A REVIEW OF SELECTED POTENTIAL ANTICANCER PLANT PRINCIPLES[1]

by

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ABSTRACT: A review of some recent work on anticancer compounds from plants is presented.

Our laboratory has been engaged in studies designed to isolate, identify, and/or determine the structure of biologically active plant constituents for several years. More recently, our major effort has been concerned with plants having confirmed *in vivo* and/or *in vitro* (cytotoxic) anticancer activity. During this period, we have discovered a broad range of active compounds, ranging from the complex dimeric indole alkaloids represented by leurosine, to the simplest active natural quinoid, jacaranone. Other types of active compounds include monomeric indole alkaloids, benzophenanthridine, and phenanthroquinolizidine alkaloids, triterpene saponins and lignans. Leurosine, the most active member of the group, has been evaluated clinically, mainly in France, whereas fagaronine and jacaranone appear to have sufficient activity to be considered as candidates for preclinical pharmacology.

In this brief review, we would like to summarize our data on active cytotoxic and antitumor compounds over the past decade.

THE *CATHARANTHUS* ALKALOIDS.

Probably no other single event gave a greater stimulation to the iso-

[1] Dedicated to Professor T. Takemoto, Pharmaceutical Institute, Tohoku University on the occasion of his retirement.

lation of anticancer principles from plants than the discovery of the potent antitumor alkaloids vincaleukoblastine (VLB) (I) and leurocristine (VCR) (II).

At this time it is relevant to recall some of these early results and to review the current status of some of the *Catharanthus* alkaloids.

The original interest in *Catharanthus roseus* arose because of a reputed use as an oral hypoglycemic agent (1). A large number of laboratory studies however, have almost invariably given negative results. Noble, Beer and Cutts (2) who were investigating the hypoglycemic activity found that extracts of *C. roseus* caused a rapid onset of peripheral granulocytopenia and bone marrow depression in rats. A chance examination of the white blood cell count revealed a dramatic reduction in leucocytes. This activity was traced to the alkaloid fraction and chromatography, with concomitant bioassay, eventually gave a small quantity of vincaleukoblastine as the sulfate (3).

Independently, at the Lilly Research Laboratories, Indianapolis, a group headed by Svoboda observed that a defatted ethanolic extract of *C. roseus* produced a dramatic and reproducible prolongation of life for DBA/2 mice infected with the P-1534 lymphocytic leukemia. Using this new *in vivo* system as a monitor, initially led to the isolation of leurosine and vincaleukoblastine (VLB). However, laboratory "cures" were observed in fractions devoid of these two compounds. As a result of this work, the dimeric antitumor alkaloids leurocristine and leurosidine were obtained (4) by the pH gradient technique.

These early experiments have been reviewed by Svoboda (5). It was these early results which resulted in a vertable army of researchers who assaulted the genus *Catharanthus* in search of additional antitumor alkaloids.

This work continues in a number of laboratories today.

Over 80 alkaloids have been isolated from *C. roseus* alone, and of these, six have demonstrated antitumor activity, i.e. leurocristine, leurosine, vincaleukoblastine, leurosidine. leurosidine, leurosivine and rovidine (5).

Vincristine (VCR) is currently used either singly, in combination or in combined modality trials against the following solid tumors, large bowel cancer, stomach cancer, breast cancer and sarcomas, but has had its greatest success in the treatment of acute leukemia in children. Vincaleukoblastine, having a more restricted application as an antitumor agent, is used primarily for methoxtrxate - resistant Hodgkin's disease and Choriocarcinoma (6).

The occurrence and availability of a number of other *Catharanthus* species led us to search for better sources of the active dimeric alkaloids and for related compounds with lower toxicity.

The first species that we examined was *Catharanthus lanceus*, indigenous to Madagascar (7). Preliminary studies indicated that the roots of *C. lanceus* elicited a pronounced inhibitory effect against the RC mammary carcinoma in mice. Subsequently, it was found that the benzene-soluble alkaloid fraction (A) from the leaves of this plant, although inactive on replicate testing against the P-1534 leukemia, afforded leurosine (8,9), a compound highly active against this system (10,11). A similar observation was made when leurosine was isolated from the leaf (C) alkaloid fraction of *C. lanceus* (12).

A summary of testing some of the *C. lanceus* alkaloids for antitumor and cytotoxic activity is available (13).

Another member of the *Catharanthus* genus which has been investigated in our laboratories is *C. pusillus*. From this plant two active principles

were obtained, lochnerinine (III) (14) and leurosine (15).

Potier and co-workers have shown that leurosine and VLB are also present in *C. ovalis* (15). Leurosine therefore appears to be the most widespread of the dimeric antitumor indole alkaloids from *Catharanthus*.

Leurosine was first isolated by Svoboda from *C. roseus* (17), and the molecular formula was suggested to be $C_{46}H_{58}O_9N_4$ by a variety of methods (18). Leurosine and VLB exhibited virtually superimposable IR spectra (18,19), thus confirming their close similarity. The molecular formula, however, was regarded as doubtful because of the tenacious inclusion of many solvents.

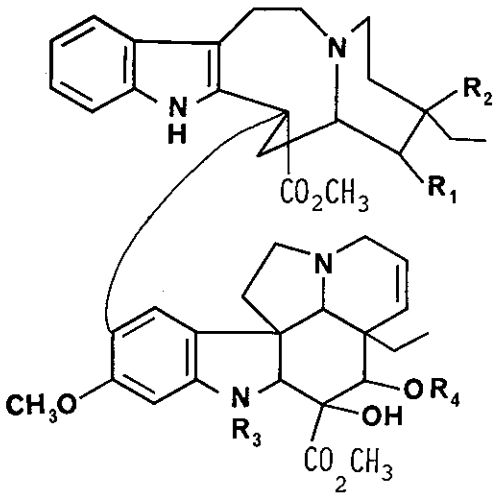
It was the structure elucidation of catharanthine and vindoline which permitted a suggestion (19) that leurosine and VLB were derived from these two monomeric alkaloids. Also, as a result of chemical evidence, both leurosine and VLB were shown to contain a vindoline "half", and from leurosine, the compound cleavamine (IV) was obtained (20), and the molecular formula of leurosine was corrected to $C_{46}H_{56}O_9N_4$. Subsequently, this molecular formula was confirmed from the examination of several derivatives (21).

In the nmr spectrum of leurosine the aromatic region was very similar to that of VLB, thereby indicating the mode of attachment to the vindoline moiety.

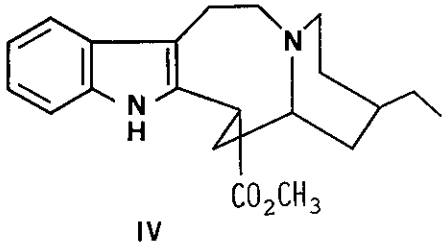
The isolation of cleavamine (IV) indicated that the additional oxygen function must have been in this "half" of the molecule, and by the molecular formula this must have been in the form of an oxide.

The correct structure of leurosine was determined by a careful examination of the high resolution mass spectrum (22).

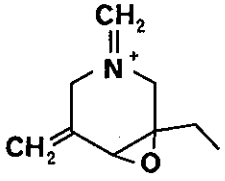
Given that one "half" of leurosine is vindoline and the other "half" an oxide of cleavamine, three series of ions should be observed; one each for the fragmentation of vindoline and cleavamine oxide, and one for the fragmentation of the dimeric species. In particular, the major fragment ions of



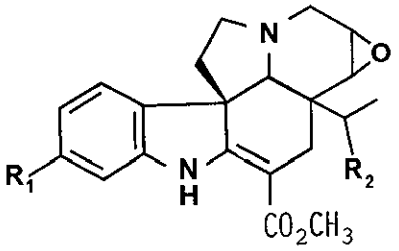
	R_1	R_2	R_3	R_4
I	H	OH	CH_3	Ac
II	H	OH	CHO	Ac
VI	-O-		CH_3	Ac
XVIII	H	OH	CH_3	H



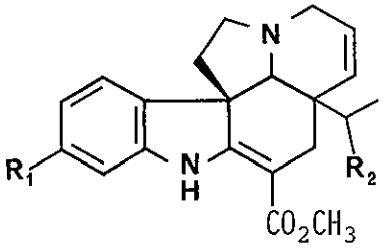
IV



V



	R_1	R_2
III	OCH_3	H
VII	H	OH
VIII	OCH_3	H
IX	H	H



	R_1	R
XI	H	=O
X	H	OH

vindoline and the cleavamine part could be added together (23) to arithmetically derive the third series of ions, all of which were found and analyzed (for their molecular formula). An important fragment ion at *m/e* 152 could, on the basis of a McLafferty-type rearrangement of the cleavamine-type alkaloids (24), be ascribed to the ion (V), $C_9H_{14}NO$, and this was confirmed by mass measurement.

A study of the nmr spectrum indicated the presence of a doublet (J = 4.1 Hz) at δ3.1 ppm in agreement with the formulation of an epoxide (22). From the magnitude of the coupling constant the stereochemistry shown could be deduced for the epoxide group. This evidence supported the structure (VI) for leurosine. This structure (VI) has recently been confirmed by CMR studies (25), but X-ray analysis of leurosine has not yet met with success (26).

Several reviews of the structure elucidation and chemistry of the dimeric *Catharanthus* alkaloids are available (26,27).

The recent completion of three independent syntheses of VLB (28-30) has stimulated research in this area, again in the effort to isolate additional active dimers which might be amenable to partial synthesis.

With the development of VLB and VCR as clinical entities it became necessary to develop a rapid, quantitative analytical technique for the estimation of these alkaloids in the dried plant material. The technique evolved (31) uses a small scale modification of the pH gradient technique (32) extracting at pH 4.0 and pH 6.1 for the total VLB content. The VLB is then separated from the remaining alkaloids by two-dimensional preparative thin-layer chromatography, and quantified by uv spectroscopy.

Prior to this study a simple thin layer chromatographic system was developed to separate VLB, VCR, leurosine and leurosidine (33).

A number of alkaloids isolated from *Catharanthus* spp. exhibit cytotoxic

activity, but are inactive in antitumor tests. All of these are monomeric and each belongs to the β-anilinoacrylate group of compounds. Two of these alkaloids were isolated from *Catharanthus lanceus*. The first of these was lochnerinine (III), which was identified (34) by its melting point uv and mass spectrum in comparison with literature (35,36) data. Subsequently, lochnerinine (III) was isolated from *Catharanthus pusillus* (14) and shown to be cytotoxic (ED_{50} 1.0×10^{-2} μg/ml) against the 9KB cell culture system.

Two new alkaloids of this general type were isolated from *C. lanceus* (37, 38). Each compound showed ir and uv spectral data in agreement with a β-anilinoacrylate structure. A characteristic chromogenic response with CAS of this chromophore, was noted, i.e. blue, changing to pale green for both compounds after 24 hours. The differences between these two compounds in the uv spectrum indicated that one was the demethoxy base of the other.

The structures of these two compounds were readily deduced (39) from an examination of the mass spectra. Both horhammericine and horhammerinine showed a typical retro Diels-Alder fragmentation and gave rise to base peaks at *m/e* 154. The other "half" of the molecules differed by 30 mu as expected. The mass spectrum also indicated that the ethyl side chain contained a hydroxyl group, (M^+-45). This group was secondary from the nmr spectrum, which showed a methyl doublet. The piperidine ring must therefore contain an epoxide group (no ketonic carbonyl) in order to account for the base peak. Hence the structure of horhammericine was deduced to be (VII). The aromatic regions of lochnerinine (III) and horhammerinine were very similar. Horhammerinine therefore has the structure (VIII).

Horhammericine (VII) was later isolated from *C. trichophyllus* (40) and evaluation against the 9KB test system, indicated that it was cytotoxic.

Also isolated at this time from *C. trichophyllus* were lochnericine (IX),

minovincinine (X) and minovincine (XI): of these, only lochnericine was cytotoxic (40). Clearly the epoxide group present in lochnericine (IX), lochnerinine (III), and horhammericine (VII) is crucial for the cytotoxic activity. Other structural requirements for cytotoxic activity have not been evaluated in this series of compounds.

In addition to our interest in the anticancer activity of *Catharanthus* spp. and their alkaloids, the availability of the various isolates has permitted biologic testing in a number of other pharmacologic screens.

The result of some of these tests on the alkaloids of *C. lanceus* are shown in Table I.

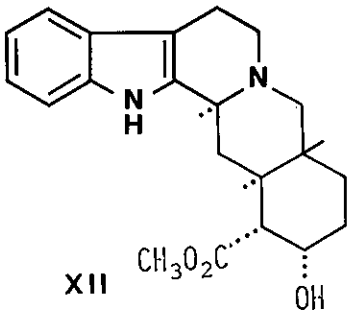
TABLE I. PHARMACOLOGIC ACTIVITIES OF ISOLATED *CATHARANTHUS* ALKALOIDS

Activity	Alkaloid
Hypotensive	Yohimbine
Hypoglycemic	Vindolinine, leurosine, catharanthine, tetrahydroalstonine
Diuretic	Catharanthine, Vindolinine
Analeptic	Pericalline
Antidiuretic	Ajmalicine
Antiviral	Pericalline, perivine, periformyline, vindolinine

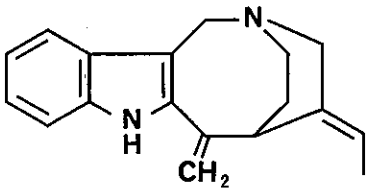
Yohimbine (XII), a potent α-adrenergic blocking agent, significantly reduced blood pressure in rats and dogs (13), whereas the other alkaloids tested were essentially inactive. Pericalline (XIII) elicited a pronounced analeptic effect (13).

Catharanthine (as the hydrochloride) (XIV) and vindolinine (as the dihydrochloride) (XV) were found to exhibit diuretic activity comparable to chlorothiazide and dihydrochlorothiazide (41).

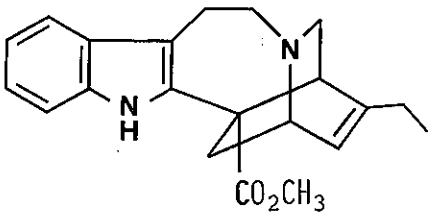
It will be recalled that the original interest in *C. roseus* was because



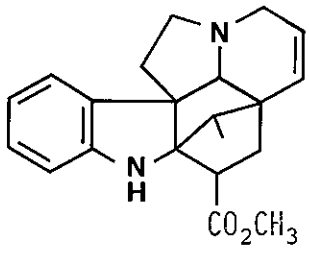
XII



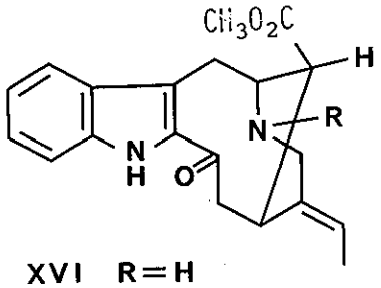
XIII



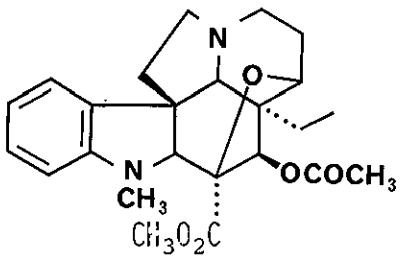
XIV



XV



XVI R=H
XVII R=CHO



XIX

of its reputed hypoglycemic activity. Of the alkaloids evaluated, leurosine sulfate (VI) and vindolinine dihydrochloride (XV) were the most active, their hypoglycemic action being intermediate between that of acetohexamide and tolbutamide (42).

Thirty-six *Catharanthus* alkaloids were evaluated *in vitro* against the polio type III virus and the vaccinia virus. Pericalline (XIII) was active against both viruses and perivine (XVI), periformyline (XVII) and vindolinine (XV) were active against only the former virus. The anticancer alkaloids leurosivine, VLB, VCR, leurosidine and desacetyl VLB (XVIII) were also active against the vaccinia virus *in vitro* (43).

Clearly, the alkaloids of *Catharanthus* have a great potential in other clinical pharmacologic areas than cancer. It is therefore not surprising to find new structures and alkaloids still being investigated in several laboratories throughout the world.

Recently we have derived the structures of a number of *Catharanthus* alkaloids which, coincidentally, all contained a tetrahydrofuran ring.

Cathanneine (Cathovaline) (44) from *C. lanceus* was shown by uv and ir spectra to be a dihydroindole containing an ester and a carbomethoxy group, but with no NH or OH functionalities. Comparison of the nmr spectrum with those of vindoline and vindorosine indicated a close similarity. In particular, the presence of three aromatic, three N-methyl, three methyl ester and three acetate protons was observed. No olefinic protons were observed and since the pattern due to the ring C-protons and the ethyl group were similar to that in vindorosine, the tertiary hydroxy group must have reacted with the 14,15-double bond. The ether bridge proton appeared as a doublet of doublets centered at δ4.08 ppm. This splitting pattern is only consistent with attachment of the ether bridge at C-15 as shown in XIX (45). The absolute config-

uration of cathovaline was determined subsequently (46).

Earlier workers had isolated the alkaloid vincoline from *C. roseus* (47) and *C. lanceus* (48). By chance the same alkaloid was isolated from *Vinca libanotica* (49) and re-isolated from *C. roseus* (50) at approximately the same time in our laboratories. Since there are only very few alkaloids isolated from both *Vinca* and *Catharanthus* species. It was therefore of some interest to elucidate the structure of vincoline.

The uv and ir spectra of vincoline indicated the presence of a dihydroindole together with ester, -OH and -NH groups and the molecular formula was found by high-resolution mass spectrometry to be $C_{21}H_{24}N_2O_4$. Since the nmr spectrum of vincoline showed the presence of two olefinic protons, the remaining oxygen must be present as an ether group (no ketone or amide carbonyl in the ir spectrum). In support of this, vincoline afforded a monoacetyl derivative following acetylation.

The position of two of the oxygen linkages was readily determined from the nmr spectrum, which showed a singlet for the C-2 proton at δ3.81 ppm and a quartet for the C-19 proton at δ3.81 ppm. The hydroxyl group was placed at C-6 on the basis of an ion XX at *m/e* 146 and an ion XXI at *m/e* 160. An ether linkage therefore can be placed at C-19 to C-16. The negative rotation and shielding of the C-18 methyl group (doublet at δ0.60 ppm) suggested a probable structure XXII for vincoline.

From a highly anticancer active fraction of *C. roseus* our group isolated an alkaloid which turned out to be identical with vincarodine (51), an alkaloid of unknown structure isolated previously by Svoboda and co-workers from *C. roseus* (52).

The molecular formula of vincarodine was established as $C_{22}H_{26}N_2O_5$, M^+ *m/e* 398 and the nmr spectrum established the presence of an aromatic methoxy

group (δ4,10 ppm) as well as a carbomethoxy group. The location of the aromatic methoxy group was determined to be at C-11 from the similarity of the aromatic region with that of vindoline. NMR spectroscopy also confirmed the presence of an ethyl group attached at a tertiary carbon.

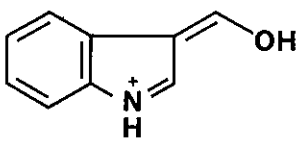
The uv spectrum was quite unlike any that we had previously encountered from *Catharanthus* species. Compounds in the 11-methoxy 16-oxygenated Eburna alkaloids were found to exhibit similar spectra, and at this point we could write a partial structure.

Vincarodine readily afforded a monoacetate derivative so that clearly the remaining oxygen function was an ether of some type, and it became important to assign the attachment of ether and hydroxyl functions. The base peak in the mass spectrum of vincarodine and its acetate was at *m/e* 200 having a composition $C_{12}H_{12}N_2O$, suggesting a probable structure XXIII for this ion. A metastable ion was observed for the transition *m/e* 398-200, indicating a very interesting loss of nine of the ten iridoid-derived carbon atoms in one process.

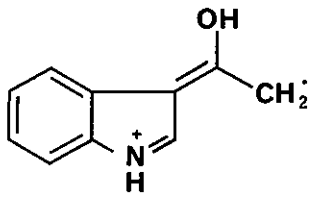
From the previous evidence it was clear that four oxygen atoms must be located in the iridoid-derived part of the molecule, including an acetylatable hydroxyl group. The retro Diels-Alder fragmentation pathway in ring C is a common primary process for the Eburna alkaloids (53). This fragmentation in the case of vincarodine leads to the ion *m/e* 297 ($C_{18}H_{19}NO_3$). This ion was not affected by acetylation, thus indicating the presence of the hydroxy group in ring E.

The ether bridge could therefore be attached from C-16 to either C-14 or C-15.

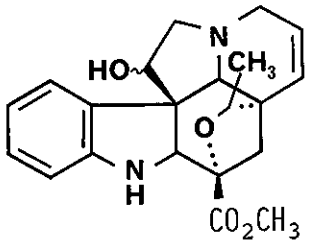
From an extensive analysis of forty-four fragment ions a number of pathways were derived, some of which served to distinguish between the two pos-



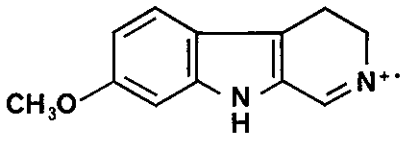
XX m/e 146



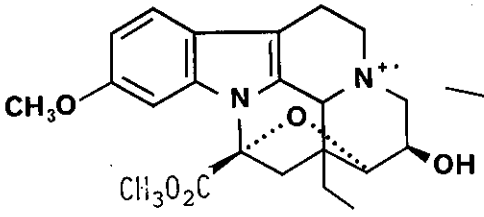
XXI m/e 160



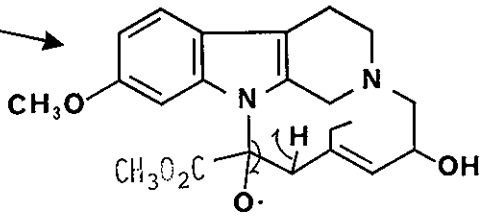
XXII

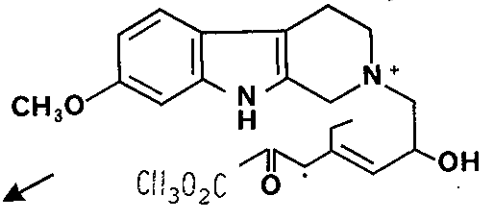


XXIII m/e 200



XXIV





Scheme 1

m/e 200

sible structures. One example is that indicated in Scheme I for the formation of the ions *m/e* 297 and 101, which suggested that vincarodine had the structure XXIV.

This structure was confirmed, and the relative stereochemistry of vincarodine deduced, from a study of the nmr spectrum of acetyl vincarodine. The proton on the carbon bearing the acetoxy group appeared at δ4.93 ppm as a broad signal. Irradiation at this position produced collapse in only two regions, δ3.87 and 2.30 ppm. Triple resonance irradiation at δ3.87 and 2.30 ppm collapsed the signal at δ4.93 ppm to a singlet.

These data are compatible only with a hydroxy group at C-14 and an ether function at C-15. Furthermore, the C-14 proton is *trans* (diaxial) to the ether bridge.

Vincarodine, exhibiting a negative rotation, therefore has the structure XXIV. Vincarodine had been isolated some twelve years earlier by Svoboda (52), yet within six months three independent groups deduced the structure (51,54,55).

Antitumor principles of *Chelidonium majus* (Papaveraceae)

The clinical use of *Chelidonium majus* (Papaveraceae) dates back to 1896 when Botkin (56) reported two cases of carcinoma which responded to treatment with *C. majus* extracts. Subsequent clinical reports include the use of chelidonine sulfate for gastric cancer (57), and *C. majus* extracts for breast cancer (58), and in other clinical trials (59,60). In addition, *C. majus* has a long history of use in other parts of Europe in the treatment of warts, papillomas and condylomas (61,62).

More recently, antitumor testing *in vivo* indicated that extracts of *C. majus* were inhibitory for sarcoma 180 and Erlich mouse carcinoma (63,64).

Our initial work on this plant (65,66) indicated that the extracts of

C. majus were devoid of antitumor activity but did exhibit significant cytotoxicity.

Extensive prior phytochemical work (for a review see ref. 67) succeeded in isolating a number of alkaloids, some of which have been shown to exhibit anticancer effects.

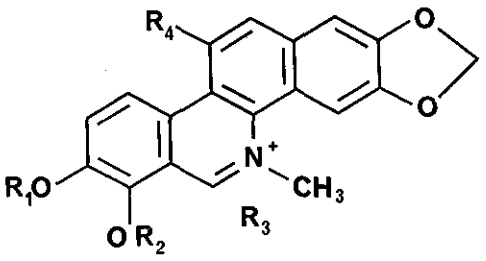
Sanguinarine (XXV) and chelerythrine (XXVI) showed antitumor activity in animals but are probably too toxic for human use (68), and chelidonine (XXVII) and protopine (XXVIII) exhibited cytotoxic activity (64).

Chromatography of the cytotoxic alkaloid fraction afforded a number of constituents, two of which proved to be cytotoxic. The first of these, on the basis of uv and mass spectral data, was identified as coptisine (XXIX) (67). Comparison with authentic sample confirmed the identity.

The second alkaloid proved to be new and was given the name chelidimerine (69). The uv spectrum indicated that chelidimerine was a member of the benzophenanthridine group of compounds, but the ir spectrum (67) showed a ketonic carbonyl (ν_{max} 1710 cm^{-1}). The mass spectrum indicated the presence of a molecular ion at *m/e* 720, with a probable molecular formula of $C_4H_{32}N_2O_9$. Important fragment peaks were observed at *m/e* 389 ($C_{23}H_{19}NO_5$) and *m/e* 332 ($C_{20}H_{14}NO_4$). This latter peak, which was also the base peak, could be attributed to the sanguinarium ion (XXX).

The nmr spectrum substantiated the presence of a sanguinarine moiety showing one N-methyl, two methylenedioxy and six aromatic protons. In addition, a multiplet was observed at δ4.88 ppm and a pair of doublets at δ2.52 and 2.20 ppm. This could be explained in terms of a $CO\text{-}CH_2\text{-}CH\text{-}N$ system where the nitrogen atom must be part of the sanguinarine nucleus.

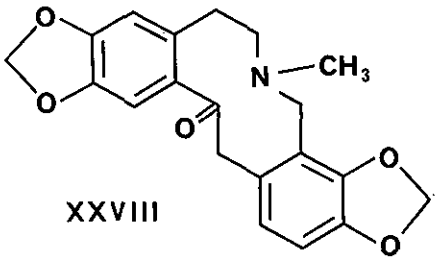
These data led to the structure XXXI for chelidimerine. Since chelidimerine showed no optical rotation, it must be either *meso* or racemic.



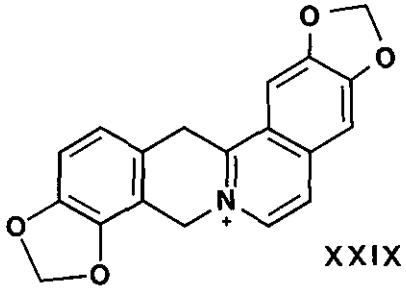
	R_1	R_2	R_3	R_4
XXV	$-CH_2-$		Cl^-	H
XXVI	CH_3	CH_3	Cl^-	H
XXVII	$-CH_2-$		-	OH
Rings B/C hexahydro and cis				
XXX	CH_3	CH_3	-	H

Single crystal X-ray analysis indicated that chelidimerine belonged to the space group P $2_12_12_1$. Chelidimerine is therefore *meso*-1,3-*bis*-(11-hydrosanguinarinyl)-acetone (XXXI).

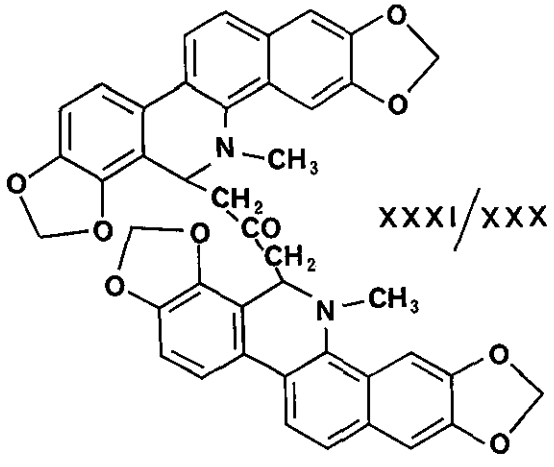
This structure assignment was confirmed by synthesis. Treatment of sanguinarine with acetone dicarboxylic acid gave chelidimerine identical with the natural product (69).



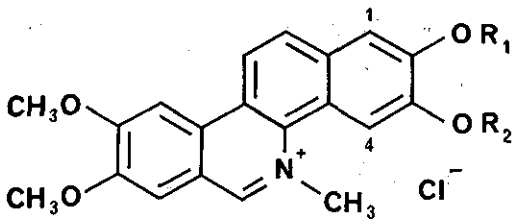
XXVIII



XXIX



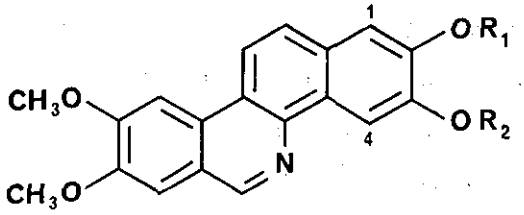
XXXI/XXXII



XXXIII $R_1R_2 = -CH_2-$

XXXIV $R_1 = H$; $R_2 = CH_3$

XXXV $R_1 = CH_3$; $R_2 = H$



XXXVI $R_1 = H$; $R_2 = CH_3$ or $R_1 = CH_3$; $R_2 = H$

Sanguinaria canadensis, like *C. majus* also has a long history in the empirical treatment of cancer (68), and a number of similar phytochemical constituents have been isolated (for a review see ref. 70) including sanguinarine (XXV) and chelerythrine (XXVI).

In addition to sanguinarine (XXV), we also isolated a new alkaloid, sanguidimerine, belonging to the benzophenanthridine series (70). Except for optical rotation and mp, the alkaloid proved to be identical in all respects to chelidimerine. Sanguidimerine therefore, has the structure (+)-1,3-*bis*-(11-hydrosanguinarinyl)-acetone (XXXII).

Surprisingly, sanguidimerine (XXXII) in addition to being inactive in the Walker carcinosarcoma 256 and P-388 lymphocytic leukemia system, was also

inactive in the 9KB system. This, in contrast to chelidimerinine (XXXI) which was active in this *in vitro* test.

Fagaronine

The benzophenanthridine alkaloids have recently proved to be one of the most promising groups of natural products from the point of view of clinical evaluation. The two most important compounds in this series are nitidine (XXXIII) and fagaronine (XXXIV).

Fagaronine was isolated as the chloride salt from the roots of *Fagara zanthoxyloides* (Rutaceae) (71). The uv spectrum indicated that it belonged to the benzophenanthridine class of alkaloids, and in particular, a resemblance to the uv spectrum of nitidine (XXXIII) was noted. A phenolic hydroxy group was demonstrated by the bathochromic shift in the uv observed on the addition of dilute alkali, and by a broad hydroxyl absorption in the ir spectrum.

The nmr spectrum showed an N^+ methyl singlet (δ5.11 ppm) and three O-methyl singlets, in addition to the aromatic protons which appeared as expected. Two structures, (XXIV) and (XXXV) were proposed for fagaronine at this point.

A clear distinction between these two possibilities was made after degradation of fagaronine to the N-demethyl derivative (XXXVI) (72). The improved solubility of this latter compound permitted a nmr investigation to be made.

Hydrogen bonding or phenolate anion formation is expected to shift *ortho* or *para* related protons upfield, due to increased shielding (73). Indeed, one aromatic proton did exhibit substantial shielding on the addition of triethylamine and sodium deuteroxide, and the magnitude of this shift (73) indicated an *ortho* relationship to the phenolic group.

On the basis of the two structures for fagaronine, this proton, originally at δ7.37 ppm, must either be at C-1 or C-4. A second aromatic proton, originally at δ8.68 ppm was also shifted on the addition of sodium deuteroxide. The magnitude of this shift (24) indicated that it had a *meta* relationship to the phenolic group. This proton must therefore be either at C_4 or C_1.

Elucidation of the structure therefore depends upon an assignment of the δ8.68 and 7.37 ppm protons to C_1 and C_4. The close proximity of the nitrogen atom is expected to give rise to a marked deshielding of the C_4 proton. On this basis, the δ8.68 ppm proton can be assigned to C_4 and fagaronine has the structure XXXIV.

Substantiating evidence for this structure assignment came from a total synthesis of fagaronine (XXXIV) (74).

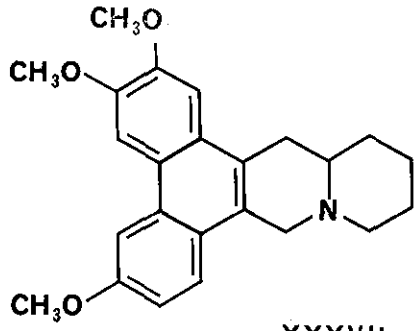
Although nitidine (XXXIII) and fagaronine (XXXIV) are structurally quite similar, they are quite interestingly different in their spectrum of anticancer activity. Fagaronine (XXXIV), for instance, although just as active as nitidine in the P388 test system, is devoid of cytotoxicity; whereas nitidine (XXXIII) is quite strongly cytotoxic.

Although fagaronine (XXXIV) offers several advantages over nitidine (XXXIII), preclinical toxicity studies have so far been limited to the latter compound.

Cryptopleurine (XXXVII).

A further class of compounds which we found to exhibit cytotoxic activity was isolated from *Boehmeria cylindrica* (Urticaceae) (75), a plant native to North America.

Chromatography of the alkaloid fraction gave a number of pure alkaloids, one of which was shown to be cryptopleurine (XXXVII), an alkaloid also ob-



XXXVII

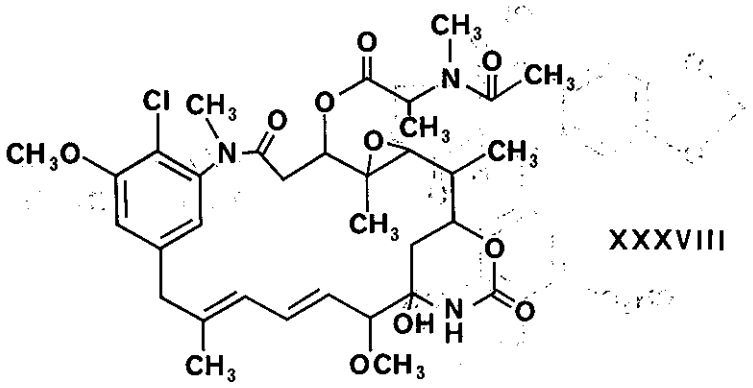
tained from an Australian *Boehmeria* species, *B. platyphylla* (76,77).

Cryptopleurine (XXXVII) was found to be inactive in several tumor systems, including the sarcoma 180, adenocarcinoma 755, Lewis lung carcinoma, L-1210 leukemia, P-388 lymphocytic leukemia and the Walker 256 intramuscular sarcoma. It was however highly active against Eagle's 9 KB carcinoma of the nasopharynx in cell culture, exhibiting an ED_{50} of approximately 1.0×10^{-5} µg/ml (75). The compound was also shown to exhibit antiviral activity (78).

The macrolide alkaloids.

One of the most significant new types of compounds, showing antitumor and cytotoxic activity, to be isolated in recent years are the macrolide alkaloids. The clinically most important of these at the present is maytansine (XXXVII) a macrocyclic alkaloid of *Maytenus senegalensis* (79).

In our laboratories, a sample of *Maytenus senegalensis* from Ghana was found to exhibit antitumor (WM-256, L-1210, and P-388) and cytotoxic (9KB) activities (80). Because of a paucity of plant material, we were forced to suspend our efforts on this plant. Dulcitol showing marginal PS *in vivo* activity, was isolated.



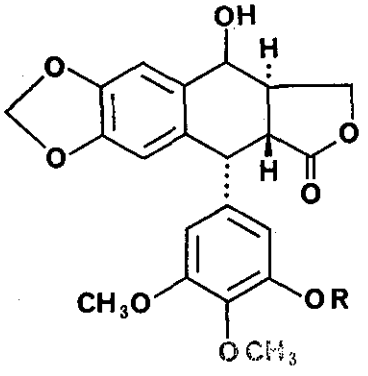
XXXVIII

Subsequently, Kupchan and co-workers have isolated a number of closely related macrolide alkaloids from other *Maytenus* spp. exhibiting marked *in vivo* and cytotoxic activity (81,82). The novel structures of these compounds and the dissimilarity to any previously known plant alkaloids gave rise to suspicions that possibly these products were fungal products derived from the soil where the plants grow. Several factors mitigate against this theory. The first is that *Maytenus* spp. from Nigeria, Ghana and Kenya show the presence of maytansinoids. Secondly, recollections of plant material contain maytansinoids. Thirdly, and probably most convincingly, related compounds have been isolated by Wall and co-workers (83) from *Colubrina texensis*, a plant native to Texas (U.S.A.).

Maytansine (XXXVIII) has proceeded through preclinical toxicology and drug formulation and is about to be evaluated in the clinic.

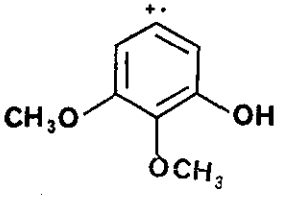
Antitumor and Cytotoxic Lignans

Probably the best known of the antitumor lignans is podophyllotoxin (XXXIX), a compound originally isolated from *Podophyllum peltatum* by Hartwell (84), and exhibiting cytotoxic and a wide range of antitumor activities (85).

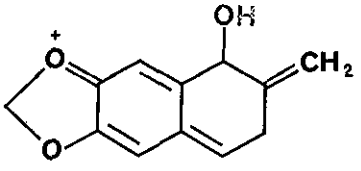


XXXIX R = CH_3

XLII R = H



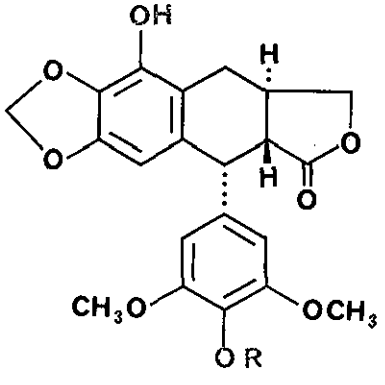
XL m/e 154



XLI m/e 201

XLIII R = H

XLIV R = CH_3



We have recently isolated podophyllotoxin (39) from a new source, and have also obtained a new lignan exhibiting both cytotoxic and antitumor effects (86).

An ethanolic extract of *Linum album* (Linaceae) exhibited both cytotoxic and antitumor effects. Application of the standard fractionation procedure gave petroleum ether and chloroform extracts each of which was active. Chromatography of the chloroform extract afforded podophyllotoxin (XXXIX) and a small quantity of a crystalline compound, which, from the mass spectrum, lacked a methyl group compared with XXXIX.

The compound showed a fragmentation pattern characteristic of the podophyllotoxin series (87). In particular, fragment ions at *m/e* 154,XL,and *m/e* 201,XLI,indicated that the fourteen mass units were missing from the lower, D, ring.

This interpretation was confirmed by the nmr spectrum which showed the presence of two methoxy groups and a methylenedioxy group. The aliphatic part of the spectrum was very similar to that of podophyllotoxin (XXXIX), but the aromatic region showed distinct differences. In particular, the asymmetric nature of ring D was established, for two doublets (J=2Hz) were observed at δ6.78 and 6.01 ppm. Double-resonance studies confirmed that these two protons were coupled to each other, and were therefore *meta*-orientated. Several possible structures could be proposed on the basis of this evidence, but treatment of the isolate with diazomethane afforded podophyllotoxin (XXXIX). The compound therefore has the structure 3'-demethyl-podophyllotoxin (XLII) (86).

The compound showed marginal cytotoxic and antitumor activity.

Podophyllotoxin (XXXIX) has proved to be somewhat disappointing in the treatment of human neoplastic disease, regressions being mainly transient and

clinically insignificant (88). Side effects (nausea, vomiting, diarrhea, oral ulcers and fever) were frequent.

Two other lignans, α-peltatin (XLIII) and β-peltatin (XLIV), were detected in *Linum album* by thin layer chromatography in comparison with authentic samples.

Although these compounds are effective in the treatment of tumors in laboratory animals (89,90), they are of no significance in the clinical setting (91,92).

Although the podophyllotoxin-type lignan aglycones have little or no clinical efficacy, considerable attention has recently been focussed on certain water-soluble glycosidic derivatives. Most important of these is 4'-demethylepipodophyllotoxin-(4,5-O-2-thenylidene)-β-D-glucopyranoside, which has been used clinically with some success.

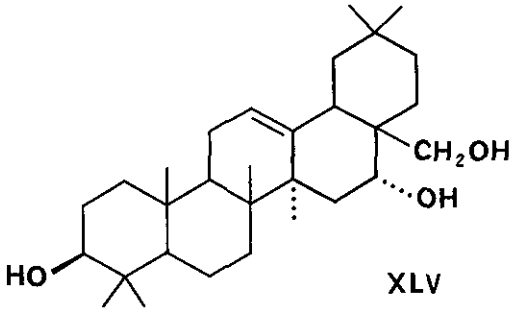
Miscellaneous Antitumor and Cytotoxic Compounds

Myrsine-saponin

Wallenia yunquensis of the family Myrsinaceae is indigenous to the Luguillo Mountains of Puerto Rico. In particular it is a common shrub in the Pico del Oeste area (93). An aqueous alcohol (1:1) extract of the whole plant material was found to be active in the Walker 256 (IM) carcinosarcoma test system. We therefore endeavored to obtain the active principle(s) of this plant (94). After the plant material had been defatted, it was extracted with ethanol. Concentration of this extract gave a precipitate which was filtered and shown by froth and hemolysis tests to be a saponin. The crude saponin was purified over magnesium oxide-celite to give the saponin in 2.1% yield. Acid hydrolysis of the saponin afforded a pure sapogenin mp 242-244°. Comparison with primulagenin A (XLV) an olean-12-ene derivative obtained previously by Kupchan *et al.* (95), indicated a close similarity and

direct comparison by mixture mp, tlc and mass spectra confirmed the identity (94).

The identity of the sugar components was determined by comparison with standards using paper chromatography and quantitated by glc comparison with trimethylsilylated sugars. These experiments (94) indicated that the sugar mixture was composed of rhamnose, glucose, galactose and glucuronic acid in the molar ratio (2:1:1:1). This is in agreement with a compound, myrsine-saponin isolated from *Myrsine africana* by Kupchan *et al.* (95), and demonstrated to be responsible for the antitumor activity of this plant. The precise structure of this saponin remains to be determined.



XLV

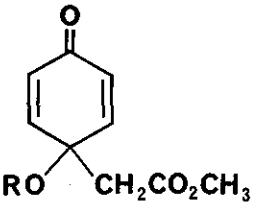
Jacaranone (XLVI)

A further new, although not heterocyclic, structure type has recently been shown to exhibit both *in vivo* and *in vitro* activity.

The isolation of jacaranone, as we have named the compound, provides a classic example of the isolation of pharmacologically active compounds by monitoring biological activity, and will therefore be described in detail.

An aqueous ethanolic extract of *Jacaranda caucana* (Bigoniaceae) showed *in vivo* antitumor activity against the P388 system. Evaluation of the ex-

XLVI R = H
XLVII R = Ac



tracts and fractions after preliminary fractionation indicated that the activity was in the chloroform-soluble fraction (T/C 163% at 200 mg/kg). The petroleum ether and aqueous fractions were devoid of activity (Scheme 2).

Chromatography of the chloroform-soluble material afforded five major fractions, and antitumor activity was now concentrated in the first two fractions (eg. T/C 153% at 100 mg/kg). Each fraction contained the same major constituent and chromatography of each fraction afforded an identical pale yellow oil, jacaranone, which crystallized slowly on standing, and which was shown to be the active constituent (T/C 165% at 2 mg/kg; ED_{50} 1.3 µg/ml).

The ir spectrum indicated the presence of hydroxyl (ν_{max} 3320 cm^{-1}), carbonyl (1745 cm^{-1}) and quinonoid (1680 and 1635 cm^{-1}) absorptions. The nmr spectrum showed that two pairs of *ortho* protons were present in addition to a methoxyl group and a deshielded methylene group. A molecular ion at *m/e* 182 was observed in the mass spectrum, and high resolution analysis indicated a molecular formula $C_9H_{10}O_4$.

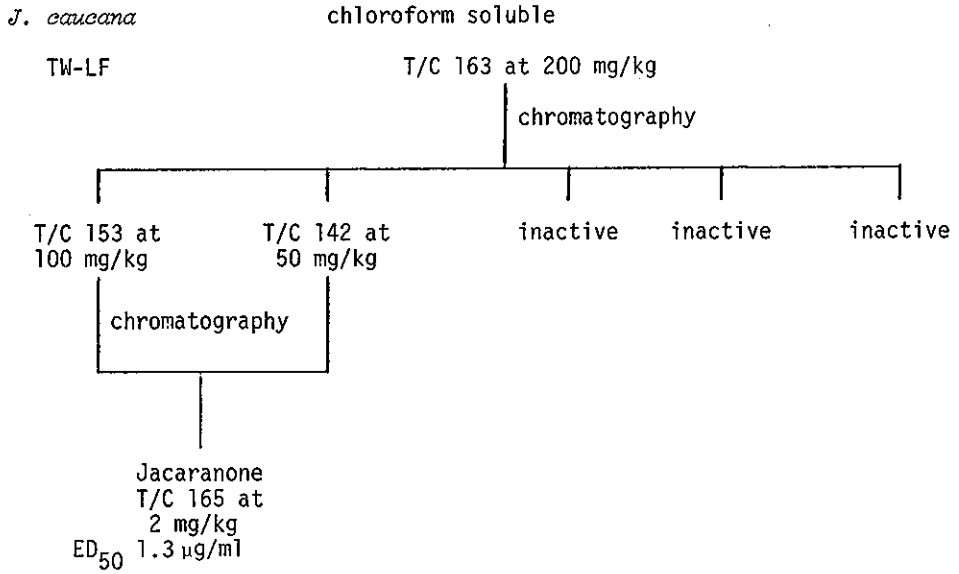
The above data can only be interpretated in terms of a single structure, and the structure (XLVI) was proposed (96).

This structure was substantiated by an examination of the cmr spectrum and by synthesis from methyl p-hydroxy-phenylacetate *via* the acetate (XLVII). The acetate (XLVII) was also prepared from jacaranone (XLVI) for anticancer evaluation. The compound was devoid of antitumor activity but maintained

cytoxicity.

Additional antitumor testing of jacaranone is presently underway.

Scheme 2. Fractionation of *Jacaranda caucana* by monitoring *in vivo* anti-leukemic activity



Tannins

The antitumor activity of many plants is due to the presence of tannins. These compounds, however, are of no interest for clinical purposes because of their erratic and toxic nature (85).

Several of the antitumor active plants with which we have worked have been found to contain substantial quantities of antitumor active tannins (97). The latter being obtained by the method of Wall *et al.* (98). Among the plants shown to contain antitumor active tannins were *Caylcogonium squamulosum*, *Cornum canadensis*, *Lespedeza capitata* var. *velutina*, and *Rubus odorata* (97).

Hydrolysis of the tannin obtained from *Calycogonium squamulosam*, followed

by chromatographic examination, indicated the presence of gallic acid and glucose in the molar ratio (3:1). On this basis a partial structure of tri-O-galloyl-D-glucose was proposed (99).

Recently, we have demonstrated that the antitumor activity of *Drimys granatensis* var. *granatensis* (Winteraceae) is also probably due to tannins (100).

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