THE ROLE OF THE ALKALOIDS OF CATHARANTHUS ROSEUS (L.) G.DON (VINCA ROSEA) AND THEIR DERIVATIVES IN CANCER CHEMOTHERAPY

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Abstract

The search for useful foodstuffs, dyes, medicinal agents, and other materials from plants, animals, or minerals is as old as civilization itself. It should not be surprising, therefore, to learn that all pharmacological prototypes originate from one of these three kingdoms.

The alkaloids, nitrogen-containing plant bases, notwithstanding the advent of antibiotics and many valuable synthetic drugs, constitute an indispensable part of our medicinal arsenal. The manufacture of alkaloids comprises an important segment of the fine chemicals industry. Their production is steeped in tradition, and processes are usually so highly specialized that it frequently takes years to establish them on an economic basis.

The author has based his pharmacognosy research on plants having reported folkloric usage and/or recorded chemical content. After conducting appropriate literature surveys, 440 plants were chosen for study. One of the plants collected and investigated was the Madagascar periwinkle, then botanically known by three names–Vinca **rosea,** *Catharanthus roseus,* and *Lochnera rosea.* This plant was reported to produce several desirable biological effects. Folkloric usage cited the plant as possessing properties that lowered blood sugar, thereby making it a possible source of an oral insulin substitute. It also was reported to contain alkaloids. Related *Vinca* species were reported to contain alkaloids and to possess both neurosedative properties and properties that lowered blood pressure.

Once obtained, extracts from *Catharanthus roseus* elicited none of the reported biological activities. (Eventually, pure compounds were isolated which did possess the reported properties.) Submission to a cancer screening program showed a profound and reproducible activity against an experimental leukemia model designed to predict clinical activity against human leukemia. One particular alkaloid, leurocristine (vincristine), was isolated. It was effective in treating not only acute lymphocytic leukemia of childhood but a wide variety of human neoplasms as well. It has become the "common denominator" in most combination therapies, serving as the synchronizing agent.

Classical methods of isolation and purification were of no value in obtaining the active antitumor agents from C. *roseus;* new techniques had to be devised. Use of selective/differential extraction, coupled with column chromatography and the gradient pH technique, has resulted in the reported isolation of 74 alkaloids from mature plants and an additional 21 chemical compounds from immature plants. This research and the results thereof constitute a classic in the annals of pharmacognosy/photochemistry. Collecting hundreds of thousands of kilograms of this plant material from the wild did not seem promising or reliable. As a result, the plant was cultivated on farms in India and Madagascar. Because a number of variables could seriously threaten the supply of this life-saving drug, the decision was made to attempt cultivation in the United States. This was achieved in Texas using modern growing and harvesting techniques.

To date, attempts for a total synthesis of the active antitumor alkaloids have not been successful. Synthetic modifications have produced active agents, none of which have yet replaced the parent compounds. The drug from C. *roseus* is one of many drugs obtained from renewable resources.

The important drug isolated from the Madagascar periwinkle is known scientifically as leurocristine, generically as vincristine, and is marketed by Eli Lilly & Co., the pharmaceutical house to which I assigned the patent, as ONCOVIN.

It had been an in-house concept that no anticancer agent would or could ever produce a profit for the marketing company. The reason was that cancer is a progressively fatal and emotional disease. (Diabetes can be placed in this category, yet the financial track record for insulin is substantial.) Pricing for the marketing of ONCOVIN was designed to recover research and development costs, not an unreasonable concept.

A company official was reported by William L. Laurence in *The New York Times* as saying that "it will market the periwinkle chemicals at a price calculated to yield no profit to the company. " Nevertheless, ONCOVIN became the highest percent profit item in the Lilly product line, carrying the relatively insignificant cost-of-sales of 12 per-

"Previously with Eli Lilly & Co.

cent. ONCOVIN provided an annual profit of several tens of millions of dollars.

It has been estimated that the world contains 500,000 to 750,000 higher order plants, less than 10 percent of which have been even cursorily investigated. The research success potential seems enormous. However, judicious plant selection must be coupled with appropriate biological test systems. In view of the not-for-profit pronouncements of the company known to have a successful phytochemical screening program, is it any wonder that other domestic firms hestitate to venture into this seemingly risky endeavor?

Private enterprise in the United States usually has been factorily innovative. When this occurs, intervention by government or others is unwarranted. However, government should expose and correct monumental profiteering. Also, when industry refuses to do the job, the government should present a choice: either do it with our cooperation, or we will do it without you. We now stand at this juncture.

Natural plant products have served as the basis of man's medicinal arsenal since time immemorial. The history of herbal medicine in the treatment of disease coincides with the history of medicine, and indeed with the history of civilization itself. Literature citations are recorded for more than 3,000 plant species that have been used or recommended in various parts of the world for the treatment of cancer (15).

It has been only within the last 20 years that any product from a higher order plant has been successfully used in cancer chemotherapy. Oddly enough, the plant yielding these agents was not included in the above-mentioned list of 3,000 plants. The isolation of two complex alkaloids—vincaleukoblastine (VLB) and leurocristine (LC) from the pantropical plant *Catharanthus roseus* (L,) G. Don *(Vinca rosea* L,) (Apocynaceae)—initiated a resurgence of interest in this area. The success of leurocristine, termed a "miracle drug" (38), in treating acute childhood lymphocytic leukemia and a wide variety of other human neoplasms is well documented.

The following discussion will be restricted primarily to *Catharanthus roseus* compounds possessing demonstrated utility in the treatment of human neoplasms, and in a few instances to compounds considered to have some potential utility on the basis of antitumor effects in experimental animals.

The true botanical name for the Madagascar periwinkle is *Catharanthus roseus* (L.) G. Don (33). It has also been referred to as *Lochnera rosea* Reichb, and *Vinca rosea* L. While the former name has no nomenclatural validity, the latter does, A text has been devoted to a study of this genus (39). It is important to recognize that *Catharanthus* alkaloids are different from *Vinca* alkaloids and ensure that information is recorded under the appropriate name.

The Madagascar periwinkle, C. *roseus,* originally a species endemic to Madagascar (Malagasy Republic), is an erect, everblooming herb or subshrub with either pink or white flowers that now has a tropical distribution. It is cultivated as an ornamental plant in gardens throughout the world.

Independent interest in this plant was generated by reports of hypoglycemic properties of certain extracts (14). The observation by Noble, Beer, and Cutts (29) of a toxic depletion of white cells and bone marrow depression produced in rats by certain fractions of these extracts eventually led to the isolation of vincaleukoblastine (VLB) sulfate. * Svoboda and Johnson, while screening selected botanicals for experimental antitumor activity, observed in certain extracts and fractions a reproducible oncolytic activity primarily against P1534 leukemia, a transplanted acute lymphocytic leukemia, in DBA/2 mice. This finding eventually led to the isolation of leurosine (35),** a new dimeric alkaloid closely related in chemical structure to VLB. Subsequently, VLB itself was isolated from the extracts (21).

Extraction of the Catharanthus Alkaloids

As with any single plant constituent, the extraction of an alkaloid is an individual problem. While several standard techniques existed for preliminary extraction of crude plant materials and subsequent separation and purification of individual alkaloids, none of these were applicable to the specific alkaloids of C. *roseus*. Consequently, a new technique of "selective" or "differential" extraction had to be devised (app. I) (36).

This technique differed from the classical approach in that a measure of purification was effected during extraction by forming salts of the stronger bases in the crude drug on addition of an aqueous solution of a naturally occurring weak organic acid. Final purification of most of the alkaloids was accomplished by column chromatography on Alcoa F-20 alumina which was partly deactivated with 10 percent acetic acid. This method-

*The United States Adopted Names Committee (USAN) has approved vinblastine as the generic name for this alkaloid. It is available as VELBAN, VELBE (vinblastine sulfate, Lilly).

* ● The USAN-approved generic name for this alkaloid is vinleurosine.

ology was responsible for the isolation of leurosine and VLB sulfate in a single purification step.

The major thrust of the Lilly investigation, however, centered around the early observation that certain fractions produced an unusually high percentage of laboratory cures. Neither leurosine nor VLB, nor any therapeutic combination thereof, was responsible for these cures. Eventually a gradient pH technique was devised which yielded leurocristine* and leurosidine, * * the two alkaloids responsible for the observed cures (35).

Use of selective or differential extraction, coupled with column chromatography and the gradient pH technique, has resulted in the reported isolation of 55 new alkaloids in our laboratories and three others which were codiscovered by other investigators. To date, 74 alkaloids, three of which we have never encountered, have been reported as having been isolated from mature plants of C. *roseus*. In addition, studies related *to* alkaloid biosynthesis involving immature plants have yielded known monomeric alkaloids, derivatives thereof, and glycosides. A total of 95 distinct alkaloidal entities have been isolated from this plant (app. II-VII).

One of the basic prerequisites for the successful isolation of the oncolytic alkaloids from C. *roseus* was the availability of a biological monitoring system—i.e., the P1534 leukemia. The use of this experimental mouse tumor as an in vivo assay was unique to the Lilly Research Laboratories and has demonstrated how important it is to use strain-specific tumors in inbred animals and to select tumors that are naturally resistant to most clinically useful agents. This screening procedure can be of great value in detecting new agents with different chemical structures. Just as a compound has a spectrum of tumor specificity, so a tumor maybe said to have a "spectrum of compound specificity. "

Structures and Pharmacologic Activities of Catharanthus Alkaloids

The determination and proof of the various indole and dihydroindole alkaloids structures proved to be an organic chemist's dream—virgin territory involving new entities and heretofore unknown combinations of ring systems, particularly those containing the dimeric indole-indoline structure. It would be remiss not to cite the imaginative and elegant efforts of German and Neuss in establishing the structures of VLB (app. VIII) and leurocristine (app. IX) *(26).* The eventual elucidation of the sterochemistry of these structures *[24)* has made it possible to follow a rational approach in studying structure-activity relationships in this series.

Leurosidine (app. X), the most experimentally active antitumor alkaloid of this group, is isomeric with VLB *(27).* The difference is in the indole ("upper") portion of the molecule; the hydroxy (OH) is attached to an adjacent carbon *(4 I).* The epoxide structure proposed for leurosine (app. XI) by Abraham and Farnsworth (1) has been accepted quite widely.

Yields of leurocristine are on the order of *0.0003* percent, the lowest yield of any medicinally useful alkaloid ever produced on a commercial basis. Because yields of VLB are considerably higher, it would be worthwhile to design an oxidative process to convert VLB into leurocristine. Although several laboratories have "proven" that this could not be done, the conversion was in fact accomplished by a method using chromic acid oxidation at *–60°* C *(22).*

While derivatives of alkaloids from higher order plants seldom, if ever, possess more therapeutic activity than the parent compound, any decrease in toxicity or side effects can be a valuable contribution. One compound, desacetyl VLB, 4-(N-N-dimethyl-aminoacetate), displayed excellent experimental antitumor activity and possessed far more benign side effects than those of VLB. Consequently, this compound was selected for clinical testing (5). While initial results seemed promising, two patients receiving prolonged therapy subsequently suffered from corneal and lens changes in the eye. Although a causal relationship was not definitely documented, clinical trial with this derivative was terminated *(18).*

N-Formyl leurosine (app. XII) has shown activity against a series of animal tumors. Some reversible white-cell suppression was observed in rats, but no signs of neurotoxicity were found in the limited data reported *(32).* The latest derivative to stimulate scientific interest in this area is desacetyl VLB amide *(Vindesine)** (app. XIII), a compound selected from a group of VLB derivatives prepared by Gerzon and coworkers (8).

Vindesine was selected because, in testing against tumor systems both sensitive and resistant to VLB and leurocristine, its spectrum of activity more closely resembled that of leurocristine than that of VLB *(37).* Its acute LD_{50} dose in mice, 6.3 mg/kg,

*The USAN-approved generic name for this alkaloid is vincristine. It is available as ONCOVIN (vincristine sulfate, Lilly).

•● The USAN-approved generic name for this alkaloid is vinrosidine.

*The USAN-approved generic name for this compound is vindesine.

is between those of VLB and leurocristine. No evidence of neurotoxicity has been observed in chronic studies in mice, rats, dogs, cats, and chickens (40).

It is a matter of record that minor molecular modifications in the basic dimeric moiety can produce dramatic differences in dose-limiting toxicity. Preliminary clinical studies validate this premise; vindesine's dose-limiting toxicity involves both bone marrow and neurological toxicity, thereby placing it between VLB and leurocristine.

The dimeric *Catharanthus* alkaloids represent a new class of oncolytic agents. Attempts have been made to study their effects on various biochemical reaction sequences to determine their mode of action. However, the mechanism of the action of these compounds is still not clearly defined or understood.

These alkaloids appear to affect cell division in various phases and to varying degrees. This phenomenon has been observed in both in vitro and in vivo studies *(30).* Such inhibition can be observed in the absence of therapeutic response. VLB and leurocristine seem to elicit similar responses, as do leurosine and leurosidine.

Studies at the cellular level indicate that many pharmacologic effects of the C. *roseus* alkaloids can be attributed to their completing with tubulin, the protein component of microtubules and the mitotic spindle. Substantial evidence indicates that microtubules are important for the substructure and probably the function of cell membranes. Of the agents studied, only these alkaloids produce microtubule crystals (7).

Data from various laboratories concerning the mechanism of action of the *Catharanthus* alkaloids appear to conflict in both the techniques and systems used (20,31). A definitive, concentrated effort at the molecular level, using drug-sensitive tumor systems, is clearly warranted.

The minor variation in the molecular structures of VLB and leurocristine has resulted in quite different spectra of tumor specificity in humans for these two alkaloids. In the relatively short time since their introduction into the clinic, the dimeric alkaloids from *Catharanthus roseus* have become some of the most valuable agents in cancer chemotherapy. They have proven to be useful as single agents in the palliative treatment of a number of advanced neoplasms, and more recently have played an important role in combination chemotherapy by heightening the action of other antitumor drugs.

The major use of VLB is in treating lymphoma, particularly in patients with Hodgkin's disease who are no longer candidates for high dose, extended field radiotherapy with curative intent. In single agent therapy, VLB rivals nitrogen mustard responses in *70* to *80* percent of patients.

VLB is highly effective in the treatment of testicular neoplasms. Objective responses also have been obtained, albeit with variable frequency, in patients with choriocarcinoma, neuroblastoma, Letterer-Siwe disease (histiocytosis X), and metastatic breast cancer,

The dose-limiting side effect of VLB is bone marrow depression. Temporary loss of hair and constipation are common.

As for vincristine, Taylor (38) says: "Judged by the usual yardstick of time for the development of new drugs and their clinical acceptance by physicians, vincristine qualifies as a miracle drug, for it was only 10 years ago that work was begun on this compound. Except for the increased activity in the field of cancer research, the work on vincristine was not abetted like the work on the miracle drug penicillin by a World War. In spite of this, vincristine is held in high regard by cancer chemotherapists, and much is known of its toxicology, pharmacology, and clinical activity in the human being. The worldwide search for plants containing substances that may inhibit cancer owes much to the incredibly successful story of vincristine. "

The most striking feature of leurocristine is its ability to induce complete bone marrow remission of 50 percent of children with acute lymphocytic leukemia. In combination with steroidal hormones, a 90-percent complete-response rate can be expected. It is also considered to be highly effective in the treatment of Hodgkin's disease, Wilm's tumor, and rhabdomyosarcoma, and is said to be somewhat effective in the treatment of choriocarcinoma, breast cancer, and primary brain tumors. Responses against carcinomas of the cervix, prostate, and kidney have also been reported (19). Despite their extremely great structural similarities, there appears to be no cross-resistance between VLB and leurocristine.

Because of its lack of white-cell suppression, leurocristine becomes an excellent candidate in combination cancer chemotherapy. Two to five drug combinations give higher response rates and more prolonged remissions than obtained from single-agent therapy. Furthermore, new clinical concepts involve synchronization of cells in metaphase and alteration of membrane transport by leurocristine as an approach to combination therapy.

The dose-limiting side effects of leurocristine are its neuromuscular manifestations. Prophylaxis of constipation is indicated. Temporary loss of hair

is more frequent than with VLB and is dependent on dosage and duration of treatment.

Children tolerate leurocristine therapy better than adults. Some treatment centers limit the adult dose to a maximum of 2 mg per injection. Toxicity is usually reversible, although its manifestations may persist for several months.

The clinical experience with leurosine, both as the sulfate *(2,13,23)* and the methiodide (16), has been more limited than with VLB and leurocristine. The methiodide was used in clinical trials before the sulfate because it had greater experimental activity against P1534 leukemia. However, little therapeutic activity was noted, while severe side effects outweighed the transient tumor shrinkage observed in two patients *(4).*

In the case of leurosine sulfate, clinical activity was evident but at a much lower level than that of VLB or leurocristine. Leukopenia was less pronounced than with VLB, but was the dose-limiting toxicity.

Noble and coworkers *(28)* observed in tissue culture studies that leurosine lysed malignant cells better than VLB and leurocristine when the alkaloids were added to the culture as sulfate salts in saline solution. However, the lytic activity of leurosine was inhibited completely in the presence of adult human plasma, but not fetal plasma. It was noted that the inhibitory factor of plasma resided in the globulins of the adult human plasma. Obviation of this protein inactivitation in human patients has not been accomplished and represents a real clinical challenge.

If the clinical predictivity of the P1534 in vivo system can be considered valid, leurosidine would be the most effective *Catharanthus* alkaloid in humans. However, its yield from the plant is lower than that of leurocristine. In addition, dose-response studies with P1534 leukemia in DBA/2 mice indicate that the optimum dose for complete cures in 100 percent of the mice is *30* times that of leurocristine, or *7.5* mg/kg compared to 0.25 mg/kg. Sufficient quantities of leurosidine have never been stockpiled to allow for a comprehensive clinical trial. Clinical evaluation had been initiated several years ago (3), but because supplies were limited was halted before conclusive efficacy was observed. Transient responses and the appearance of some apparent neuropathy were noted in patients receiving high doses of the drug. It cannot be considered as having been adequately evaluated by current standards. Sufficient quantities would have to be provided by an alkaloid modification program, which has yet to be accomplished.

N-Formyl-N-desmethyl leurosine would not have been chosen as a clinical candidate on the basis of experimental antitumor activity in screening programs used in the United States today. However, it has been reported as producing complete remissions in acute leukemia and partial remissions in malignant lymphomas, chronic lymphocytic leukemia, and multiple myeloma (10,11). These preliminary clinical studies reveal no neurotoxicity and indicate well-tolerated effects on hemopoietic tissues, except for transient effects on mature cells of the granulocytic series. Personal communications regarding its clinical efficacy indicate that cardiovascular complications will preclude its utility as a single agent entity.

Desacetyl VLB amide (Vindesine) has been in Phase I clinical trial. In one study involving *32* adults with far-advanced neoplasia, it produced partial remissions in two of nine patients with acute leukemia, one of four with squamous carcinoma, and one of four with renal cell carcinoma. No responses were seen in patients with malignant melanoma, colorectal carcinoma, or several other types of advanced solid tumors. Sixty percent of the patients in this study previously had received *Catharanthus* alkaloid therapy (6). In a second study, minor responses were seen in acute lymphocytic leukemia, acute myelogenous leukemia, hypernephroma, lymphoma, and Ewing's sarcoma. No signs of neurotoxicity were observed in children (9).

A recent Phase II single agent study achieved two complete remissions and four partial responses in 21 patients with advanced breast carcinoma (overall response rate of 29 percent). Results in a pilot study involving patients with advanced multiple myeloma resistant to alkylating agents appeared to be promising. Phase II studies in non-small lung cancer showed frequent major objective responses (17).

In the adult patient, vindesine has myelosuppressive activity resembling that of VLB. While the neurotoxicity produced by vindesine is similar to that of leurocristine, it is less severe and does not progress even with continued therapy. Pharmacological studies indicate that vindesine has a larger volume of distribution, longer serum half-life, and lower rate of renal clearance than VLB, all of which may account for the longer duration of marrow suppression induced by this drug (25).

Cancer chemotherapy has evolved over the past 20 years so that it now ranks with surgery and radiation as a palliative, and in many instances curative, mode of treating malignant disease. Much of the improving efficacy of chemotherapy is the

result of development of multiple drug regimens. Initially these drug combinations were derived empirically by selecting agents that had different mechanisms of action and showed as little additive toxicity as possible. This empirical approach has progressed in sophistication to the point where it is now even possible to use multidrug regimens that block different discrete segments of the cell cycle. The *Catharanthus roseus* alkaloids play an especially important role in these multidrug regimens. Now that chemotherapy is being developed to augment surgery on both a pre- and post-operative basis, the prognosis for many types of human malignancy appears increasingly bright.

Problems and Potentials

Many problems were encountered during the course of this research, and corresponding solutions were devised. A number have been described; others follow.

Supplying the hundreds of thousands of kilograms of raw material required per year could have become a monumental problem, Adequate supply of the natural plant material had to be maintained. Original work was performed on the whole plant. Determination that the desired alkaloids were found in the leaves allowed for stripping of the plant, thereby affording a healthier and more profuse regrowth. Collection from the wild eventually progressed to farm or plantation cultivation, allowing for greater control over growth.

In anticipation that politics would threaten what had been a reliable crude drug supply, experimental plantings were begun in the United States. New crop introduction is always risky. The economics involved are always of major concern. Hand collection and "native" wages are out of the question. Despite the risk, plantations were started in Texas; India and Madagascar had lost a viable cash crop. A forage harvester replaced hand labor and, a single planting was able to provide several harvests per season, surviving harvester-cutting with relatively rapid regrowth.

periwinkle cultivation represents a renewable resource, The chemical complexities of the antitumor alkaloids have to date defied practical total synthesis. When realized, it certainly will require the ready availability of petroleum-derived starting materials,

The American pharmaceutical industry was built on and sustained by natural products from the higher order (chlorophyll-containing) plants. It is an enigma that little work in this realm is being pursued by U.S. industry, particularly since approximately *25* percent of new and refilled prescriptions from community pharmacies contain plant products (12).

There is no dearth of plants to be collected and screened for specific or general biological activity. Of the Earth's 500,000 to 750,000 higher order plants, less than 10 percent have been investigated phytochemically. The investigator has an almost unlimited choice for selection.

Success potential should be extremely high. However, one must select plants judiciously and must have appropriate biological test systems available for monitoring isolation and purification progress. Plant selection can be based on reported folkloric usage, but said usage must be rational in both an investigative and practical sense.

The use of *Catharanthus roseus* as an oral insulin substitute previously has been cited. Reports of usage against hemorrhage, scurvy, as a mouthwash for toothache, and for healing and cleaning chronic wounds (36) did not stimulate either scientific or pratical adrenalin. And yet, these uses could well prove valuable.

Selection of plants to be tested based on reported chemical constituents may also be useful. The author selected alkaloids for research, not only because of familiarity therewith but also recognition that most medicinal agents of plant origin fall into this category.

A third, and very valid, category for selection is a plant which never before has been investigated. This obviously represents the bulk of selective material. Here again, prudence must be exercised. Botanical and chemotaxonomic relationships must be considered.

The fact that major U.S. pharmaceutical houses are no longer engaged in pharmacognostical/phytochemical research, particularly as it relates to palliative/curative measures against human cancer, may well stem from the erroneous concept that any product would be a "not-for-profit" item. It may also stem from ignorance on the part of research administrators who conceive, and most certainly maintain, that antibiotics will treat or cure all ills, mainly those designated as "profits."

The marketplace has been extremely kind to the periwinkle alkaloids, particularly leurocristine (vincristine, ONCOVIN). It has become the highest percent profit item in the product line of Eli Lilly & Co., bearing the almost insignificant cost-of-sales of 12 percent. Yearly sales running into tens of millions of dollars indicate the absolute success of this natural product, Perhaps public pronouncements

that the item was to be sold "at a price calculated to yield no profit to the company" misled other companies that were considering entering the field.

The greatest story **never** told could well be the search for an ethereal entity often referred to as "super leurocristine." This tentative title was bestowed upon it by virtue of experimental antileukemic activity. Typical leurocristine/leurosidine fractions exhibited activity on the order of three survivors at a dosage of 0.3 mg/kg. "Super" fraction activity was of the order of three survivors at 0.0009 mg/kg, representing a 300-percent increase in activity (or decrease in dosage).

Workup of "super" material failed to yield any new crystalline compounds. The press of readying leurocristine for clinical trial and subsequent marketing prevailed over investigative matters. Besides, equivalent material was being stockpiled for later investigation, thereby affording larger quantities for workup, Unbelievable as it seems now, and most certainly at the time, these stockpiled materials were discarded in an unsanctioned and misguided cleanup effort.

Because of processing changes which were constantly being initiated, no such "super" material could again be stockpiled. Efforts to repeat the original process for stockpiling purposes were denied, as certain cost projections for "experimental processing" were prohibitive. If the problem of human cancer were solved, the search for "super leurocristine" would probably be a matter of scientific semantics, but the problem has not been solved, and the challenge must be met.

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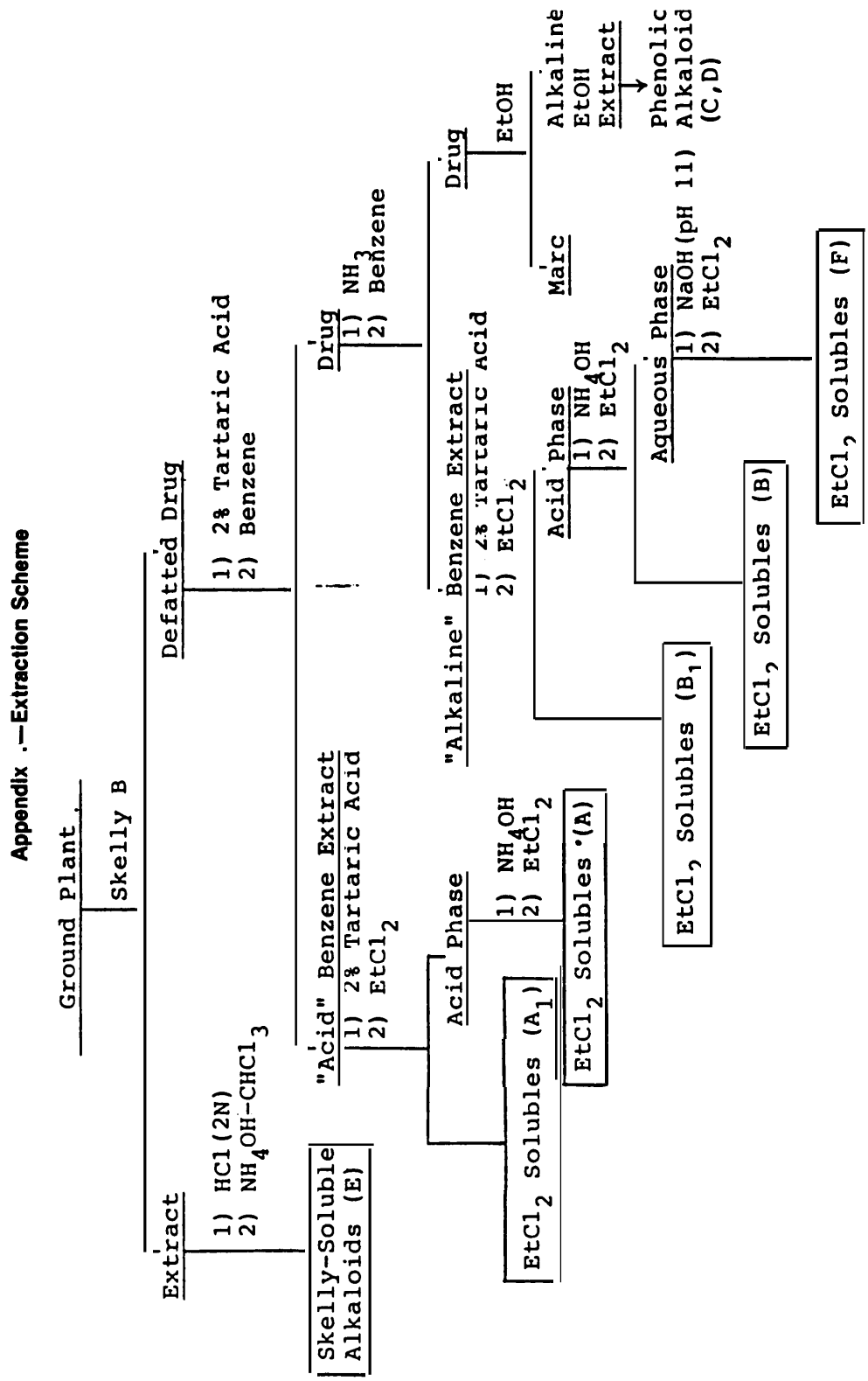
Appendix .—Extraction Scheme



Appendix II.—Alkaloids Previously Reported

Name	Empirical formula	M.P., °C
Ajmalicine	$C_{21}H_{24}N_2O_3$	253-254
Tetrahydroalstonine	$C_{21}H_{24}N_2O_3$	230-231
Serpentine	$C_{21}H_{22}N_2O_3$	156-157
Lochnerine	$C_{20}H_{24}N_2O_2$	202-203
Akuammine[a]	$C_{22}H_{26}N_2O_4$	258-260
Reserpine[a]	$C_{33}H_{40}N_2O_9$	264-265

Appendix III.—Monomeric Alkaloids

	Formula	pK'_a	M.P. °C	Source[b]
Indoles				
1. Alstonine[a] (·HCl)	$C_{21}H_{20}N_2O_3 \cdot HCl$	--	281-282	Rb
2. Ammorosine		7.30	221-225	R
3. Catharanthine	$C_{21}H_{24}N_2O_2 \cdot H_2O$	6.8	126-128	L , R
4. Cathindine (·½H_2SO_4)	----	7.25	239-245 (dec.)	R
5. Cavincidine (·½H_2SO_4)	----	7.85	236-239 (dec.)	R
6. Cavincine (·½H_2SO_4)	$C_{20}H_{24}N_2O_2 \cdot ½H_2SO_4 \cdot ½H_2O$	6.90	275-277 (dec.)	L , R
7. Dihydrositsirikine	$C_{21}H_{28}N_2O_3$	--	215	L , R
8. Isositsirikine (·½H_2SO_4)	$C_{21}H_{26}N_2O_3 \cdot ½H_2SO_4$	--	263.5	L , R
9. Pericyclivine	$C_{20}H_{22}N_2O_2$	--	228	L
10. Sitsirikine (·½H_2SO_4)	$C_{21}H_{26}N_2O_3 \cdot ½H_2SO_4$	7.6	239-241 dec.)	L , R
11. Vinaspine	----	7.85	235-238	L
2-Acyl Indoles				
1. Perividine	$C_{20}H_{22}N_2O_4$	neutral	271-279 (dec.)	L
2. Perivine	$C_{20}H_{24}N_2O_3$	7.5	180-181	L , R
3. Perosine (·½H_2SO_4	----	7.60	219-225	L , R
Oxindoles				
1. Mitraphylline	$C_{21}H_{26}N_2O_4$	6.20	269-270	L , R

Appendix IV.—Monomeric Alkaloids

	Formula	pK'_a	M.P., °C	Source[a]
a-Methylene Indolines				
1. Akuammicine	$C_{20}H_{22}N_2O_2$	7.98	181-182	R
2. Lochnericine	$C_{21}H_{24}N_2O_3$	4.2	190-193 (dec.)	L
3. Lochneridine	$C_{20}H_{24}N_2O_3$	5.5	211-214 (dec.)	L
4. Lochnerinine	$C_{22}H_{26}N_2O_4$	----	168-169	L
5. Lochnerivine	$C_{24}H_{28}N_2O_5$	neutral	278-280	R
6. Lochrovicine	$C_{20}H_{22}N_2O_3$	4.50	234-238	L
7. Lochrovidine	$C_{22}H_{26}N_2O_4$	5.60	213-218	L
8. Lochrovine	$C_{23}H_{30}N_2O_3$	neutral	258-263	L
Dihydroindoles				
1. Catharosine	$C_{22}H_{28}N_2O_4$	----	141-143	L
2. Desacetylvindoline	$C_{23}H_{30}N_2O_5$	----	163-165	L
3. Maandrosine ($\cdot \frac{1}{2}H_2SO_4$	----	6.90	160-173	R
4. Vincolidine	$C_{23}H_{26}N_2O_3$	5.45	165-170	L
5. Vincoline	$C_{21}H_{24}N_2O_4$	6.1	230-233	L
6. Vindoline	$C_{25}H_{32}N_2O_6$	5.5	154-155	L
7. Vindolinine 2HCl	$C_{21}H_{24}N_2O_2 \cdot 2HCl$	7.1	210-213 (dec.)	L
8. Vindorosine	$C_{24}H_{30}N_2O_5$	----	167	L
Miscellaneous				
1. Ammocalline	$C_{19}H_{22}N_2$	7.30	> 335 (dec.)	R
2. Pericalline (Tabernoschizine) (Apparicine) (Gomezine)	$C_{18}H_{20}N_2$	8.05	196-202	R
3. Perimivine	$C_{21}H_{22}N_2O_4$	indeterminate	292-293 (dec.)	L
4. Virosine	$C_{22}H_{26}N_2O_4$	5.85	258-261 (dec.)	R

Appendix V.—Dimeric Indole-Indoline Alkaloids

		Formula	pK_a	M.P., °C	Source[a]
1.	Carosine	$C_{46}H_{56}N_4O_{10}$	4.4, 5.5	214-218	L
2.	Catharanthamine	$C_{46}H_{56}N_4O_9$	-	-	L
3.	Catharicine	$C_{46}H_{52}N_4O_{10}\cdot CH_3OH$	5.3, 6.3	231-234 (dec.)	L
4.	Catharine	$C_{46}H_{52}N_4O_9$	5.34	271-275 (dec.)	L
5.	N-Demethyl VLB	$C_{45}H_{56}N_4O_9$	-	-	L
6.	Desacetoxy VLB	$C_{44}H_{56}N_4O_7$	-	-	L
7.	Desacetyl VLB ($\cdot H_2SO_4$	$C_{44}H_{56}N_4O_8\cdot H_2SO_4$	5.40, 6.90	>320 (dec.)	L
8.	Isoleurosine	$C_{46}H_{58}N_4O_8$	4.8, 7.3	202-206 (dec.)	L
9.	Leurocolombine	$C_{46}H_{58}N_4O_{10}$	5.05, 6.3	-	L
10.	Leurocristine	$C_{46}H_{56}N_4O_{10}$	5.0, 7.4	218-220 (dec.)	L , R
11.	Leurosidine	$C_{46}H_{58}N_4O_9$	5.0, 8.8	208-211 (dec.)	L , R
12.	Leurosidine N_b -oxide	$C_{46}H_{58}N_4O_{10}$		215-218	L
13.	Leurosine	$C_{46}H_{56}N_4O_9$	5.5, 7.5	202-205 (dec.)	L , R
14.	Leurosivine ($\cdot H_2SO_4$)	$C_{41}H_{54}N_3O_9\cdot H_2SO_4$	4.80, 5.80	>335 (dec.)	R
15.	Neoleurocristine	$C_{46}H_{56}N_4O_{12}$	4.68	188-196 (dec.)	L
16.	Neoleurosidine	$C_{48}H_{62}N_4O_{11}$	5.1	219-225 (dec.)	L
17.	21^1-Oxoleurosine	$C_{46}H_{54}N_4O_{10}$	-	215	L
18.	Pleurosine	$C_{46}H_{56}N_4O_{10}$	4.4, 5.55	191-194 (dec.)	L
19.	Pseudovincaleukoblastine-diol	$C_{46}H_{56}N_4O_8$	-	-	L
20.	Rovidine ($\cdot H_2SO_4$)	$C_{46}H_{56}N_4O_{10}$	4.82, 6.95	>320 (dec.)	L
21.	Vinamidine	$C_{46}H_{56}N_4O_{10}$	-	-	L
22.	Vinaphamine	$C_{46}H_{56}N_4O_9$	5.15, 7.0	229-235	L
23.	Vincadioline	$C_{46}H_{58}N_4O_{10}$	-	-	L
24.	Vincaleukoblastine	$C_{46}H_{58}N_4O_9\cdot(C_2H_5)_2O$	5.4, 7.4	201-211	L , R
25.	Vincathicine ($\cdot H_2SO_4$)	$C_{46}H_{58}N_4O_9\cdot(C_2H_5)_2O$	5.10, 7.05	> 320 (dec.)	L

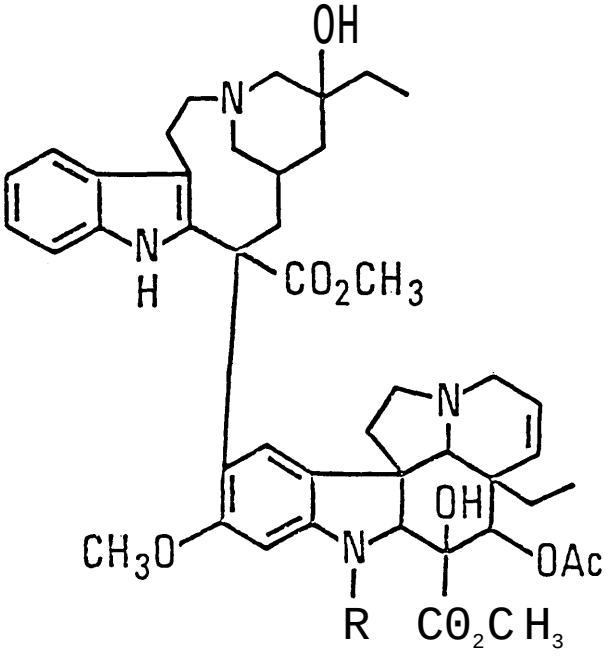
Appendix VI.—Miscellaneous Dimeric Alkaloids

	Formula	pK'_a	M.P., °C	Source[a]
1. Carosidine	----	indeterminate	263-278, 283 (dec.)	L R
2. Vincamic ne	⊢⁼⊢⊢	4.80, 5.85	224-228 (dec.	L
3. Vincarodine	$C_{44}H_{52}N_4O_{10}$	5.8	253-256 (dec.)	L
4. Vindolicine	$C_{25}H_{22}N_2O_6$ 2	5.4	248-251 (melts, recryst.)	L
			265-267 (dec.)	
5. Vindolidine	$C_{48}H_{64}N_4O_{10}$	4.7, 5.3	244-250 dec.	L
6. Vinosidine	$C_{44}H_{52}N_4O_{10}$	6.80	253-257 (dec.	R
7. Vinsedicine	Mol. wt. 780	4.45, 7.35	206	S
8. Vinsedine	Mol. wt. 778	4.65, 7.0	198-200	S

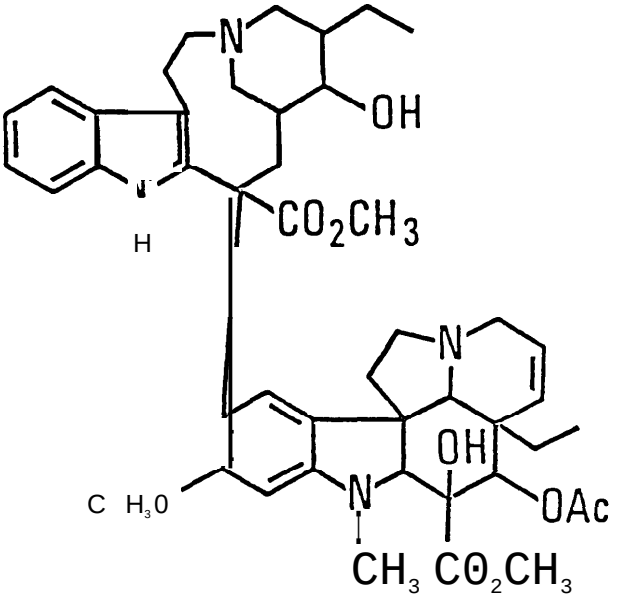
Appendix Vii.—Alkaloids isolated From C. *roseus* From Biosynthesis Experiments

Alkaloid
19-Acetoxy 11 hydroxy tabersonine
19-Acetoxy 11 methoxy tabersonine
N-Acetylvincoside
Ajmalicine
Akuammicine
Catharanthine
Cathenamine
Coronaridine
Corynantheine
Corynantheine Aldehyde
4,21-Dehydrogeissoschizine
19-Epi-ajmalicine
19-Epi-vindolinine
Gelssoschizine
Horhammericine
Horhammerinine
Isovincoside
11-Methoxytabersonine
Preakuammicine
Stemmadenine
Strictosidine lactam
Tabersonine
Vallesiachotamine
Vincoside
Vindoline

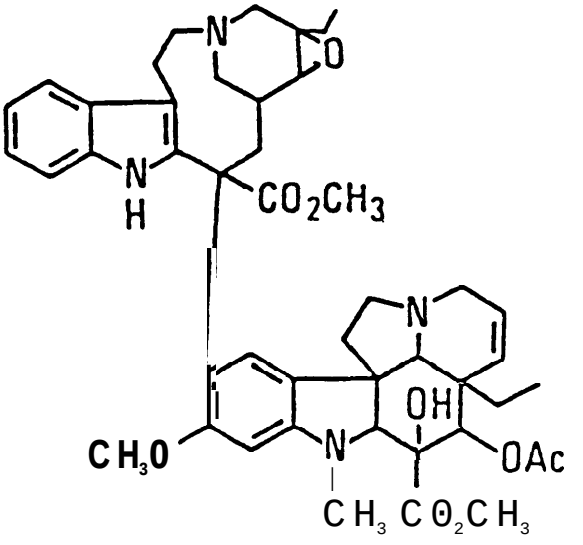
Appendix Viii



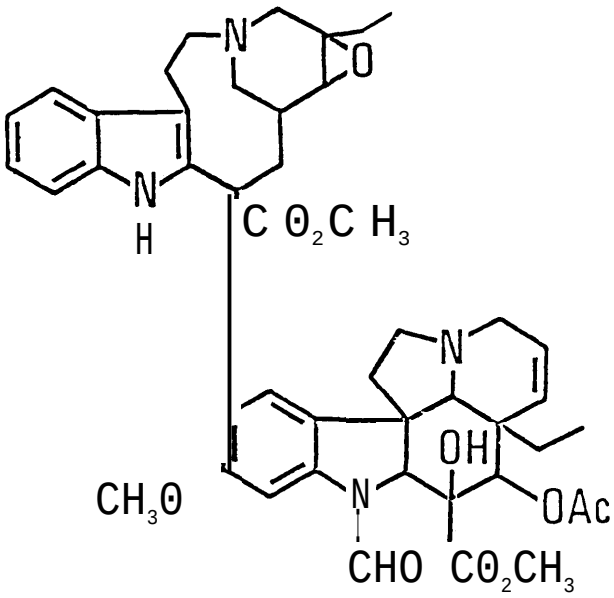
Appendix IX



Appendix X



Appendix XI



Appendix XII