Structure Elucidation and Chemistry of *Catharanthus* Alkaloids III: Structure of Leurosine, an Active Anticancer Alkaloid

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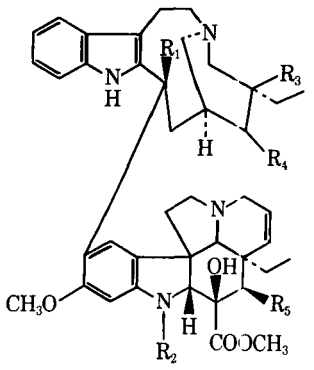
Abstract □ Evidence for the structure of the antitumor alkaloid leurosine is presented, together with the explanations for the mass spectral fragmentation patterns observed. Nuclear magnetic resonance spectroscopic data support the proposal that leurosine contains an epoxide moiety, and is closely related to the other *Catharanthus* antitumor alkaloids vincaleukoblastine and leurosidine.

Keyphrases □ *Catharanthus* alkaloids—leurosine structure, chemistry □ Antitumor alkaloid—leurosine □ IR spectrophotometry—structure □ NMR spectroscopy—structure □ Mass spectroscopy structure

Six antitumor alkaloids have been isolated from the madagascan periwinkle, *Catharanthus roseus* (L.) G. Don (*Vinca rosea* L., *Lochnera rosea* Reichb.), family Apocynaceae. They include the dimeric indole alkaloids vincaleukoblastine (vinblastine, VLB) (1, 2), leurocristine (vincristine, VCR) (3), leurosine (vinleurosine) (2), leurosidine (vinrosidine) (3), leurosivine (4), and rovidine (5). In addition, leurosine has been isolated in these laboratories from the related sub-shrub *Catharanthus lanceus* Boj. ex A.DC. (6). Vincaleukoblastine and leurocristine are now available for the clinical management of several neoplastic diseases (7).

At least 24 of the 72 alkaloids isolated from *Catharanthus* species of plants (*C. roseus*, *C. lanceus*, *C. pusillus*, *C. trichophyllus*) are dimeric indoles, and the structures for vincaleukoblastine (I) (8–11), leurocristine (II) (8–11), leurosidine (III) (12), desacetyl VLB (IV) (5, 8–11), and vindolicine (13) have been established.



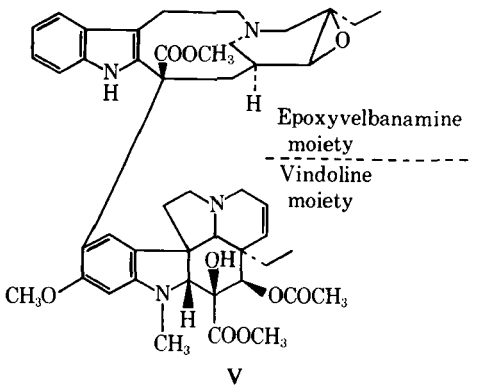
Alkaloid		R_1	R_2	R_3	R_4	R_5
Vincaleukoblastine	I	$COOCH_3$	CH_3	OH	H_2	Ac
Leurocristine	II	$COOCH_3$	CHO	OH	H_2	Ac
Leurosidine	III	$COOCH_3$	CH_3	H	HOH	Ac
Desacetyl VLB	IV	$COOCH_3$	CH_3	OH	H_2	OH

The partial structure for catharine was recently deduced in these laboratories (14).

The fact that in these studies leurosine had been isolated as one of the active antitumor entities of *C. lanceus* (6, 15) prompted investigation of its structure. Partial structures have been advanced for this alkaloid by Neuss *et al.* (16–19), and they have presented three possible structures for this base (19); however, their chemical degradative studies could not be rationalized with only one proposed structure (19). Evidence is presented at this time, which is in agreement with one of the structures advanced by the Lilly group for the structure of leurosine. The detailed mass spectral studies reported herein also show some new correlations hitherto unreported.

DISCUSSION

Leurosine, as the free base, is a very unstable substance which is decomposed by light, heat, or upon standing in the dark over a period of a few days. It is considerably more stable under these conditions as the sulfate salt. Due to a lack of a large quantity of leurosine, evidence has been obtained (primarily by analytical methods—IR, NMR, and mass spectrometry) which is in agreement with structure V for leurosine. The structure of leurosine can be divided into its two individual units, namely vindoline, and what is termed epoxyvelbanamine.



V

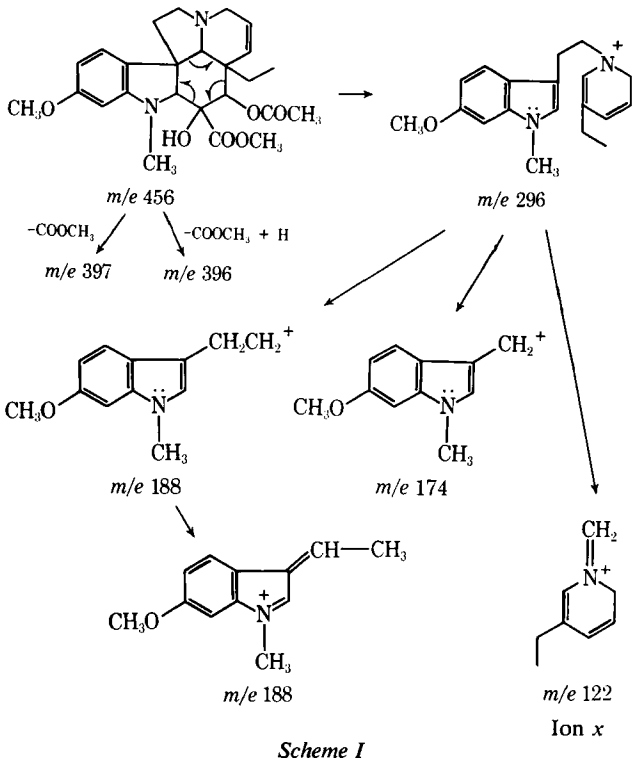
Mass Spectral Study of Leurosine—The mass spectrum[1] of V (Table I) exhibits the same type of thermal additions as those reported for vincaleukoblastine (I) by Biemann *et al.* (10). Three possible molecular weight peaks were found at *m/e* 836, 822, and 808, thus the molecular weight for V was postulated as 808, with higher peaks being attributed to CH_2 addition as reported above (10). By taking a spectrum of V almost immediately after insertion of the probe, *m/e* 808 was verified. This 808 molecular weight was also confirmed by the fact that all characteristic degradations of the vindoline moiety arise from the 808 peak, and are not found to arise from either *m/e* 822 or 836. The 808 molecular weight is in agreement with a molecular formula of $C_{46}H_{56}N_4O_9$ for Structure V, which is verified by the high-resolution measurement in Table I. Thus, the elemental formula of V differs by only two hydrogens from that of the active antitumor alkaloid vincaleukoblastine (I).

The mass spectrum of V showed some intense peaks at the *m/e* values (*i.e.*, 120, 121, 122, 135, *etc.*) that are also present in the spectrum of vindoline and vindoline-containing dimers. An interesting phenomenon was discovered on combination of various known vindoline *m/e* values with *m/e* values of ions predicted to arise by

[1] Measurements were taken on an MS-9, double-focusing, high-resolution mass spectrometer.

normal fragmentation of the epoxyvelbanamine moiety. Various combinations of these sums appeared as major peaks in the spectrum.

To illustrate this, the known degradations of vindoline are presented in Scheme I (20, 21). Several depicted stable vindoline



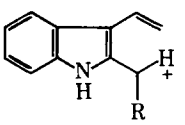
Scheme I

ions are 456, 397, 396, 296, 188, and 174. If these ions were bonded to the epoxyvelbanamine moiety, they would then be present as one mass unit less, since one hydrogen is missing for the bonding of the second alkaloid moiety of the dimer. Therefore, masses of 455, 396, 395, 295, 187, and 173 would be expected to arise in combination with the mass for a stable epoxyvelbanamine moiety. Thus, when the stable vindoline ions were combined with the predicted stable epoxyvelbanamine ions 353, 215, 214, 156, and 155, various combinations resulted in *m/e* values which were evident as peaks in the mass spectrum. The composition of a large number of these ions was then verified by high-resolution mass spectrometry (Table I). These combinations are discussed separately.

The elemental formula for the molecular weight ion is derived from the following reasoning. If it is assumed that the epoxyvelbanamine molecular ion 353 is composed of $C_{21}H_{25}N_2O_3$, and that of vindoline is 455 $C_{25}H_{31}N_2O_6$, then the molecular weight should be 808, and be analyzed as $C_{46}H_{56}N_4O_9$. The calculated value of 808.4044 proved it to be in agreement with the observed 808.4117.

Structural formulas for the stable ions of the epoxyvelbanamine moiety were proposed by using the similar degradative mechanisms published for this type of ring system.

The structure of a velbanamine ion (Ion *a*) has been published by Biemann *et al.* (10) as

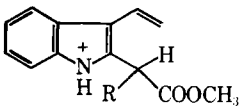


Ion *a* R = vindoline

where R represents some ion from the vindoline moiety from Scheme I. This Ion *a*, derived from the fragmentation of I would not differ in formation from that obtained from V since the only difference between V and I is attachment of the hydroxy as an epoxide, which is not involved in the fragmentation of this portion of the molecule. The *m/e* for Ion *a* is *m/e* 155 + R (R = vindoline ion from Scheme I) and it has the formula $C_{11}H_9N$ + R. Therefore, Ion *a* should be present in the epoxyvelbanamine degradation. Looking at Scheme I, and subtracting one mass unit for loss of hydrogen on bonding to the aromatic moiety, shows that the ions from vindoline (*m/e* 455, 396, 395, 295), when combined with the *m/e* 155 epoxyvelbanamine ion, give *m/e* values of 610, 551, 550, and 450 (Ions *b*, *c*, *d*, *e*). High-resolution measurements (Table I) of these ions are in agreement with this hypothesis. The following formulas are proposed for these ions (see Table I).

The elucidation of other stable epoxyvelbanamine ions, singly and when combined with the stable vindoline ions in Scheme I (*vide supra*), appeared to account for the majority of the peaks in the mass spectrum of V.

An additional and expected epoxyvelbanamine ion would also be Ion *f*, similar to Ion *a*, except that the carbomethoxy group has not been lost.



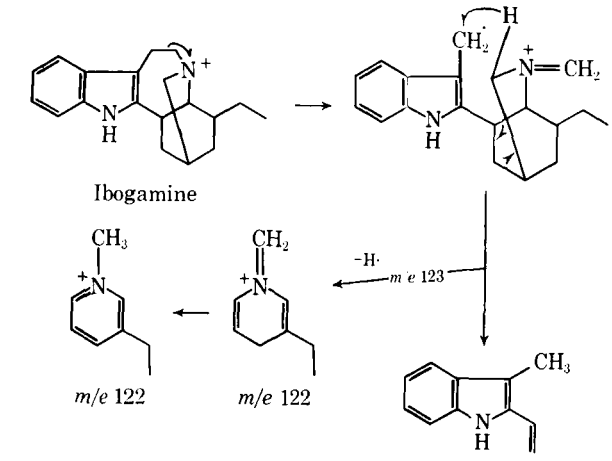
m/e 214 + R

Ion *f* R = vindoline

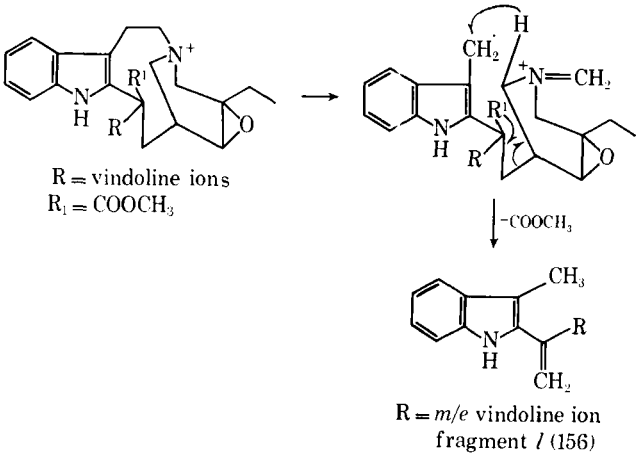
Table I—High-Resolution Measurements on Leurosine

Formula	Observed *m/e*	Calculated *m/e*	Formula	Observed *m/e*	Calculated *m/e*
C_7H_8N	106.0656	106.0657	$C_{25}H_{28}N_2O_3$	404.2070	404.2098
C_7H_9N	107.0734	107.0735	$C_{30}H_{32}N_3O$	450.2552	450.2543
$C_8H_{10}N$	120.0814	120.0816	$C_{30}H_{33}N_3O$	451.2618	451.2622
$C_8H_{11}N$	121.0894	121.0892	$C_{30}H_{34}N_3O$	452.2714	452.2700
$C_8H_{12}N$	122.0953	122.0970	$C_{29}H_{31}N_3O_3$	469.2342	469.2367
$C_9H_{13}N$	135.1053	135.1048	$C_{32}H_{35}N_3O_3$	509.2673	509.2676
$C_9H_{14}N$	136.1107	136.1126	$C_{32}H_{36}N_3O_3$	510.2747	510.2755
$C_{10}H_{10}N$	144.0814	144.0813	$C_{32}H_{37}N_3O_3$	511.2797	511.2833
$C_9H_{14}NO$	152.1071	152.1075	$C_{32}H_{32}N_3O_4$	522.2427	522.2391
$C_{11}H_8N$	154.0652	154.0656	$C_{33}H_{36}N_3O_3$	522.2759	522.2754
$C_{11}H_{11}N$	157.0880	157.0891	$C_{33}H_{38}N_3O_4$	540.2876	540.2860
$C_{11}H_{12}N$	158.0963	158.0969	$C_{33}H_{39}N_3O_4$	541.2960	541.2938
$C_{11}H_{10}NO$	172.0772	172.0761	$C_{34}H_{36}N_3O_4$	550.2733	550.2704
$C_{12}H_{14}NO$	186.0898	186.0918	$C_{34}H_{37}N_3O_4$	551.2787	551.2782
$C_{22}H_{21}N_2O$	329.1655	329.1653	$C_{34}H_{38}N_3O_4$	552.2852	552.2860
$C_{22}H_{23}N_2O$	331.1814	331.1809	$C_{36}H_{40}N_3O_6$	610.3005	610.2915
$C_{23}H_{24}N_2O$	344.1885	344.1887	$C_{36}H_{41}N_3O_6$	611.3044	611.2993
$C_{23}H_{25}N_2O$	345.1954	345.1965	$C_{36}H_{42}N_3O_6$	612.3149	612.3071
$C_{21}H_{25}N_2O_3$	353.1876	353.1858	$C_{36}H_{43}N_3O_6$	613.3214	613.3149
$C_{25}H_{23}N_2O_2$	383.1778	383.1759	$C_{38}H_{44}N_3O_8$	670.3077	670.3121
$C_{25}H_{25}N_2O_3$	401.1877	401.1864	$C_{38}H_{45}N_3O_4$	671.3170	671.3204
$C_{25}H_{26}N_2O_3$	402.1914	402.1941	$C_{46}H_{56}N_4O_9$	808.4117	808.4044
$C_{25}H_{27}N_2O_3$	403.2049	403.2020			

If 455, 396, 295, and 187 are substituted for R in Ion *f*, another series of peaks is seen at 669, 610, 509, and 401 (Ions *g*, *h*, *i*, *j*) in the spectrum which correlate with the proposed formula by high-resolution measurements (Table I). Of the above ions, Ion *g* (*m/e* 669) does not have a high-resolution measurement; however, there is a peak in the mass spectrum at that molecular weight. In searching for known fragmentation routes of this type of moiety it was found that the epoxyvelbanamine skeleton has a similarity to the ibogamine ring system, except that the one ring is opened and a carbomethoxy group is substituted in the epoxyvelbanamine moiety (see Schemes II and III). The mechanism of degradation of ibogamine and its derivatives has been shown by two groups (22, 23) to proceed as shown in Scheme II. The neutral fragment in Scheme II would also be expected from epoxyvelbanamine by the same type of mechanism to give Fragment *l* as shown in Scheme III. This is observed since a combination of 156 plus the vindoline ions 455, 396, 395, 295, and 173 from Scheme I give rise to *m/e* 611, 552, 551, 451, and 329 (Table I).

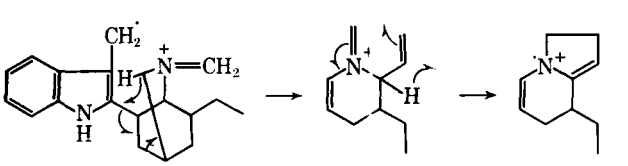


Scheme II

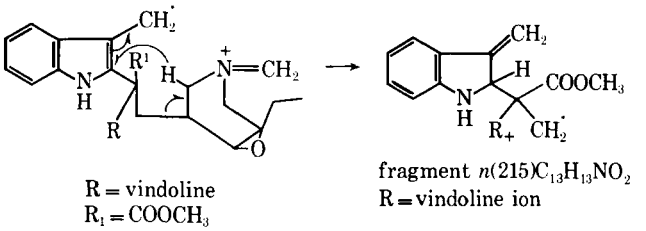


Scheme III

An additional mechanism of fragmentation in this type of ring system has been advanced by the same authors and confirmed by deuterium labeling (22) as described in Scheme IV. Note the hydrogen transfer to the indole nucleus. By a similar transition then, V should be expected to fragment in this manner by the same type fragmentation as in Scheme IV, without loss of the carbomethoxy, and then cleavage, which results in a neutral fragment (*n*) (215) $C_{13}H_{13}NO_2$ as shown in Scheme V.



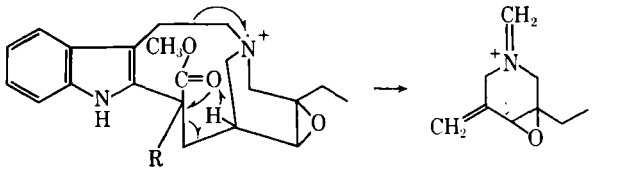
Scheme IV



Scheme V

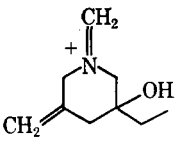
The combination of *m/e* 215 with 455, 396, 395, 295, 187, and 173 was observed to give *m/e* 670, 611, 610, 510, 402, and 388, all appearing in the spectrum and the majority of which were measured again by high resolution mass spectrometry for confirmation (Table I).

Another fragmentation pattern that might arise in this case would be the well-known β-cleavage with γ-hydrogen transfer mechanism, since it is a favored fragmentation route of carbonyl with γ-hydrogens (2) (Scheme VI). The above fragmentation produces Ion *p* at *m/e* 152 and does agree with the correct molecular formula (Table I).



Scheme VI

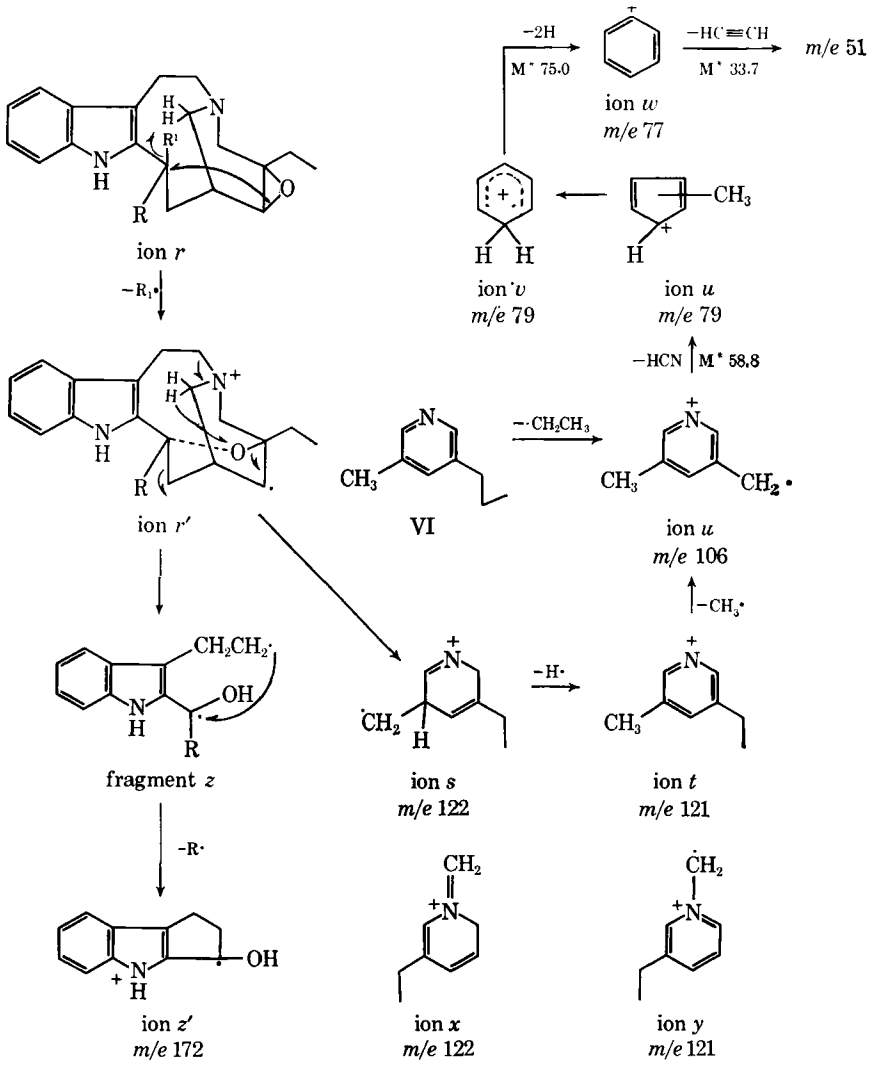
The positions of eight of the nine oxygens in V have thus been easily discerned from the spectrum. The significant Ion *p* then suggests the location of the ninth oxygen, or epoxy oxygen. This ion would be similar to the Ion *q* obtained from I (11) which occurs at *m/e* 154.



m/e 154

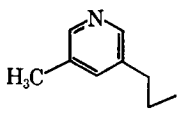
Ion *q*

Another fragmentation pathway is proposed in Scheme VII to explain several strong ions in the spectrum. The first step of this mechanism involves rearrangement of the epoxy across the ring. In the second step of Scheme VII, the hydrogen transfer (Ion *r'*) to the oxygen seems plausible since this hydrogen is observed in the literature (see Schemes II and IV) to migrate to either of the two indole carbon atoms (22) through a five-member ring transfer, and this mechanism shows a six-member ring transition, which would be feasible. Opening of this system would then show an *m/e* 122 ion (Ion *s*) with the same elemental composition C, H, N as that Ion *x* obtained from vindoline (see Scheme I), but of different molecular structure. In this case there are two sets of ions with the same elemental formulas, but different structures. Ions *x* and *y* of the vindoline moiety at *m/e* 122 and 121 have the identical molecular weights as the corresponding Ions *s* and *t* from epoxyvelbanamine, but have different structures. The additional Ions *s* and *t* are proposed to explain the very abundant series of ions at *m/e* 106, 79, 77, and 51 (Ions *u*, *v*, *w*) which appear in leurosine, and not in the spectrum of vindoline. The Ions *u*, *v*, and *w* have been established by Djerassi *et al.* (24) to have the structures and fragmentation patterns as shown in Scheme VII, when one is dealing with a



Scheme VII

system such as 3-propyl-5-methyl pyridine (VI) or similar pyridines



VI

substituted in the same positions. The fact that Ions *u*, *v*, and *w* occur in very abundant amounts, and that the corresponding metastable ions are present as shown, is good evidence for the proposed mechanism in Scheme VII. The other pathway in Scheme VII might be observed if the oxygen transfer takes place with the positive charge on the indole portion. One might then expect an ion at *m/e* 172 (Ion *z'*) which contains the epoxy oxygen. Ion *z'* at *m/e* 172 is observed which is found by high-resolution measurements to be of that elemental composition ($C_{11}H_{10}NO$).

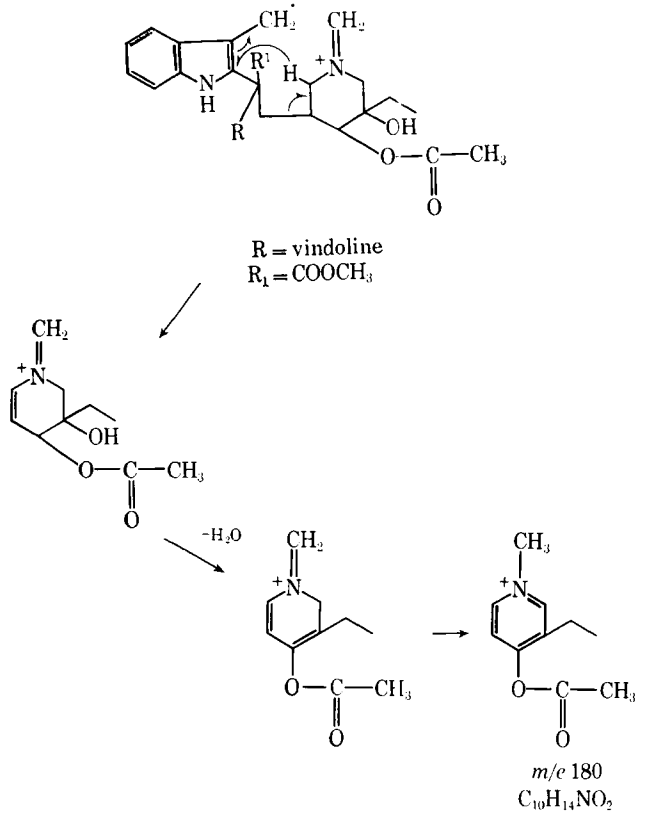
It was thought that the dimeric leurosine (V) molecule may cleave into separate units in the mass spectrograph. An ion was observed at 353 in the spectrum of V, which could be due to cleavage of vindoline, leaving the epoxyvelbanamine moiety $C_{21}H_{25}N_2O_3$ with a positive charge. High-resolution measurements verified that this fragment had the elemental composition of $C_{21}H_{25}N_2O_3$.

NMR Studies of Leurosine—The NMR spectrum of leurosine is very similar to that of VLB (1). An observable difference is that of a peak centered around 6.9 τ. This peak was shown to be a doublet with J equal to 4.1 ± 0.2 c.p.s., both at 60 and 100 Mc. The observed position for an epoxy methine proton of similar type is reported to be at 6.9–7.2 τ with a J_{cis} of 3.3–4.1 c.p.s. (25, 26). If one looks at the hydroxyl group in I, whose stereochemistry was determined by X-ray analysis (8, 9), one can reasonably assume that if the hydroxyl of velbanamine was involved in this epoxy linkage, the methine hydrogen of the epoxy would be *cis* to the bridge proton, and the J_{cis} coupling constant and chemical shifts would agree with the proposed structure. This evidence also would eliminate the other possible attachments of oxygen to any place in the epoxyvelbanamine structure since either the chemical shifts, multiplicities, and/or coupling constants would be different. The integral of this epoxy proton also proves to be equivalent to one proton when matched against the six methoxyl protons at 6.2 τ.

Other Experiments—The IR spectrum of leurosine (V) and VLB (I) are almost superimposable, with V having a small side band at 3035 cm.$^{-1}$ (epoxy proton for a strained ring at about 3,030 cm.$^{-1}$), and I having a small band at about 3,560 cm.$^{-1}$ (hydroxyl of the velbanamine moiety) which is not present in V.

Acetolysis experiments were conducted by heating V with anhydrous acetic acid at 100° for 2 hr. The resulting product was shown by TLC on silica gel G plates (250 μ), using ethyl acetate–absolute ethanol (3:1) as an eluent, to be a one-spot material having R_f 0.23, and exhibiting a color reaction to the ceric ammonium sulfate reagent similar to that of V (28). This compound was subjected to mass spectrometry and some preliminary high-resolution measurements were obtained. The results of this experiment are incomplete at the present time, but they are consistent with the conclusion that V is an epoxide-containing alkaloid.

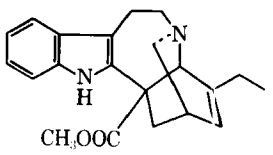
Proof of reaction under mild conditions was observed, as would be predicted, by changes of peaks in the 100–250 *m/e* spectral range. The mass spectrum showed an intense peak at *m/e* 180, which could possibly form by the fragmentation pattern shown in Scheme V.



Scheme VIII

This transformation is shown in Scheme VIII and the high-resolution measurement is consistent for the ion at *m/e* 180 ($C_{10}H_{14}NO_2$).

Biogenic Relationships—Catharanthine (VII) has been shown to give rise to cleavamine, which is closely related to velbanamine (27). Since it is possible, biogenetically, that the double bond of VII is hydroxylated *en route* to I, it also seems reasonable then, that this vinyl linkage could be oxidized to an epoxy group.



VII

The structure of leurosidine, a related dimeric alkaloid, was recently established (12), and this structure also fits the biogenetic argument presented above. Thus, all four of the six active antineoplastic *Catharanthus* alkaloids, whose structures are now known, are closely related (I–III, V).

At this time, the authors are engaged in confirming the structure of V by single crystal X-ray analysis.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 22, 1968, from the *Department of Pharmaceutical Chemistry and the Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213*

Accepted for publication February 11, 1969.

This research was supported, in part, by research grants CA 08509, FR 05455 and CA 08228, from the National Institutes of Health, U. S. Department of Health, Education and Welfare, Bethesda, MD. The NMR studies were supported, in part, under grant FR 00292, and the mass spectrum work, in part, under grant FR 00273, both from the National Institutes of Health.

The authors are grateful to Dr. C. Griffin, Department of Chemistry, University of Pittsburgh, for his assistance with the NMR phases of this investigation. For the previous paper in this series see *Reference 14.*