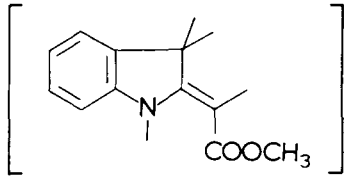
XXX R = CH_3
XXXIII R = CHO



XXXV



XXXVII



XXXVIII

As mentioned above, spectral evidence strongly suggested that the dimeric alkaloids were composed of a molecular combination of catharanthine (I) and vindoline (XIV). This assumption could be further substantiated by the products of acid cleavage (concentrated hydrochloric acid, stannous chloride, tin, reflux) carried out on the dimeric alkaloids. In each case there was obtained upon chromatography of the reaction mixture an indole compound (*vide infra*), followed by vindoline derivatives. Vincaleukoblastine (XXX) and leurosine (XXXI) afforded desacetylvindoline (XV), thus proving the identity of the dihydroindole portion of these alkaloids. The corresponding fraction from the cleavage of leurocristine yielded des-N(*a*)-methyldesacetylvindoline (XXXII), M = 400, $C_{22}H_{28}N_2O_5$. The mass spectrum, as well as ultraviolet and infrared spectra, demonstrate the relationship of this compound to desacetylvindoline. Both VLB (XXX) and leurocristine (XXXIII) yield the same tetracyclic indole derivative velbanamine (XXXIV), $C_{19}H_{26}N_2O$, the infrared spectrum of which clearly shows the oxygen to be present as a hydroxyl (72).

A related tetracyclic indole derivative, cleavamine (XXXV), $C_{19}H_{24}N_2$ was obtained from the cleavage of leurosine. (The correct formula of leurosine is still in doubt due to difficulty of preparing a solvent-free sample.) Isolation of cleavamine demonstrates the relationship of the indole portion of C_{46} alkaloids to catharanthine (I) since it was also obtained by a similar acid treatment of the latter. Cleavamine is believed to possess structure (XXXV) and velbanamine appears to be its dihydrohydroxy derivative (XXXIV). The mass spectra indicate the same carbon skeleton for the two compounds and are consistent with the proposed structures (72).

The cleavage reaction demonstrates that leurocristine (XXXIII) differs from VLB (XXX) only in the vindoline portion of the molecule. The nature of this difference was demonstrated in the following manner.

Analyses of leurocristine base (XXXIII), sulfate, and monomethiodide, are consistent with a formulation of $C_{46}H_{54}N_4O_{10}$. Its ultraviolet spectrum $\lambda_{max.}^{EtOH}$ 220 mμ (log a_M 4.65), 255 mμ (log a_M 4.21), 296 mμ (log a_M 4.18) and $\lambda_{max.}^{EtOH}$ 275 mμ (log a_M 4.02) is quite different from that of VLB (XXX) and also indicates a different substitution on $N_{(a)}$ of the dihydroindole moiety (16).

The infrared spectra of VLB and leurocristine (Fig. 6) are quite similar with the exception of the presence of a strong additional band, $\lambda_{max.}^{CHCl_3}$ 5.94 μ in the spectrum of the latter. The NMR spectra differ in that the N—CH_3 proton resonance at 2.73 δ present in VLB is missing in leurocristine; and, conversely, in place of only one low field, 9.8 δ proton in VLB, there are two in leurocristine at 9.5 and 8.9 δ.

Lithium aluminum hydride reduction of the two alkaloids afforded good yields of the same pentahydroxy derivative, $C_{42}H_{54}N_4O_6$ (XXXVI). This formulation is consistent with the reduction of two methyl esters, one acetate and an *N*-formyl group in the case of leurocristine. The NMR spectrum of (XXXVI) shows, accordingly, only two methyl signals, aromatic OCH_3 at 3.8 δ and N—CH_3 at 2.73 δ. The N—CHO of leurocristine is therefore at the anilino nitrogen in place of the N—CH_3 in VLB and further, since the indole cleavage products (velbanamine) are identical, then leurocristine is des-$N_{(a)}$-methyl $N_{(a)}$-formyl VLB (72).

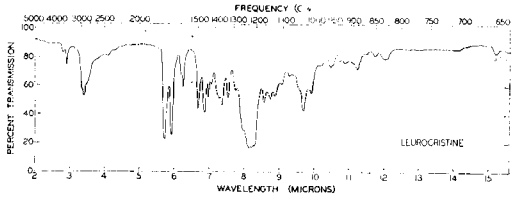


Fig. 6.—Infrared spectrum of leurocristine.

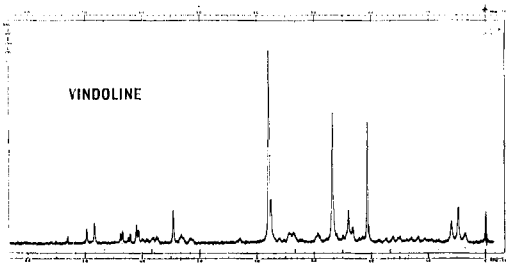


Fig. 7.—NMR spectrum of vindoline. Low field-OH not shown; peak at 7.32δ due to chloroform.

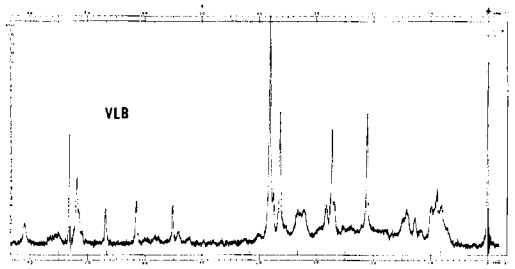


Fig. 8.—NMR spectrum of VLB. Low field-OH not shown; peak at 7.32δ due to chloroform.

The mode of attachment of the indole portion of the dimeric alkaloids to the dihydroindole fragment must be through the C-15 aromatic carbon since the NMR spectrum shows only two protons in a 1 : 4 relationship in the aromatic portion of the molecule rather than the 1, 2, 4 three-proton pattern present in vindoline (Figs. 7 and 8).

These data clearly indicate the partial structures (XXX) and (XXXIII) for VLB and leurocristine, respectively. On the basis of biogenetic considerations (free radical photosynthesis), as well as the mode of cleavage of these alkaloids, we are prompted to propose either C-3′ or C-4′ as the most likely site of attachment in the indole moiety and possible positions of the tertiary hydroxyl. The elucidation of this problem is at present in progress in these laboratories.

Little is known of the chemistry of the remaining alkaloids in Table II; however, they can be classified into three general groups. A number of the bases are closely related to the VLB-leurosine group of alkaloids (1, 27). The resemblance is seen in their infrared, ultraviolet, and NMR spectra, as well as from some results of acidic cleavage of several of these. Thus, leurosidine and isoleurosine both yield desacetylvindoline and an as yet unidentified indole derivative when treated as described above. The members of this group are listed in Table VIII.

The second group consists of monomeric alkaloids which range from C_{19} to C_{25} compounds (Table IX).

The infrared and ultraviolet spectra of perivine indicate that it is closely related to vobasine (XXXVII) (73), a 2-acyl indole alkaloid.

TABLE VIII.—VLB-LEUROSINE-LIKE ALKALOIDS

1. Vincaleukoblastine
2. Leurosine
3. Leurocristine
4. Leurosidine
5. Isoleurosine
6. Catharine
7. Carosine
8. Pleurosine
9. Catharicine
10. Neoleurocristine
11. Neoleurosidine

TABLE IX.—MONOMERIC ALKALOIDS

1. Perivine
2. Virosine
3. Catharanthine
4. Vindoline
5. Lochnericine
6. Vindolinine
7. Lochneridine
8. Sitsirikine

Perivine also contains an ethylidene group, N-methyl ester, and a 2-acyl indole chromophore.

Sitsirikine appears to be an as yet undescribed yohimbine isomer.

The remaining alkaloids are present in trace quantities and little is known about them. Lochnericine and lochneridine are related and possess the chromophore shown in (XXXVIII).

The third group consists of dimeric alkaloids which are not indole-indoline in nature (Table X).

TABLE X.—MISCELLANEOUS DIMERIC ALKALOIDS

1.	Vindolicine
2.	Vindolidine
3.	Vincamicine
4.	Vincarodine
5.	Carosidine

Of these only the first two are fairly well characterized, and they appear to be symmetrical dimers of vindoline.

Vincarodine appears to be related to vincine obtained from *V. minor* Linn. (74, 75).

Further work is planned in these laboratories toward structural elucidation of these alkaloids, with particular emphasis being placed on the interrelationships of the growing number of indole-indoline compounds.

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