305. Total Synthesis of Indole and Dihydroindole Alkaloids. IX[1],[2] Studies on the Synthesis of Bisindole Alkaloids in the Vinblastine-Vincristine Series. The Biogenetic Approach

by **James P. Kutney, Toshihiko Hibino, Edwin Jahngen, Tetsuya Okutani, Arnold H. Ratcliffe, Adi M. Treasurywala** and **Stephen Wunderly**

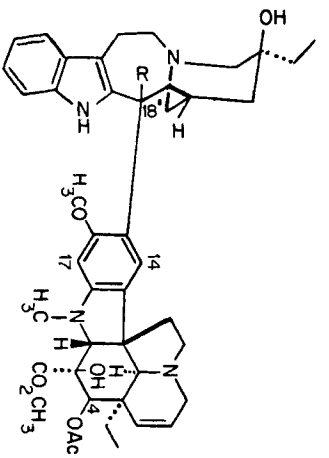
Department of Chemistry, University of British Columbia,
2075 Wesbrook Place, Vancouver, Canada V6T 1W5

(18. II. 76)

Summary. A detailed study of the reaction of catharanthine N-oxide and vindoline has been carried out employing various conditions. Under optimum conditions, which involve low temperatures and trifluoroacetic anhydride as reagent, 3′,4′-dehydrovinblastine (XIII, R = $COOCH_3$), in reasonable yields is essentially the exclusive product. However two additional products, 18′(*epi*)-3′,4′-dehydrovinblastine (XIV, R = $COOCH_3$) and 1′-hydroxy-3′,4′-dehydrovinblastine (XVI, R = $COOCH_3$) are also often isolated.

The reaction, which follows the course of a *Polonovski*-type fragmentation process, has been extended to the N-oxide derivatives of dihydrocatharanthine and decarbomethoxycatharanthine to provide again a series of bisindole alkaloid derivatives, also vinblastines. A mechanistic rationale is provided to explain the various results obtained.

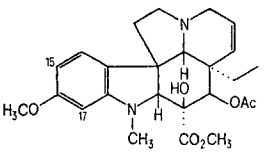
The wealth of structural diversity [3a] and clinical importance [3b] [4] of the *Catharanthus* alkaloids continues to provide a challenge for devising a general synthetic approach to them. In [2] we provided a detailed discussion of the use of chloroindolenine intermediates for the synthesis of the bisindole alkaloids in the vinblastine (I) series. We now present results relating to a completely different synthetic approach.



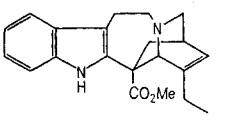
I, R = $COOCH_3$

1) For a preliminary report on a portion of this work, see [1].
2) Part VIII, see [2].

In considering the structures of the large number of alkaloids isolated from *Catharanthus roseus* G. DON (*Vinca rosea* L.) [3a] several features are of particular interest. Vindoline (II) and catharanthine (III) are the major components present while the bisindoles, exemplified by vinblastine, represent a minor portion of the alkaloid mixture. Furthermore, analysis of the structural features inherent in this latter family reveals that whereas vindoline represents the dihydroindole unit in



II

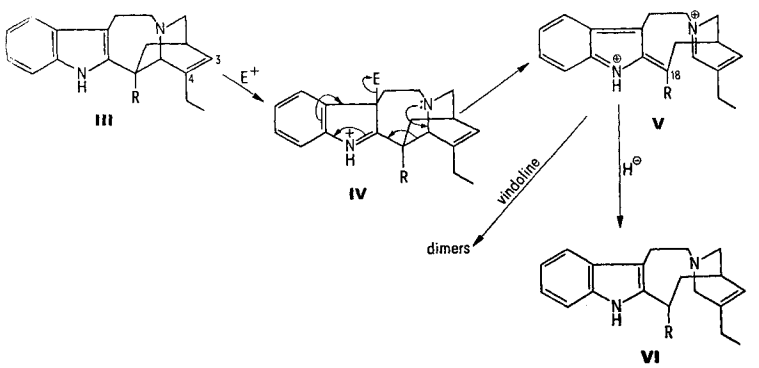


III

these systems, the indole portion, normally referred to as the velbanamine or cleavamine unit, is represented by a 'cleaved' catharanthine system. Obviously biogenetic speculations, with regard to the bisindole alkaloids, must consider the possibility that the plant enzymes utilize the catharanthine system, perform an appropriate fragmentation to a biointermediate with the nine-membered ring skeleton of the cleavamine-velbanamine series, and the latter then reacts with vindoline to provide the natural products isolated. If such were the case, clearly enzymatic activation of the catharanthine skeleton for such a fragmentation must occur at some stage. Based on experiments performed here and elsewhere, we were led to consider three possible sites in the catharanthine molecule where such activation with the enzyme (E^+) appeared feasible.

The first of these (*Scheme 1*) involves electrophilic attack by the enzyme at the β-position of the indole ring in catharanthine to provide the intermediate IV which, *via* V, can then react with vindoline to yield the 'dimeric' bisindoles. Ample support for such a fragmentation process has been furnished. Acid-catalysed ($E^{\oplus} = H^{\oplus}$) fragmentation of catharanthine has been studied in the *Lilly* laboratories [5] as well as in our group [6] [7]. In our more recent investigations employing glacial acetic acid and sodium borohydride, for example, we have effected fragmentation of a catha-

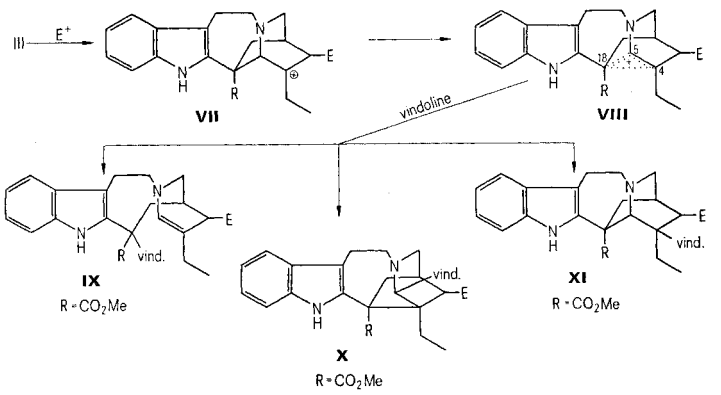
Scheme 1



ranthine to a cleavamine (VI, R = CO_2CH_3) in about 90% yield. As *Scheme 1* indicates, the crucial step in the pathway, as it relates to the synthesis of the dimeric alkaloids, involves the nucleophilic attack of vindoline at the C(18) of the generated intermediate V. Such a process has already been considered, in effect, in the recently published chloroindolenine approach [2] where it has been demonstrated that the unnatural configuration at C(18') of the dimers resulted has been obtained. Obviously if such a process is operative in the plant system some strict enzymatic control of stereochemistry is mandatory.

Enzymatic activation prior to fragmentation of the catharanthine system could also involve electrophilic attack at C(3), C(4) double bond (*Scheme 2*). The resulting carbenium ion thus formed (VII), in the form of the non-classical ion VIII, could react with vindoline to yield the possible dimers IX–XI; obviously only IX would be desirable for our bisindole synthesis. The process portrayed in *Scheme 2*, involves,

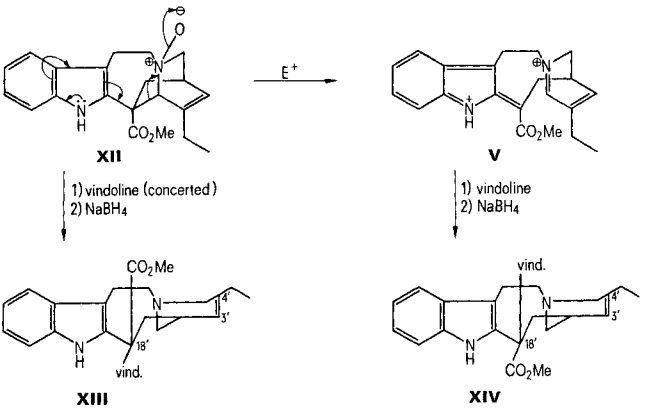
Scheme 2



in effect, the fragmentation of the bicyclic quinuclidine system in catharanthine for which there is as yet no known evidence. The analogous bicyclo[2.2.2]octane system has been investigated in some detail (see chief references in [8]) and if such results can be extended, at least approximately, to the present case, the favoured fragmentation would provide X rather than IX. Investigations in this direction are under way and will be presented later.

The third possible alternative for an enzymatic fragmentation of III could involve initially an oxidative attack at the basic nitrogen (*Scheme 3*) and the resulting intermediate, *e.g.* XII, could then react as in the *Polonovski*-type fragmentation [9]. The formation of N-oxides is well known in alkaloid chemistry and their presence in plant alkaloid mixtures is also well established. As *Scheme 3* indicates, the fragmentation of catharanthine N-oxide (XII) could, in principle, proceed along several plausible mechanistic pathways and these in turn could provide dimers with different configuration at C(18'). Thus fragmentation of XII could provide V, an intermediate postulated in *Scheme 1*, and generally considered in the chloroindolenine approach [2]

Scheme 3



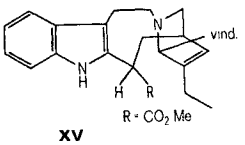
from which dimer XIV, with unnatural configuration at C(18'), was isolated. On the other hand, if the fragmentation of the C(18),C(5) bond in XII were to proceed in a 'concerted' fashion, the resultant configuration would be as shown in XIII. Molecular models reveal that vindoline in displacing the C(18),C(5) bond in a *trans* coplanar fashion would provide the dimeric product with natural configuration at C(18'). In view of a great deal of previous data [2] relating to the role of intermediates similar to V in formation of bisindole alkaloids, it was logical to systematically evaluate the approach shown in *Scheme 3*.

Investigations of the 'biogenetic' approach according to *Scheme 3* were initiated in our laboratories in 1973, and involved the reaction of catharanthine (or its hydrochloride salt) with MCPBA[3]) and subsequent reaction of the so-formed intermediates, with vindoline in presence of methanolic hydrochloric acid, as the latter had been employed successfully in earlier studies [2]. Although the desired dimers did not result, important information was obtained which formed the basis for the subsequent successful approach, so that a brief discussion of these experiments is warranted.

It was clear from the initial investigations that the catharanthine N-oxide (XII) or other intermediates formed from the reaction of III with MCPBA were *very sensitive* to reaction conditions (temperature, solvent, reaction work-up, *etc.*); *e.g.* chromatographic purification of the peracid reaction product mixture provided a substance which was believed to be a rearrangement product of XII although its structure could not be established with certainty. In view of the apparent instability of these substances minimum handling and purification of the reaction mixture appeared to be appropriate. On this basis, the hydrochloride of III was treated with MCPBA, the resultant product mixture rapidly chromatographed, and the chromatographic fractions immediately underwent reaction with vindoline (II) in the presence of hydrochloric acid. After 10 days standing, the reaction mixture was treated with sodium borohydride to remove the anticipated iminium salt intermediates and finally

[3]) For abbreviations see beginning of Experimental Part.

purified to provide a dimeric product in low yield. Spectroscopic data (see Exper. Part) did not establish conclusively the structure but a tentative assignment as shown in XV (vind = vindoline) could be made.



XV

Although the isolated product did not possess all of the required features of the vinblastines, its dimeric nature provided considerable stimulus for further related study. Clearly its mode of formation must involve fragmentation of the catharanthine system in some fashion and subsequent reaction with vindoline; for which one attractive pathway could involve a *Polonovski*-type fragmentation as shown in *Scheme 3* (XII → V → XV). If appropriate refinement of reaction conditions could be achieved this approach might provide an attractive synthetic access to the bisindole alkaloids. The results presented below reveal that this is indeed the case.

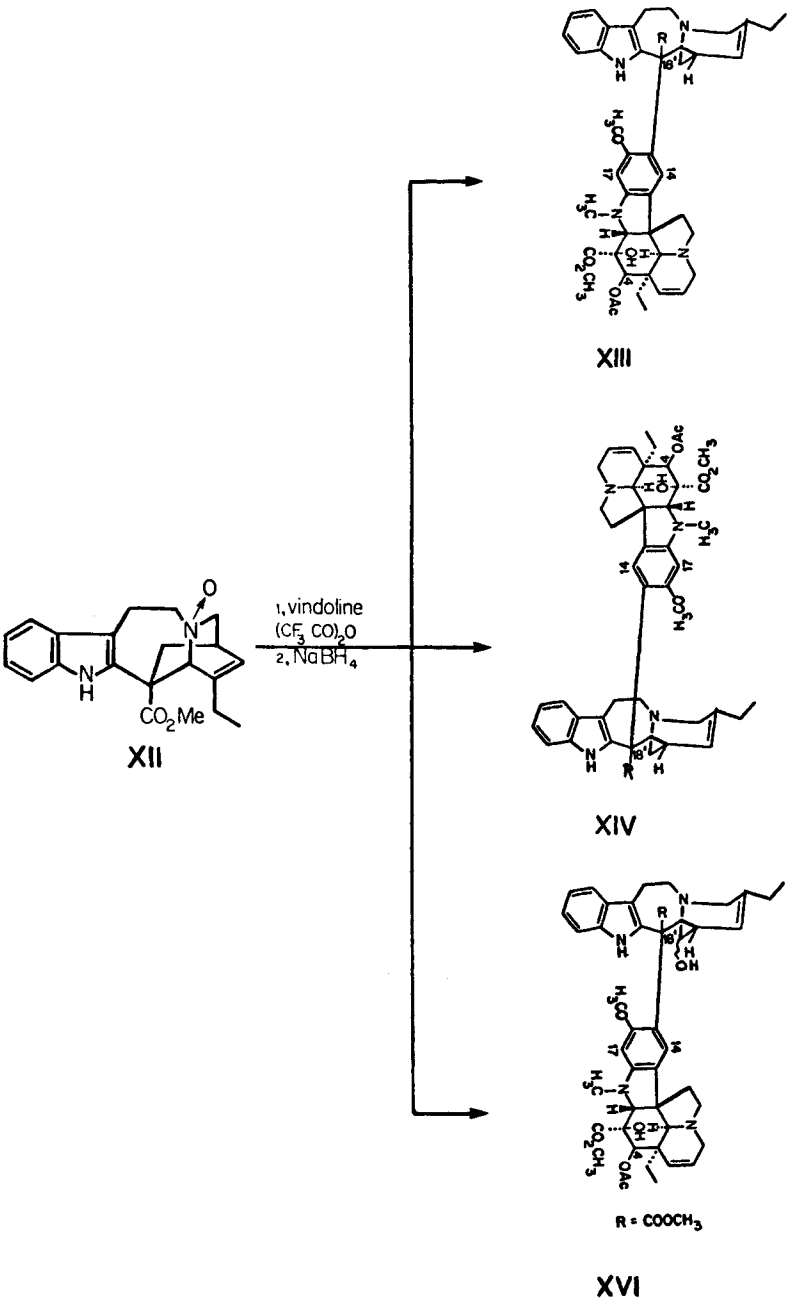
For a more successful 'biogenetic' approach to the bisindole alkaloids, as summarized in *Scheme 3*, it was necessary to determine the optimum conditions for the preparation of XII and related N-oxides, and in particular, the rearrangement and/or decomposition of these reactive substances must be minimized. It could be shown from the numerous experiments conducted that catharanthine and its various derivatives could be converted to the N-oxides by reaction with MCPBA acid in CH_2Cl_2-solution. Optimum yields and purity of the product are generally attained at low temperatures (−30° to 0°) and although XII has been obtained by careful purification (see Exper. Part) it is generally preferable to generate the N-oxides *in situ*.

A large number of experiments involving the N-oxide intermediates and vindoline or its derivatives were performed. For the sake of clarity the coupling of XII with II at low temperature is considered as the 'standard' reaction and differences in yields, configuration of dimers obtained, *etc.* are compared to this reaction. *Scheme 4* shows the products obtained.

When XII, generated *in situ*, reacted with vindoline in CH_2Cl_2 at low temperature (−50°) in the presence of trifluoroacetic anhydride, three products could be recognized after chromatographic purification. Based on very numerous spectroscopic data obtained previously in the bisindole field [2] it was possible to assign conclusively the structures for two of these products.

The major product, obtained consistently in about 50% yield, was crystalline, m.p. 171–173°, and could be assigned structure XIII. The molecular formula, $C_{46}H_{56}N_4O_8$, obtained by high resolution mass spectrometry (mol. wt.: 792.410; found: 792.405), while the NMR. spectrum, virtually on its own, established the postulated structure. As already detailed in the previous publication [2] NMR. spectra for the bisindoles are extremely informative since the signals arising from the indole and dihydroindole units are well separated and the spectrum of the dimer is, in effect, a summation of the spectra for the two monomeric units involved. The point of attachment, C(18′)–C(15), is immediately apparent from two one-proton signals at δ 6.11, H–C(17) and 6.59, H–C(14). Furthermore the configuration at C(18′)

Scheme 4. *Coupling of catharanthine N-oxide* (XII) *with vindoline* (II)



is also suggested from the chemical shift of the C(14) proton signal. It had been demonstrated previously [2] that this proton signal is sensitive to the configuration at C(18′) (δ 6.58 in vinblastine *vs.* δ 6.98 in XIV). Conclusive evidence for this stereochemical assignment was furnished from the CD. measurement (227 nm, $\Delta\varepsilon + 27$; 210 nm, $\Delta\varepsilon - 30$). A separate detailed study of the application of the CD. method has been recently published [10]. The UV. spectrum of XIII was, as expected, a superposition of the indole and indoline absorptions, and in excellent agreement with that reported for vinblastine. On this basis it was clear that the major product obtained in this reaction contained the desired natural configuration at C(18′) and was therefore an isomer of XIV, the latter having been established previously by chemical and spectroscopic evidence, including X-ray analysis [2] [11]. Product XIII was therefore 3′4′-dehydro-vinblastine. The unnatural configuration isomer XIV [2] [11] was generally not observed in the 'standard' coupling procedure.

A second dimeric product also often obtained, in 8–18% yield, could be assigned the interesting structure XVI, after extensive spectroscopic analysis. Its dimeric nature was clear from the mass spectrum (*m/e* 790, $M^+ - 18$) although the molecular ion peak could not be observed. The UV. spectrum (312, 295, 285, 263 and 213 nm) was in agreement with that obtained for the other dimers, thereby revealing that both indole and dihydroindole chromophores were intact. The point of linkage of the two monomeric units, C(18′)–C(15), was established from the NMR. spectrum which revealed two one-proton singlets at δ 6.13, H–C(17) and 6.47, H–C(14). In addition a new one-proton doublet at δ 3.99 ($J = 14$ Hz), never previously observed in the dimers, was assigned to a proton of a carbinolamine system ($>$N–C*H*OH).

During the various studies in our laboratory on indole and dihydro-indole alkaloids, we have also pursued an extensive investigation of the ^{13}C-NMR. spectra of these substances [12]. These data were invaluable in completing the structural assignment of dimer XVI. Thus in the various dimers examined, *e.g.* XIII, the C(19′) signal is seen at 46 ppm (in TMS), in good agreement with that reported for this carbon atom in vinblastine (47.5 ppm) [13]. However in the case of XVI this signal is lacking and is replaced by a new signal downfield at 76.5 ppm, a chemical shift which is in excellent agreement with that obtained in our studies on two known carbinolamine systems in the catharanthine series (C(19) signal at 76.6 ppm for 19-hydroxycatharanthine and 77.8 ppm for 19-hydroxy-decarbomethoxy-catharanthine) [12]. Conclusive chemical evidence supporting the structural assignment XVI was obtained when the latter was treated with tin and hydrochloric acid to provide the previously described dimer XIII. To summarize, these data allowed the assignment of XVI as 19′-hydroxy-3′,4′-dehydrovinblastine; the configuration of C(19′) remains uncertain.

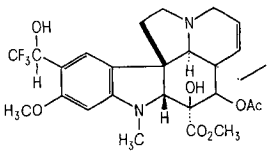
Obviously XVI could be considered as a further oxidation product of the initially formed dimer XIII. Support for this suggestion is obtained by investigating the reaction of XII and II under 'standard' conditions *but* in an oxygen atmosphere, when XVI is isolated from the mixture in much higher yield (33%), and dimer XIII in only 28% yield. On the other hand if the coupling is performed in an inert atmosphere (*e.g.* argon) the formation of XVI is diminished. These results were available only after the studies discussed below were performed, with the result that the various experiments were not conducted with rigorous exclusion of oxygen from the medium, so that variations in the yields of XVI may be simply due to this.

We can now present the various investigations conducted with the intention of evaluating the various reaction parameters in terms of yields and configuration of the isolated dimers. It was hoped that they would provide information relating to the optimum conditions for obtaining these compounds and might in turn also shed some light on the mechanism of the coupling process.

In the first series of coupling reactions of XII with II variation of temperature was investigated; all other parameters (unless noted) were identical with those for the above 'standard' reaction.

Temperature has a pronounced effect on the course of the reaction, the most noteworthy feature being the increased amount of the dimer with unnatural configuration at C(18') (XIV) with increase in temperature, indicating attack of vindoline at the β-face of the indolic intermediate generated, as previously observed in the chloroindolenine reaction [2]. (See Table 1 in Exper. Part for details.)

In addition to the dimers XIII, XIV and/or XVI formed during the reaction: at various temperatures, another product was produced in all studies conducted at $-10°$ and above. This substance was subsequently shown to be a mixture of two isomers, of which the less polar had a retention time, on thin layer chromatoplates, which was very similar to that of XVI. By spectroscopic analysis (see Exper. Part) both isomers could have structure XVII but different configuration of the C(15) side chain. Clearly acylation of a *Friedel-Crafts* type, involving trifluoroacetic anhydride and the highly reactive aromatic system of vindoline, provides the initial product and the latter undergoes reduction with sodium borohydride to provide the two possible isomers.



XVII

The effect of solvent for coupling under 'standard' conditions was also studied in some detail and the results indicate that CH_2Cl_2 is the best solvent to employ if the natural configuration dimer XIII is desired. (See Table 2 in Exper. Part for details.)

The reaction course is also very sensitive to the manner in which the N-oxide is generated, *i.e.* the molar equiv. of MCPBA employed and its mode of addition. Initial experiments showed that only a very slight excess of the peracid should be employed to obtain optimum yields of dimers. The results of further experiments are curious. If catharanthine N-oxide is first isolated from the peracid reaction and then exposed to reaction with vindoline in the presence of trifluoroacetic anhydride, *i.e.* approx. normal *Polonovski* conditions, the yields of the isolated dimers are very low. Addition of a catalytic amount of the peracid (Expt. 17) to the whole mixture increases significantly the overall yields of dimers. It is perhaps pertinent that in the latter experiments the N-oxide was more rigorously purified by column chromatography whereas earlier the N-oxide was obtained by solvent extraction and then employed immediately for reaction with vindoline, but whether this difference in

procedure is responsible for the difference in yields is not known with certainty. (For details see Table 3, Exper. Part.)

The *Polonovski* reaction has been studied over the years by numerous investigators [9] [14] using various reaction conditions and reagents. In addition to trifluoroacetic anhydride utilized above, originally employed by a French group in a modified *Polonovski* reaction [9], it was of interest to evaluate other reagents with a view to improved results. It was clear that trifluoroacetic anhydride was the most efficient reagent for effecting the desired transformation to the bisindole series. (For details, see Tables, Exper. Part.)

Finally the overall effect of altering the molar ratios of III and II was ascertained in terms of the yields and nature of the dimeric products. The pertinent data are presented in Table 5 (see Exper. Part).

As sequel to the various investigations above it was of interest to apply this procedure to other catharanthine derivatives.

Dihydrocatharanthine N-oxide (XVIII) obtained in the usual manner from dihydrocatharanthine was selected for the next series of investigations. As in the previous studies, the number and nature of the dimeric products were markedly dependent on reaction conditions; a summary of the results is provided in *Scheme 5*.

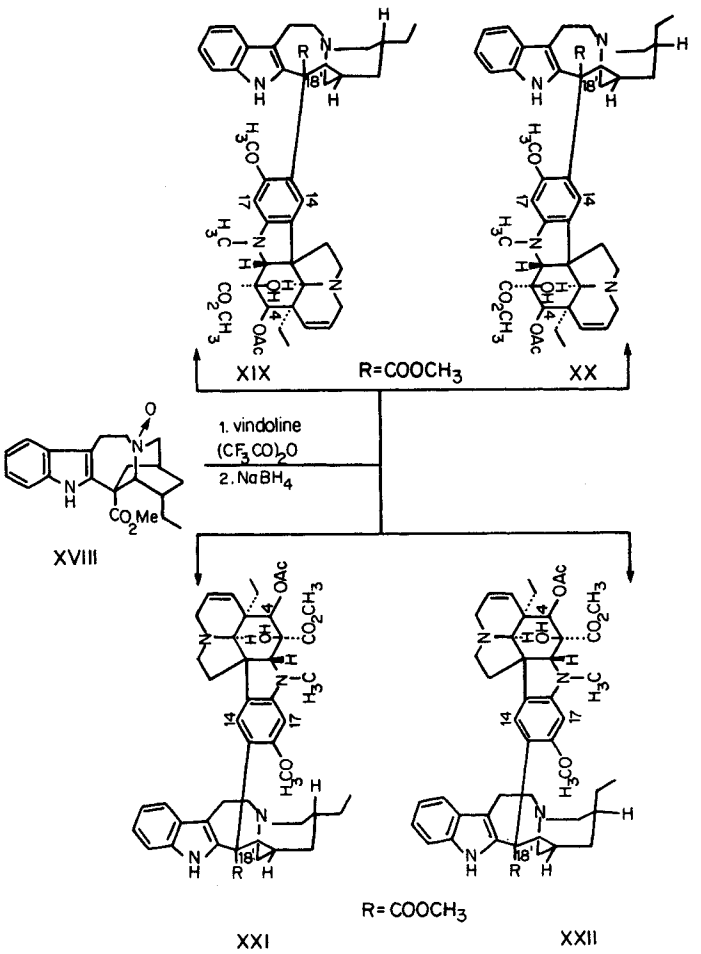
A notable first point is that for dihydrocatharanthine the yields of the desired dimers are significantly lower (usually 5–20%), presumably due to the absence of the double bond which would tend to stabilize the intermediate resulting from the fragmentation of the catharanthine system.

Reaction of XVIII with vindoline at $-10°$, or above, gave four recognizable dimeric products (XIX–XXII). At -50 to $-30°$ only the two dimers XIX and XX with the natural configuration at C(18′) were obtained. As before, extensive spectroscopic analysis allowed definitive assignments of structure to all of these products.

The major component was obtained crystalline, m.p. 190–194°, and its molecular formula, $C_{46}H_{58}N_4O_8$, obtained by high resolution mass spectrometry (mol. wt.: calc.: 794.425; found: 794.422), differed from that of the previously studied dimers by the expected 2 mass units. The two centres, C(18′) and C(15), involved in the attachment of the indole and dihydroindole units, were established by NMR. spectroscopy (δ 6.58, *s*, H–C(14) and 6.20, *s*, H–C(17)), and the latter spectrum was again extremely informative in regard to the overall structure. The circular dichroism measurement (227 nm, $\Delta\varepsilon$ + 26; 209 nm, $\Delta\varepsilon$ – 47) established the configuration at C(18′) as portrayed in XIX.

The question of configuration at C(4′) the ethyl bearing in XIX, and in the isomeric XX, was settled in an interesting manner. It had been already established [2] [11] that catalytic hydrogenation of the C(3′),C(4′) double bond in XIV proceeds with hydrogen adsorption from the α-face of the indole residue to produce exclusively the dimer XXII. This accords with expectation since molecular models readily reveal that adsorption of XIV onto the catalyst surface must occur from the side *opposite* to that occupied by the large vindoline molecule. Thus, it is obvious that a similar reduction of XIII must produce exclusively the dimer XIX, exactly as obtained. When XIII was exposed to controlled conditions (Pt as catalyst) the pro-

Scheme 5. *Products resulting from dihydrocatharanthine N-oxide coupling with vindoline*



duct isolated was identical in every respect with the major component obtained in the coupling studies with XVIII. To summarize these data established the structure of XIX as 4'-deoxovinblastine, which had been isolated earlier by the *Lilly* group [15] but unfortunately a suitable sample could not be obtained for comparison.

The other product, XX, obtained in lower yield but still with natural configuration C(18'), was shown to be the C(4') epimer of XIX, readily revealed from the mass spectrometric ($C_{46}H_{58}N_4O_8$ requires 794.425; found, 794.421), NMR. (δ 6.13, *s*, H–C(17) and 6.61, *s*, H–C(17)) and CD. data (226 nm, $\Delta\varepsilon$ + 17; 212 nm, $\Delta\varepsilon$ – 36). Again comparison could not be made with a previously isolated sample [15].

The two other products obtained in the above studies, if carried out above –10°, were the dimers XXI and XXII with unnatural configuration at C(18'). The latter

isomer was shown to be identical with the dimer obtained previously [2] [11] for which a complete X-ray analysis had been performed.

The isolation of four dimeric products, two in each series, differing at the C(4') ethyl bearing site may, at the outset, seem curious. In fact, the results are readily explicable in terms of the well-known imine-enamine equilibrium established previously in the fragmentation reactions of the catharanthine series [5] [6] [16]. Thus if the fragmentation of XVIII generates an enamine intermediate, the subsequent borohydride reduction would be expected to lead to the isomeric products isolated.

The next series of investigations, in terms of extending the *Polonovski*-type fragmentation approach to other dimeric systems, concerned decarbomethoxycatharanthine, originally obtained during the structural studies on catharanthine [5]. Unfortunately in this latter study only low yields of this compound were obtained so an improved procedure had to be developed. After numerous experiments, a refinement of reaction conditions has led to consistently high yields (about 85%).

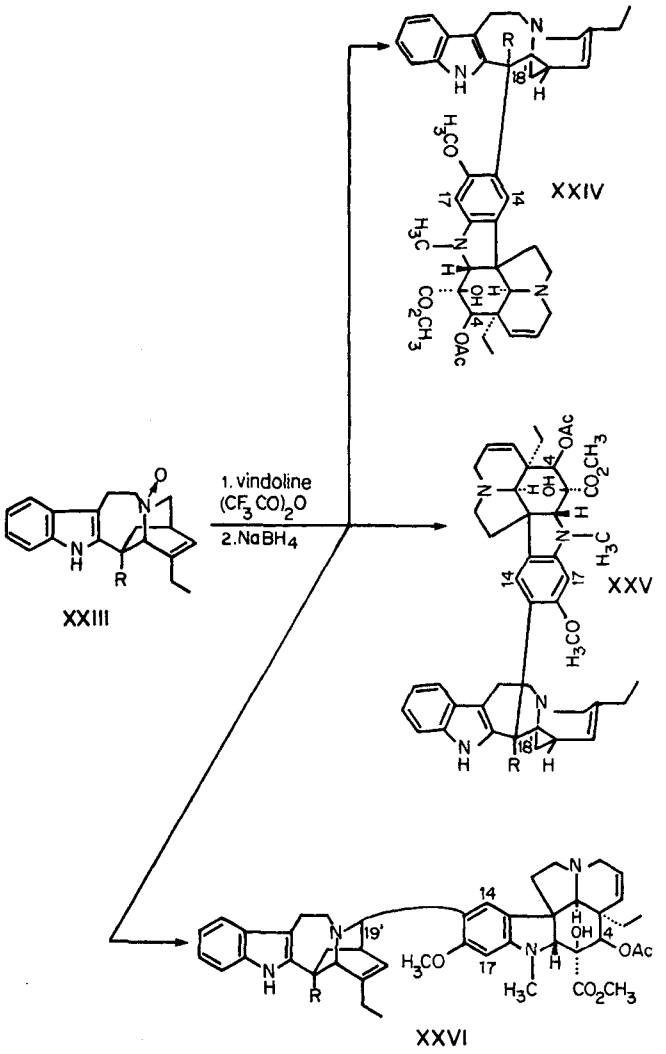
When decarbomethoxycatharanthine N-oxide (XXIII, R = H) reacted with vindoline at low temperature (−15 to −30°) three dimeric products were recognized; *Scheme 6* illustrates their structures.

The major component, obtained in 27% yield, possessed the natural configuration at C(18') (CD.: 224 nm, $\Delta\varepsilon + 23$; 257 nm, $\Delta\varepsilon - 3$) and from other spectral data it was clear that it was 18'-decarbomethoxy-3',4'-dehydrovinblastine (XXIV, R = H). Chemical evidence in support of this assignment was obtained on treatment with tin and hydrochloric acid as described previously [2] which gave vindoline, deacetylvindoline and cleavamine. A second component (11% yield) was the known dimer XXV (R = H) characterized previously [2] [11].

The third dimeric product (32% yield) isolated from this reaction exhibited spectral properties which were somewhat different from those of the dimers studied previously. The molecular formula, $C_{44}H_{52}N_4O_6$, determined by high resolution mass spectrometry ($C_{44}H_{52}N_4O_6$ requires 732.928; found 732.926) differed from that of XXIV (and/or XXV) by 2 mass units and suggested that intact decarbomethoxycatharanthine had coupled with vindoline. The NMR. spectrum, as before, was in effect a summation of the signals expected for the two monomeric units, while the CD. spectrum was completely different from that obtained previously (215 nm, $\Delta\varepsilon - 14$; 250 mm, $\Delta\varepsilon + 16$). Cleavage of this dimer with tin and hydrochloric acid provided vindoline and decarbomethoxycatharanthine. On this basis, this dimeric product is assigned the structure XXVI (R = H). It is considered to arise from a normal *Polonovski* elimination occurring with XXIII thereby generating the iminium intermediate $>C(19') = N^+<$, which then reacts with vindoline in the expected fashion. It is interesting to note that this was the only case in which this type of dimer was recognized although this does not exclude the presence of such compounds (in minute amounts) in some of the other reaction mixtures. The complexity of the reaction mixtures often precluded isolation of very minor components.

Gerzon et al. [17] have recently reported that vinblastine and vincristine derivatives in which the carbomethoxy group of the vindoline unit is replaced by an amide function possess very interesting anti-tumor properties. Consequently it was appropriate to evaluate the above method with vindoline-N-methyl-amide (XXVII,

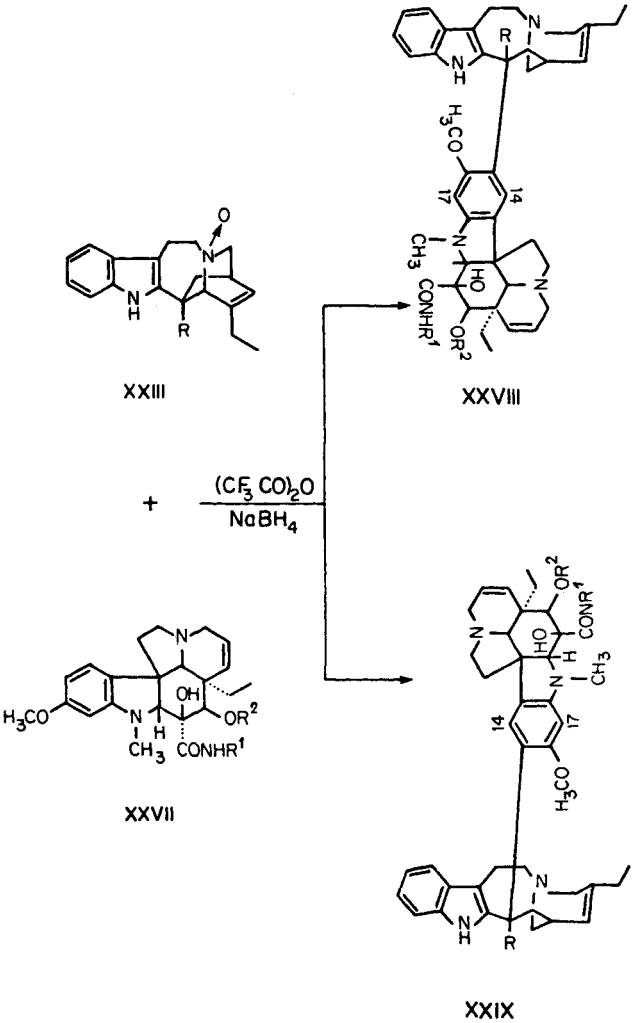
Scheme 6, *Coupling of decarbomethoxycatharanthine N-oxide* (XXIII) *with vindoline* (II)



$R_1 = CH_3$; $R_2 = CH_3CO$) and either catharanthine N-oxide (XII) or decarbomethoxy-catharanthine N-oxide (XXIII, R = H) since these should provide novel dimers which closely resemble the amido-vinblastines.

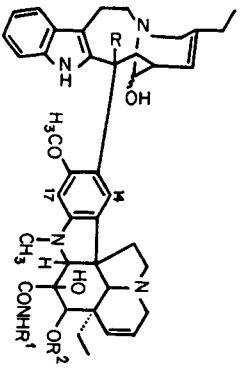
By reaction of XXIII (R = H) and vindoline N-methyl-amide under *Polonovski*-type conditions, both the natural (XXVIII) and unnatural (XXIX) ($R = H$; $R^1 = CH_3$; $R^2 = CH_3CO$) configuration dimers were obtained (*Scheme 7*). However an interesting phenomenon was observed and it was evaluated further since it was obviously another reaction parameter, in addition to those detailed earlier, which should be considered for providing optimum conditions for preparation of the required dimers. It appears

Scheme 7. *Coupling of decarbomethoxycatharanthine N-oxide* (XXIII, R = H) *and/or catharanthine N-oxide* (XXIII, R = COOMe) *with vindoline N-methylamide* (XXVII, R = Me)



that in this coupling reaction the volume of solvent employed also has a rather significant effect on the yields and nature of the products formed. Details concerning the effect of temperature and concentration in this coupling process are given in Table 6 (Exper. Part).

Finally the reaction of catharanthine N-oxide (XII) with vindoline N-methyl amide was conducted according to the procedure already described. The major component generally obtained in 25–50% yield was the desired dimer XXVIII possessing the natural configuration at C(18′) (XXVIII) and, in minor amounts (2–7%, depending on conditions), the carbinol amide dimer XXX, (for both: R = CO_2CH_3; $R^1 = CH_3$; $R^2 = CH_3CO$), corresponding to dimer XVI obtained in earlier studies.



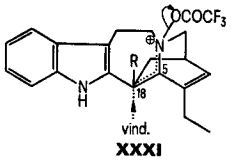
XXX

To summarize, the investigations discussed above, employing various substrates and reaction conditions, have demonstrated the application of the *Polonovski*-type fragmentation in the synthesis of a series of bisindoles closely related to the vinblastines. The following generalizations with respect to these studies are possible: 1) catharanthine is the best substrate and generally leads to highest yields of dimers; 2) the absence of a double bond, for example, dihydrocatharanthine, does not prevent fragmentation of the N-oxide derivative but, in general, the products are obtained in lower yield and are isomeric at the C(4′) ethyl bearing centre; 3) the absence of an ester group, as in decarbomethoxycatharanthine, appears to reduce reactivity toward fragmentation and generally leads to lower yields of dimers; 4) the ratio of natural *vs.* unnatural configuration at C(18′) in the resultant dimers is markedly dependent on reaction conditions, particularly temperature. In all cases studied, the highest ratio of natural configuration dimers is obtained when very low temperatures are employed. Obviously if the reactivity of the indole-N-oxide intermediate toward *Polonovski* fragmentation is low, such low temperatures are precluded so the yields of desired dimers become minimal.

It is appropriate at this stage to put forward a rationale which attempts to explain the various results obtained. It seems likely that the *Polonovski* fragmentation process may proceed according to several routes: 1) a truly 'concerted' or S_N2'-like mechanism in which there is a simultaneous fragmentation of the C(18),C(5) bond and formation of the C(18′),C(15) bond in the resulting dimer; 2) a step-wise process in which fragmentation of the C(18),C(5) bond generates an intermediate (XII → V, *Scheme 3*) and the latter then reacts with vindoline to provide the dimer, and 3) both mechanisms occur depending on the particular *Iboga* skeleton employed.

With respect to the 'concerted' mechanism, as mentioned earlier, electronic and steric requirements are such that the vindoline molecule must enter from the α face of the molecule thereby generating the *natural* configuration at C(18′) (see XXXI). It is difficult to imagine how a 'concerted' process could explain the dimeric products with unnatural configuration at C(18′).

Regardless of mechanism, catharanthine is expected to be the preferable substrate among those studied. Thus the orbital being formed at C(5) could interact with the

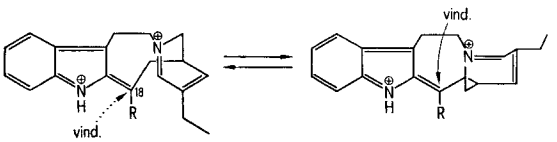


XXXI

π system of C(3),C(4) double bond, thus providing stabilization for the intermediate and lowering the activation energy. Also since build-up of some charge is required at C(18) during the fragmentation process, the C(18) carbomethoxy group will aid in this process as well. On this basis, it is expected that dihydrocatharanthine, which lacks the double bond, and/or decarbomethoxycatharanthine which does not possess the C(18) ester group, would be less favourable substrates for the desired fragmentation. Indeed as shown, both of the latter generally provide lower yields of dimeric products and, at least in the case of decarbomethoxycatharanthine, an alternative fragmentation occurs (see dimer XXVI).

The alternative step-wise process schematically represented by the conversion XII→V, also provides an attractive rationalization of the obtained results. Conformational considerations of the intermediate thus formed allow an explanation for the formation of the various dimers isolated under the various reaction conditions (temperature, *etc.*) studied.

It is obvious that for V various conformations are possible of which only two will be considered since they suffice for the required explanation. Thus if fragmentation of the rigid structure inherent in the catharanthine system occurs, the initially formed intermediate possesses what we will term the '*Iboga*' conformation V. If reaction conditions (temperature, solvent, *etc.*) allow, V can undergo alteration to conformation XXXII, the latter having been established as that for the indole unit in vinblastine (I) and our previous synthetic dimers, from three independent X-ray studies [2] [11]. Molecular models reveal that if the initially formed intermediate is maintained in



V XXXII

the '*Iboga*' conformation V, preferential attack of the vindoline molecule during the coupling process, would occur from the α face thereby producing the natural configuration dimers. On the other hand if conformational conversion to XXXII occurs, the β face of the indolic unit becomes more accessible and the coupling results in an unnatural configuration at C(18'). Obviously reaction conditions, particularly temperature for example, will play a dominant role in establishing any conformational mobility between the above-mentioned conformations (as well as others). As Table 1 reveals, the relative proportion of unnatural configuration dimer XIV increases with temperature and similar results are noted in the dihydrocatharanthine and decarbomethoxycatharanthine series. It is therefore tempting to postulate that at low tem-

peratures (−50°) where only the natural configuration is observed *e.g.* (Expt. 1), the initially formed intermediate retains the '*Iboga*' conformation and provides the natural dimer XIII while higher temperatures allow conversion to XXXII and, in turn, dimer XIV. In the studies involving decarbomethoxycatharanthine, for example, the required fragmentation can only be achieved at higher temperatures, when a mixture of natural and unnatural configuration dimers is always obtained. Thus the rationalization involving a step-wise process can provide an explanation for all of our results whereas the 'concerted' mechanism seems limited to only the natural configuration dimers. Whether both mechanisms are operative in the various studies remains an open question.

This 'biogenetic' approach does indeed provide an attractive alternative to the chloroindolenine method employed earlier [2] and further studies are under way.

An independent investigation of the *Polonovski* reaction for the synthesis of these types of compounds has been recently reported by the French group [18].

Experimental Part

General m.p.s. were determined on a *Kofler* block and are uncorrected. UV. spectra (λ_{max} (log ε)) were measured in 95% ethanol or methanol on *Cary* model 11, 14 or 15 spectrophotometers. IR. spectra were recorded in $CHCl_3$ on a *Perkin-Elmer* 21 or 137 spectrophotometer. NMR. spectra were taken in deuteriochloroform solution on *Varian* spectrometers, models HA-100 or XL-100. Line positions are given in the δ scale, with TMS as internal standard. The types of protons, integrated areas, multiplicity and spin coupling constant J (in Hz) are indicated in parentheses. MS. (main peaks, *m/e*) were recorded on an *Atlas* CH-4B or AEI MS-902 spectrometer, high resolution measurements being determined on the latter instrument.

Silica gel G and aluminia *Woelm* containing 2% by weight of a fluorescent indicator were used for thin-layer chromatoplates (TLC.). As spraying reagent a solution of 1:2 of antimony pentachloride in carbon tetrachloride, or a solution of ceric sulfate in aqueous sulfuric acid were used extensively. Unless otherwise specified column chromatography was performed using either *Woelm* grade silica or neutral alumina, and deactivated as required with the correct amount of water.

Abbreviations: MCPBA = *m*-chloroperbenzoic acid; TFA = trifluoroacetic anhydride; CH_2Cl_2 = methylene chloride; TMS = tetramethylsilane; RT. = room temperature; TLC and TkLC = thin and thick (resp.) layer chromatography.

Elemental analyses were performed by Mr. *P. Borda* of the Microanalytical Laboratory, University of British Columbia.

Reaction of Catharanthine (III) with MCPBA. Isolation of Catharanthine N-Oxide (XII). – a) *Reaction at room temperature.* To the clear colourless solution of III (250 mg) dissolved in CH_2Cl_2 (35 ml), 1.1 mmol solid MCPBA were added. After stirring for 1 h at RT. the clear colourless solution was shaken with ice cold 10% aqueous $NaHCO_3$-solution (25 ml), the organic phase separated, dried over anhydrous sodium sulfate, filtered and taken to dryness under vacuum to yield a white glass (262 mg). This was quickly chromatographed on deactivated alumina (neutral *Woelm* III, 20 g) by elution with CH_2Cl_2 (2 × 300 ml) and methanol (300 ml). The first two contained a product (35 mg) believed to be a rearrangement product of XII although its structure is not yet known with certainty. – IR.: 3450, 2950, 2920, 1725 cm^{-1}. – UV.: 293 (3.8), 285 (3.9), 276 (sh, 3.8), 226 (4.5) nm. – NMR.: 8.8 (br.*s*, 1H); 7.7–7 (*m*, 4H, aromatic); 6.25 (*s*, 1H); 4.58 (br.*d*, J = 9, 1H); 3.79 (*s*, 3H, CO_2CH_3); 1.20 (*t*, J = 7, 3H, CH_2CH_3); – MS.: 119, 121, 135, 204, 222, 248, 254, 352.

The major compound isolated was the desired XII. – IR.: 3460, 2960, 1730, 1740 cm^{-1}. – UV.: 291 (3.8), 283 (3.9), 275 (sh, 3.8), 224 (4.5) nm. – NMR.: 8.37 (br.*s*, 1H, NH); 7.58–7.10 (*m*, 4H, aromatic); 6.15 (*m*, 1H, H–C(3)); 4.73 (br.*s*, 1H, H–C(5)); 3.74 (*s*, 3H, CO_2CH_3); 1.12 (*t*, J = 7, 3H, CH_2CH_3). – MS.: 130, 135, 144, 218, 222, 248, 254, 336, 352 (base peak). The

structure of the N-oxide was substantiated by submitting it immediately to hydrogenation in ethanol over *Adam*'s catalyst (PtO_2); the sole product was identified by IR. and MS. as dihydrocatharanthine. The N-oxide XII underwent rearrangement, even when dried to a white powder and stored in an evacuated dessicator for 48 h, to yield a 1:1 mixture of XII and the above rearrangement product, the latter being stable to chromatography.

b) *Reaction at low temperature.* To 0.83 mmol of III, dissolved in CH_2Cl_2 (10 ml) and cooled to $-30°$, 0.985 mmol MCPBA was added and the solution stirred for 10 min. The solvent was carefully removed *in vacuo* and the residue chromatographed rapidly on a short column of neutral alumina (20 g, activity III). Initial elution with ethyl acetate (55 ml) removed unreacted peracid, and subsequent elution with ethyl acetate/methanol 4:1 provided XII, 91% yield. Since this procedure provided a product of superior quality it was employed in all cases where isolated N-oxide was required.

Reaction of catharanthine hydrochloride with MCPBA. To III-HCl (62.2 mg) dissolved in CH_2Cl_2 (7 ml), 1.1 mol-equiv. of MCPBA in CH_2Cl_2 solution (5 ml) were added over 15 min with stirring, washed in with a further 2 ml of solvent and the reaction mixture stirred at RT. for 32 h. The clear colourless solution was then washed with 10% aqueous $NaHCO_3$-solution (2×10 ml) and the organic phase dried over anhydrous sodium sulfate, filtered and evaporated to yield the crude reaction product as a white foam (64.4 mg). This was purified by TkLC. on alumina (2 plates 20 cm × 20 cm, 0.3 cm thickness) using chloroform/methanol 20:1 for elution. The major product was III (36.9 mg) identical by NMR., IR., UV., and MS. to authentic material; minor products included its N-oxide XII (9.9 mg) and baseline material (4.6 mg). The total weight recovery was 83.5%.

Reaction of catharanthine (III) hydrochloride with vindoline (II) and MCPBA. Isolation of dimer [XV. To 710 mg of III-HCl dissolved in CH_2Cl_2 (30 ml), cooled to 0°, a solution of excess MCPBA (2.2 g) in CH_2Cl_2 (30 ml) was added dropwise over 40 min, the reaction mixture warmed to RT. and stirred for 39.5 h. Solid sodium sulfite was then added and the resulting suspension was stirred for a further 10 min, after which it was partitioned between ice cold water and CH_2Cl_2. The organic phase was washed with 10% aqueous $NaHCO_3$-solution (2×25 ml) and then dried over anhydrous sodium sulfate, filtered and the solvent removed under reduced pressure to yield a whitish-yellow foam (702 mg) which was chromatographed on alumina (neutral *Woelm* III, 100 g). Benzene elution furnished a compound (31 mg) (in fractions 4, 5 and 6) which was different from the starting material or its N-oxide. – NMR.: 7.8–7.0 (*m*, 4H, aromatic); 6.0 (br.*d*, 1H, olefinic); 4.78 (br.*s*, 1H, H—C(5)); 3.62 (*s*, 3H, CO_2CH_3); 1.03 (*t*, J = 8, 3H, CH_2CH_3).

This material was dissolved in methanol (5 ml) containing II (35 mg) and 1 M hydrochloric acid (7 ml) was added. The reaction flask sealed under nitrogen and the mixture stirred at RT. for 10 days, during which it changed from a light yellow colour to a deep, almost opaque, emerald green. It was then poured into a beaker containing water (10 ml) and CH_2Cl_2 (30 ml) with stirring, when all remained in the aqueous phase. The addition of small amounts of sodium borohydride (0.5 g in small portions over 20 min) with rapid blending of the two phases resulted in the sudden loss of colour and concomitant transfer into the organic phase, which was separated and the aqueous phase washed with further portions (2×25 ml) of CH_2Cl_2. The combined organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent removed *in vacuo* to yield a yellowish glass-like material (60 mg) which fluoresced either in solution (methanol) or as a solid when exposed to UV. light. Column chromatography on alumina (neutral *Woelm* III, 20 g) yielded, on elution with benzene/ethyl acetate 4:1 dimeric material (in fractions 7 to 10) which proved to be the fluorescent principle in the reaction mixture (~30% yield). – UV.: 296 (3.8), 285 (3.9), 265 (4.0), 215 (4.5) nm. – NMR.: 8.0 (br.*s*, 1H); 7.7–7.0 (*m*, 5H, aromatic+H—C(14)); 6.06 (*s*, 1H, H—C(17)); 6.0 (br.*d*, J = 7, 1H); 5.82 (*d*×*d*, J = 10 and 4, 1H, H—C(7)); 5.48 (*s*, 1H, H—C(4)); 5.22 (*d*, J = 10, 1H, H—C(6)); 4.76 (br.*s*, 1H); 4.28 (*m*, 1H); 3.80 (*s*, 3H, CO_2CH_3); 3.76 (*s*, 3H, CO_2CH_3); 3.58 (*s*, 3H, OCH_3); 2.65 (*s*, 3H, N—CH_3); 1.05 (*s*, 3H, OAc—C(4)); 1.21 (*t*, J = 7, 3H, CH_2CH_3); 0.71 (*t*, J = 7, 3H, CH_2CH_3). – MS.: 121, 122, 135, 136, 158, 174, 188, 222, 266, 282, 296, 309, 415, 457, 469, 521, 523, 617, 624, 683, 791, 792. Mol. wt.: 792.412. Calc. for $C_{46}H_{56}N_4O_8$: 792.410. On the basis of this data this dimeric product was tentatively assigned structure XV.

Reaction of Catharanthine N-oxide (XII) with Vindoline (II) in presence of TFA. – *The 'Standard' Coupling Conditions.* (Expt. 1 of Table 1). The necessary XII was first prepared *in situ* by addition to III (0.188 mmol) dissolved in CH_2Cl_2 (2 ml) cooled to $-3°$, 0.213 mmol of MCPBA (36.7 mg) and stirring for 10 min. For subsequent coupling 0.203 mmol of II in CH_2Cl_2 (2 ml) was added, the mixture purged with argon and cooled to $-50°$. Anhydrous TFA (1.1 mmol, distilled from P_2O_5) was added as rapidly as possible by means of a microsyringe, causing colour change from pale yellow to dark burgundy in about 10 min. The reaction mixture was stirred for 3 h while maintaining the temperature at $-50°$, and then added rapidly to a prepared solution of sodium borohydride (500 mg) in ethanol (10 ml) at RT. This mixture was then treated with water and CH_2Cl_2, the organic layer separated, dried over sodium sulfate, and the solvent removed *in vacuo*. Purification of the resultant residue by TkLC. (silica, ethyl acetate/methanol, 85:15) provided four products.

The first product (least polar on TLC.) to be isolated (12.4 mg, 8%) was identified as 19'-hydroxy-3',4'-dehydrovinblastine (XVI). – IR.: 3460, 2900, 1730 cm^{-1}. – UV.: 307 (3.86), 293 (4.11), 283 (4.17), 262 (4.28), 213 (4.78) nm. – CD.: 305 (+8), 259 (+22), 226 (+43) nm. – NMR.: 8.07 (br. *s*, 1H, OH or NH); 7.65 (*m*, 1H, H—C(14')); 7.15 (*m*, 3H, H—C(11', 12', 13')); 6.47 (*s*, 1H, H—C(14)); 6.13 (*s*, 1H, H—C(17)); 5.87 (*m*, 1H, H—C(7)); 5.54 (*m*, 1H, H—C(3')); 5.46 (*s*, 1H, H—C(4)); 5.29 (*d*, J = 10, 1H); 3.97 (*d*, J = 12, 1H, H—C(19')); 3.80 (*s*, 3H, OCH_3); 3.83 (*s*, 3H, OCH_3); 3.65 (*s*, 3H, OCH_3); 2.72 (*s*, 3H, N—CH_3); 2.11 (*s*, 3H, $COCH_3$); 1.03 (*t*, J = 7, 3H, CH_2CH_3); 0.77 (*t*, J = 8, 3H, CH_2CH_3). This substance consistently occluded solvent which could not be removed without decomposition.

$C_{46}H_{56}N_4O_8 \cdot 2CH_3OH$	Calc.	C 66.06	H 7.39	N 6.42%
(872.4)	Found	,, 66.09	,, 7.26	,, 6.42%

The next product (34%) was simply recovered II while the third substance (50%) was characterized as 3',4'-dehydrovinblastine (XIII), m.p. 171–173° (dec). – IR.: 3462, 2900, 1735 cm^{-1}. – UV.: 292 (4.08), 285 (4.13), 261 (4.24), 212 (4.74) nm. – CD.: 305 (+6.7), 262 (+9.1), 227 (+26.7), 210 (−29.8) nm. – NMR.: 9.76 (br. *s*, 1H, NH); 8.02 (br. *s*, 1H, OH); 7.50 (*m*, 1H, H—C(14')); 7.10 (*m*, 3H, H—C(11',12',13')); 6.59 (*s*, 1H, H—C(14)); 6.11 (*s*, 1H, H—C(17)); 5.72 (*m*, 1H, H—C(7)); 5.44 (*m*, 1H, H—C(3')); 5.44 (*s*, 1H, H—C(4)); 5.27 (*d*, J = 10, 1H, H—C(6)); 3.78 (*s*, 3H, OCH_3); 3.76 (*s*, 3H, OCH_3); 3.58 (*s*, 3H, OCH_3); 2.68 (*s*, 3H, N—CH_3); 2.07 (*s*, 3H, $COCH_3$); 0.98 (*t*, J = 7, 3H, CH_2CH_3); 0.79 (*t*, J = 8, 3H, CH_2CH_3). – MS.: 106 (base peak), 108, 120, 121, 136, 282, 610, 716, 733, 792. Mol. wt.: 792.405. Calc. for $C_{46}H_{56}N_4O_8$: 792.410. As for XVI occlusion of solvent is serious.

$C_{46}H_{56}N_4O_8 \cdot 2CH_3OH$	Calc.	C 67.27	H 7.52	N 6.54%
(856.41)	Found	,, 67.13	,, 7.28	,, 6.53%

The final product, which appeared to be a dimer of vindoline, was of little interest and was not studied further.

Reduction of 19'-Hydroxy-3',4'-dehydrovinblastine (XVI) to 3',4'-Dehydrovinblastine (XIII). To a solution of tin (62.1 mg) and stannous chloride (60.4 mg), dissolved in aqueous hydrochloric acid (7%, 5 ml), a solution of dimer XVI (13.2 mg) in methanol (2 ml) was added. The mixture

Table 1. *Effect of temperature on reaction of catharanthine N-oxide XII with vindoline II* 'standard' conditions: expt. 1 (see text)

Expt.	Temp.°	Time	Reactions in mmol				Dimer products, yield %		
			III	MCPBA	II	TFA	XIII	XIV	XVI
1	−50	3 h	0.188	0.213	0.203	1.1	50	0	8
2	−10	overnight	0.147	0.162	0.154	0.733	30	14	[b]
4	− 4	3 h	0.198	0.216	0.20	1.027	38	18	[b]
5	+42	3 h	0.212	0.23	0.216	2.20	17	29	[b]
6[a]	+61	20 min	0.924	0.985	0.927	5.87	–	34	[b]

[a] $CHCl_3$ used as solvent.

[b] XVI could not be detected as in TLC. the spot is obscured by that of an impurity, a vindoline derivate XVII, obtained at this temperature. See Expt. 4 in text.

was then refluxed for 2.5 h, after which it was carefully made basic with ammonium hydroxide, filtered and extracted with CH_2Cl_2. The combined organic extracts were concentrated *in vacuo* and the resulting residue was purified by chromatography (silica gel, ethyl acetate/methanol 9:1) to yield pure dimer XIII (7.7 mg).

Experiment 2: see Table 1.

Experiment 3 differed from those in Table 1 by isolation of the intermediate N-oxide XII. To III (0.297 mmol), dissolved in CH_2Cl_2 (2.5 ml) and cooled to $-10°$, a solution of 0.321 mmol MCPBA in CH_2Cl_2 (1 ml) was added dropwise, over 15 min. The mixture was then washed with 10% aqueous $NaHCO_3$-solution, the aqueous layer extracted several times with ethyl acetate and the combined organic layers dried over anhydrous sodium sulfate. Careful removal of solvent *in vacuo* provided XII (61%). This experiment was repeated and the total amount of isolated XII (0.334 mmol) was employed in the coupling step as follows. After solution in CH_2Cl_2 (5 ml) vindoline (0.298 mmol) was added, the mixture purged with nitrogen and cooled to $-10°$. TFA (0.733 mmol) was added and the reaction allowed to proceed overnight at $-10°$, after which the mixture was allowed to react with a solution of sodium borohydride (1 mg) in methanol (5 ml) at RT. for 30 min. The solvent was removed *in vacuo* and the residue purified by TkLC. (silica gel, ethyl acetate/methanol 3:1) to provide dimer XIV in 31% and XIII in 14% yield.

Experiment 4. Supplement to data in Table 1: The yield of XVI also noted as a component in the reaction mixture, was difficult to establish accurately since the less polar epimer of XVII had very similar retention times on TLC. Separation of the XVII isomers was accomplished by TkLC. (silica gel, ethyl acetate/methanol 95:5).

For the less polar component: IR.: 3560, 3400, 2900, 1720, 1600 cm^{-1}. – UV.: 304 (3.96), 254 (4.18), 211 (4.67) nm. – NMR.: 6.94 (*s*, 1H, H—C(14)); 6.04 (*s*, 1H, H—C(17)); 5.83 (*m*, 1H, H—C(7)); 5.36 (*s*, 1H, H—C(4)); 5.15 (*m*, 2H, H—C(6) and CF_3CH)); 3.81 (*s*, 3H, OCH_3); 3,74 (*s*, 3H, OCH_3); 2.65 (*s*, 3H, NCH_3); 2.00 (*s*, 3H, $COCH_3$); 0.43 (*t*, $J = 7$, 3H, CH_2CH_3). – MS:. 121, 122, 135 (base peak), 158, 188, 266, 282, 286, 287, 394, 395, 426, 456, 554.

For the more polar component. IR.: 3560, 3400, 2900, 1720, 1600 cm^{-1}. – UV.: 304 (3.99), 254 (4.21), 211 (4.70) nm. – NMR.: 7.16 (*s*, 1H, H—C(14)); 6.09 (*s*, 1H, H—C(17)); 5.88 (*m*, 1H, H—C(7)); 5.42 (*s*, 1H, H—C(4)); 5.30 (*m*, 2H, H—C(6)+CF_3CH)); 3.84 (*s*, 3H, OCH_3); 3.79 (*s*, 3H, OCH_3); 2.71 (*s*, 3H, NCH_3); 2.05 (*s*, 3H, $COCH_3$); 0.48 (*t*, $J = 7$, 3H, CH_2CH_3). – MS.: 121, 122, 135 (base peak), 188, 282, 286, 287, 394, 395, 554.

Experiments 5 and 6: see Table 1.

Experiments 7–11: see Table 2.

Table 2. *Effect of solvent on reaction of catharanthine N-oxide (XII) with vindoline (II)*

Expt.	Solvent (vol. in ml)	Reactants in mmol				Yields of Dimers (%)			Reactants recovered %	
		III	MCPBA	II	TFA	XIII	XIV	XVI	III	II
1	CH_2Cl_2	0.188	0.213	0.203	1.1	50	0	8	–	–
7	$CHCl_3$	0.145	0.174	0.136	0.953	27	0	23	–	42
8	DMF (3)	0.134	0.142	0.138	0.908	0	0	0	small amounts	
9	THF (3)	0.175	0.187	0.177	1.10	0	0	0	63	94
10	CH_3CN	0.159	0.186	0.150	1.32	33	0	23	–	–
11[a])	toluene	0.143	0.161	0.200		0	0	0	–	–

[a]) Red gelatinous ppt. formed on addn. of TFA; reaction terminated.

Experiments 12–19: see Table 3.

Table 3. *Effect of varying MCPBA amounts on reaction of catharanthine N-oxide (XII) with vindoline (II)*

Expt	MCPBA mmol	Reactions conditions	other reactants			Dimers (% yield)		Recovered reactants[2]	
			III	II	TFA	XIII	XVI	III	II
12	0.405	standard	0.215	0.217	1.25	–	18	–	–
13	0.355	1/3 removed after 3 h	0.347	0.358	2.34	46	18	–	46
14	0.364 addl.	2/3 addl. 2 h (A)				25	18	–	–
15[a]	–	standard	0.182[a]	0.184	1.17	9	–	49	61
16[a]		standard	0.150	0.162	1.026	5	–	49	63
17[a]	0.027	standard	0.190[a]	0.199	1.17	23	5	–	53
18[a]	0.192	standard	0.198[a]	0.237	1.77	13	6	–	80
19	0.126	standard	0.153	0.136	0.88	30	12	–	46

[a] In these experiments N-oxide XII was used, (not III), prepared as in Expt. I.

Experiments 20–26: see Table 4.

Table 4. *Effect of various reagents employed in* Polonovski *reactions, in the case of catharanthine N-oxide (XII) with vindoline (II)*

Expt.	Other reactants in mmol			Reagent ml	Time	Temp. °C	Dimers (% yield)			Recovered (%)	
	III	MCPBA	II				XIII	XIV	XVI	III	II
20[a]	0.154	0.203	0.175	Ac_2O 0.15[a] +0.30	overnight +2 days	RT.	–	–	–	–	–
21[b]	0.131	0.164	0.164[c]	4 ml MeOH +4% HCl	12 days	−15					100
22	0.152	0.18	0.159[d]	SO_2	5 h	−11	–	–	–	–	–
23	0.184	0.203	0.188	p Tol SO_2Cl 0.955 mmol	2 days	− 5 to −10	–	–	–	–	100
24	0.195	0.206	0.209	CCl_3COCl[d] 0.15	3 days	− 4	–	20	–	–	–
25[e]	0.204	0.204	0.195	CF_3SO_2Cl 0.20	4 h	−40	–	–	–	36	85
26	0.163[f] XII		0.153	Fe_2SO_4 2.5 ml H_2O	12 h	0	–	–	–		

[a] 4 ml CH_2Cl_2 also added on 2[d] addition
[b] Reaction under standard conditions.
[c] Added only after 1.75 h; under N_2.
[d] II added after 0.5 h with SO_2 (used as solvent).
[e] Acetonitrile as solvent.
[f] XII isolated as in exp. 3 and dissolved in CH_3OH (2.5 ml).

Experiments 1, 27 and 28: see Table 5.

Table 5. *Effect of molar ratio the initial reactants on the reaction of catharanthine N-oxide (XII) with vindoline (II).* At –50° under standard conditions

Expt.	Reactants				Ratio	Dimer of Yield (%)	
	III	MCPBA	II	TFA	III:II	XIII	XVI
1	0.188	0.213	0.203	1.1	1:1	50	8
27	0.162	0.192	0.351	1.10	1:2	21	7
28	0.407	0.417	0.215	2.05	2:1	56	–

Reaction of Dihydrocatharanthine N-oxide (XVIII) with Vindoline (II) in presence of TFA. – Various experiments were performed; depending upon reaction conditions only C(18′) natural configuration dimers (XIX and XX) or else a mixture of natural and C(18′) unnatural (XXI and XXII) dimers were obtained. Two such experiments, A and B, are detailed here.

A) To dihydrocatharanthine (0.287 mmol), obtained by catalytic reduction of III [5], dissolved in CH_2Cl_2 (4 ml) and cooled to –15° MCPBA (0.333 mmol) was added and the mixture stirred for 0.5 h at –15°. After addition of II (0.316 mmol), the mixture was purged with argon, cooled to –50° TFA (1.46 mmol) was then added by means of a microsyringe and the mixture stirred for 3 h, the temperature being maintained at –30 to –50°. The reaction was quenched by adding a solution of sodium borohydride in methanol. Separation of the organic layer, drying the latter over anhydrous sodium sulfate and careful evaporation of the solvent *in vacuo* provided the crude product which, by TkLC. (silica gel, ethyl acetate/methanol 3:1), provided two dimeric products. The less polar dimer XX was obtained in 20% yield while the more polar dimer XIX in 20% yield. For their characterization data see B).

B) To 0.15 mmol dihydrocatharanthine dissolved in CH_2Cl_2 (10 ml) and cooled to –15° was added dropwise, over a period of 15 min, a solution of MCPBA (0.17 mmol) in CH_2Cl_2 (1 ml), after which TLC. indicated that all the dihydrocatharanthine had reacted. 0.16 mmol of II and 0.7 mmol of TFA were then added and the mixture allowed to stand under a nitrogen atmosphere at –15° for a 18 h. The reaction mixture was then added to a solution of sodium borohydride (1 g) in methanol (10 ml) and the whole stirred for 15 min at 0°, the organic solvents then removed *in vacuo*, the residue taken up in water (25 ml) and the resulting solution extracted with acetate. After drying, the solvent was removed *in vacuo* and the crude product subjected to TkLC. (silica gel, ethyl acetate/methanol 65:35) which gave three bands cleanly separated.

The first product (Rf 0.013, 13%) obtained crystalline, m.p. 190–194. (dec.), was identified as 4′-deoxovinblastine (XIX) on the following basis. – IR.: 3470, 2900, 1730 cm^{-1}. – UV.: 315 (sh, 3.70), 297 (4.02), 285 (4.08), 261 (4.15), 225 (4.55) nm. – CD.: 303 (+6.6), 260 (+20.5), 227 (+26.5), 209 (–47.0) nm. – NMR.: 9.73 (*s*, 1H); 7.94 (*s*, 1H); 7.54–7.14 (*m*, 4H, aromatic); 6.58 (*s*, 1H, H–C(14)); 6.20 (*s*, 1H, H–C(17)); 5.86 (*m*, 1H, olefinic); 5.48 (*s*, 1H, *H*COCOCH$_3$); 3.77 (*s*, 6H, 2×CO_2CH_3); 3.59 (*s*, 3H, OCH_3); 2.69 (*s*, 3H, NCH_3); 2.07 (*s*, 3H, $OCOCH_3$); 0.85 (*m*, 6H, 2×CH_2CH_3). – MS.: 794, 792, 764, 736, 734, 469, 338, 297, 282, 149, 142, 138 (base peak), 135, 124, 122, 121. Mol. wt.: 794.428. Calc. for $C_{46}H_{58}N_4O_8$: 794.425.

Another product (Rf 0.042, 5%), obtained as an amorphous solid, was identified as 4′(*epi*)-4′-deoxo-vinblastine (XX) on the following basis. – IR.: 3470, 2900, 1730 cm^{-1}. – UV.: 310 (sh, 3.54), 295 (3.81), 285 (3.84), 257 (3.97), 225 (4.41) nm. – CD.: 303 (+4.7), 260 (+12.5), 226 (+17.3), 212 (–35.7) nm. – NMR.: 8.16 (*s*, 1H), 7.53–7.16 (*m*, 4H, aromatic); 6.61 (*s*, 1H, H–C(14)); 6.13 (*s*, 1H, H–C(17)); 5.88 (*m*, 1H, olefinic); 5.48 (*s*, 1H, *H*COCOCH$_3$); 5.31 (*m*, 1H, olefinic); 3.81 (*s*, 3H, CO_2CH_3); 3.80 (*s*, 3H, CO_2CH_3); 3.64 (*s*, 3H, OCH_3); 2.71 (*s*, 3H, NCH_3); 2.10 (*s*, 3H, $OCOCH_3$); 0.85 (*m*, 6H, 2×CH_2CH_3). – MS.: 794, 792, 764, 734, 338, 282, 214, 195, 182, 168, 154, 144, 138, 124 (base peak), 122, 121. Mol. wt.: 794.421. Calc. for $C_{46}H_{58}N_4O_8$: 794.425.

Finally the third band (Rf 0.031, 12%) represented a mixture of the two C(18′) unnatural dimers XXI and XXII. The latter substance was shown to be identical with the previously isolated dimer for which X-ray analysis is available [2] [11] (TLC., IR., NMR. and MS.). XXI was

characterized as follows. – IR.: 3460, 2900, 1735 cm^{-1}. – UV.: 311 (sh, 3.82), 295 (3.98), 285 (3.98), 260 (4.05), 214 (4.62) nm. – CD.: 307.5 (+6.4), 277 (+5.8), 260 (−8.3), 223 (−28.9) nm. – NMR.: 9.0 (*s*, 1H); 7.2 (*m*, 4H, aromatic); 6.94 (*s*, 1H, H—C(14)); 5.91 (*s*, 1H, H—C(17)); 5.36 (*s*, 1H, *H*COCOCH$_3$); 3.87, 3.77 (2*s*, 9H, 2×CO_2CH_3+OCH_3); 2.6 (*s*, 3H, NCH_3); 2.05 (*s*, 3H, $OCOCH_3$); 0.91 (*t*, *J* = 7, 3H, CH_2CH_3); 0.64 (*t*, *J* = 7, 3H, CH_2CH_3). – MS.: 794, 792, 764, 750, 736, 734, 591, 589, 482, 469, 353, 336, 295, 282, 188, 138 (base peak), 135, 122, 121. Mol. wt.: 794.422. Calc. for $C_{46}H_{58}N_4O_8$: 794.425.

Catalytic reduction of 3',4'-dehydrovinblastine (XIII). To the dimer XIII (0.127 mmol) dissolved in 95% ethyl alcohol (50 ml) *Adams* catalyst (PtO_2 on charcoal, 49.5 mg) was added and the mixture was hydrogenated at RT. and atmospheric pressure for 1 h. The catalyst was then removed by filtration through celite, the solvent removed *in vacuo* and the crude product was immediately to TkLC. (silica gel, ethyl acetate/methanol 6:4). The major component isolated was crystallized from methanol and shown to be dimer XIX (64%).

Preparation of decarbomethoxycatharanthine. To 30 ml of 12% aqueous sodium hydroxide a solution of III (0.89 mmol) in ethyl alcohol (30 ml) was added and the mixture was then refluxed under nitrogen for 4 h. The reaction mixture was cooled, extracted with benzene to remove unreacted starting material, and the aqueous phase was made acidic (pH = 1.0) and stirred for 30 min during which time CO_2 was evolved. Extraction with chloroform, drying over anhydrous sodium sulfate and careful removal of the solvent *in vacuo* provided a foam (224 mg), which could be crystallized from methanol/ethyl acetate 1:4 to provide the known [5] decarbomethoxycatharanthine, m.p. 92–92.5 (85%).

Reaction of Decarbomethoxycatharanthine N-oxide with Vindoline (II) in presence of TFA. – To decarbomethoxycatharanthine (0.72 mmol), dissolved in CH_2Cl_2 (10 ml) and cooled to 0°, MCPBA (0.76 mmol) was added and the mixture stirred at 0° for 15 min, after which TLC. indicated that N-oxide formation was complete. The mixture was cooled to −30°, II (0.72 mmol) and TFA (3.60 mmol) were added and the whole was stirred under a nitrogen atmosphere and at −30 to −15° for 5 h. The reaction mixture was treated with a solution of sodium borohydride (100 mg/10 ml) until the pH of the mixture was 8.0, and after dilution with water, the mixture was extracted with CH_2Cl_2, the organic layer washed with $NaHCO_3$-solution (saturated), dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to provide a crude product (578 mg) as a brown foam. Purification of the latter by TkLC. (silica gel, methanol/ethyl acetate 1:4) allowed the separation of three dimeric products, of which the first (Rf 0.25, 11%) was quickly recognized as the known [2] dimer XXV (R = H).

The second product (Rf 0.50, 27%) was the desired dimer XXIV (R = H) having the natural configuration at C(18'). – IR.: 3460, 3400, 2920, 1730 and 1610 cm^{-1}. – UV.: 300 (3.19), 291 (3.94), 283 (3.94), 250 (4.15), 220 (4.63) nm. – CD.: 257.5 (−3.2), 224.5 (+22.6) nm. – NMR.: 8.7 (*s*, 1H, NH); 7.6–7.0 (*m*, 4H, aromatic); 6.86 (*s*, 1H, H—C(14)); 6.10 (*s*, 1H, H—C(17)); 6.0–5.8 (*m*, 1H, olefinic); 5.6 (*d*, 1H, olefinic); 5.48 (*s*, 1H, *H*COAc); 5.25 (*m*, 2H, H—C(18')+olefinic); 3.95 (*s*, 3H, OCH_3); 3.80 (*s*, 3H, CO_2CH_3); 2.65 (*s*, 3H, NCH_3); 2.11 (*s*, 3H, $OCOCH_3$); 1.05 (*t*, 3H, CH_2CH_3); 0.6 (*t*, 3H, CH_2CH_3). – MS.: 83, 85, 91, 97, 121, 135 (base peak), 734. Mol. wt.: 734.944.

$C_{44}H_{54}N_4O_6$ (734.944) Calc. C 72.00 H 7.41 N 7.33% Found C 71.52 H 7.36 N 6.89%

The third product (Rf 0.55, 168 mg, 32%) is considered to be 19'-vindolyl-decarbomethoxycatharanthine (XXVI, R = H) on the basis of the following data. – IR.: 3440, 3030, 2920, 1730 and 1605 cm^{-1}. – UV.: 300 (3.89), 292 (3.89), 285 (3.59), 250 (3.99), 222 (4.53) nm. – CD.: 250 (+16.3), 215 (−14.2) nm. – NMR.: 7.6–7.0 (*m*, 4H, aromatic); 6.62 (*s*, 1H, H—C(14)); 6.13 (*s*, 1H, H—C(17)); 6.13 (*m*, 1H, olefinic); 5.84 (*m*, 1H, olefinic); 5.41 (*s*, 1H, *H*COAc); 5.41–4.90 (*m*, 3H, H—C(18')+olefinic+H—C(2)); 3.90 (*s*, 3H, OCH_3); 3.80 (*s*, 3H, CO_2CH_3); 3.60 (*s*, 1H); 2.71 (*s*, 3H, NCH_3); 2.08 (*s*, 3H, $OCOCH_3$); 1.12 (*t*, 3H, CH_2CH_3); 0.25 (*t*, 3H, CH_2CH_3). – MS.: 97, 121, 122, 135 (base peak), 732. Mol. wt.: 732.926. Calc. for $C_{44}H_{52}N_4O_6$: 732.928.

Cleavage of dimer XXIV (R = H). A mixture of dimer XXIV (R = H, 10 mg) with a solution of tin (50 mg) and stannous chloride (50 mg) in 10% hydrochloric acid (10 ml) was refluxed for 1 h, the mixture cooled, made basic and extracted with chloroform. The organic extract was dried over anhydrous sodium sulfate, and the solvent evaporated to provide a crude product, which by

TLC. (silica gel, methanol/ethyl acetate, 1:4) showed II, deacetyl-II and cleavamine by comparison with authentic samples; some unreacted XXIV was also present.

Cleavage of dimer XXVI ($R = H$). Procedure as for cleavage of XXIV above. The products II and decarbomethoxycatharanthine were identified by comparison with authentic samples. In addition, unchanged XXVI and polar material, which remained on the baseline of the chromatoplate, were also observed.

Experiments using Vindoline-N-methyl-amide (XXVII, $R^1 = CH_3$; $R^2 = COCH_3$) (see Table 6). – *Preparation of vindoline N-methyl amide.* A mixture of vindoline (60 mg) and methylamine (333 mg) was heated at 60° for 3 days in a sealed tube, the crude product then treated directly with pyridine (1.3 ml) and acetic anhydride (1.3 ml) and allowed to stand overnight at RT. The reaction mixture was poured on to ice water and extracted with chloroform, the organic extract dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The crude product was purified by TLC. (silica gel, ethyl acetate/methanol 2:1) to provide XXVII ($R^1 = CH_3$; $R^2 = COCH_3$) (80%) as an amorphous powder. – IR.: 3660, 1736, 1675 and 1240 cm^{-1}. – UV.: 304 (3.63), 250 (3.85), 211 (4.49) nm. – NMR.: 6.78 (*d*, $J = 4$, 1H, H–C(14)); 6.26 (*d*×*d*, $J = 1$ and 4, 1H, H–C(15)); 6.08 (*d*, $J = 1$, 1H, H–C(17)); 5.85 (*d*×*d*, $J = 0.5$ and 2.5, 1H, olefinic); 5.54 (*s*, 1H, H–C(2)); 5.24 (br. *d*, $J = 2.5$, 1H, olefinic); 4.64 (*s*, 1H, *H*COCOCH$_3$); 3.78 (*s*, 3H, OCH$_3$); 2.8 (*d*, $J = 4$, 3H, CONCH$_3$); 2.72 (*s*, 3H, NCH$_3$); 2.00 (*s*, 3H, OCOCH$_3$); 1.48 (*t*, $J = 8$, 3H, CH$_2$*CH*$_3$). – MS.: 183, 197 (base peak), 251, 293, 301, 400, 404, 455. Mol. wt.: 455.239. Calc. for $C_{25}H_{33}N_3O_5$: 455.242.

Reaction of Decarbomethoxycatharanthine N-oxide with Vindoline-N-methyl-amide (XXVIII) in presence of TFA (Expt. 30). – To 0.5 mmol decarbomethoxycatharanthine dissolved in CH_2Cl_2 (15 ml) and cooled to –10°, a solution of MCPBA (0.53 mmol) in CH_2Cl_2 (7.5 ml) was added and the whole stirred at –10° under nitrogen for 20 min; 0.5 mmol of XXVIII and TFA (2.5 mmol) were then added and the reaction mixture was stirred at –10° overnight under a nitrogen atmosphere. The mixture was then treated with an excess (1 mg) of sodium borohydride dissolved in methanol (10 ml), diluted with water and extracted with CH_2Cl_2. After drying over anhydrous sodium sulfate, the solvent was removed *in vacuo* and the residue purified by TkLC. (silica gel, ethyl acetate/methanol 2:1) to provide two dimers.

The least polar (Rf 0.85, 21.6%) was recrystallized from methanol as needles, m.p. 256–259° (dec.), and was assigned the structure XXVIII (R = H), *viz.* 18′-decarbomethoxy-3′,4′-dehydrovinblastine-N-methyl-amide, based on the following data. – IR.: 3610, 3420, 1730 and 1670 cm^{-1}. – UV.: 303 (3.68), 280 (3.73), 263 (3.71), 252 (3.86), 237 (4.28), 225 (4.30) nm. – CD.: 270 (+7.1), 247 (+2.4), 229 (+11.9) nm. – NMR.: 8.77 (br. *s*, 1H, NH); 7.44–6.90 (*m*, 4H, aromatic); 6.78 (*s*, 1H, H–C(14)); 6.10 (*s*, 1H, H–C(17)); 5.84 (*d*×*d*, $J = 10$ and 3, 1H, olefinic); 5.52 (*s*, 1H, *H*COCOCH$_3$); 5.50 (*d*, $J = 8$, 1H, H–C(18′)); 5.22 (*d*, $J = 10$, 1H, olefinic); 5.20 (*m*, 1H, olefinic); 3.90 (*s*, 3H, OCH$_3$); 2.80 (*d*, $J = 5$, 3H, CONCH$_3$); 2.66 (*s*, 3H, NCH$_3$); 2.00 (*s*, 3H, OCOCH$_3$); 1.0 (*t*, $J = 8$, 3H, CH$_2$*CH*$_3$). – MS.: 187, 281 (base peak), 286, 309, 394, 406, 453, 493, 551, 733. Mol. wt.: 733.420. Calc. for $C_{44}H_{55}N_5O_5$: 733.415. Removal of solvent without decomposition was impossible.

$C_{44}H_{55}N_5O_5 \cdot CH_3OH$	Calc.	C 70.56	H 7.76	N 9.14%
(465.41)	Found	,, 69.58	,, 7.10	,, 8.83%

The more polar product (Rf 0.60, 18%) was obtained as an amorphous powder, and from the following, was assigned the structure XXIX (R = H, $R^1 = CH_3$; $R^2 = COCH_3$) *viz.*, 18′(*epi*)-18′-decarbomethoxy-3′,4′-dehydrovinblastine-N-methyl-amide. – IR.: 3440, 1730, 1675 and 1610 cm^{-1}. – UV.: 300 (3.93), 293 (3.98), 287 (3.96), 256 (4.15) nm. – CD.: 262 (+6.3), 226 (–26.6) nm. – NMR.: 7.73 (br. *s*, 1H, NH); 7.42–6.90 (*m*, 4H, aromatic); 6.94 (*s*, 1H, H–C(14)); 6.07 (*s*, 1H, H–C(17)), 5.84 (*d*×*d*, $J = 10$ and 3, 1H, olefinic); 5.47 (*s*, 1H, *H*COCOCH$_3$); 5.46 (*m*, 1H, olefinic); 5.24 (*d*, $J = 10$, 1H, olefinic); 4.56 (*m*, 1H, H–C(18′)); 3.78 (*s*, 3H, OCH$_3$); 2.78 (*d*, $J = 5$, 3H, CONCH$_3$); 2.70 (*s*, 3H, NCH$_3$); 2.00 (*s*, 3H, OCOCH$_3$); 0.92 (*t*, $J = 8$, 3H, CH$_2$*CH*$_3$). – MS.: 187, 281, 286, 309, 365, 376, 453, 443, 551, 733. Mol. wt.: 733.420. Calc. for $C_{44}H_{55}N_5O_5$: 733.418.

$C_{44}H_{55}N_5O_5 \cdot 3H_2O$	Calc.	C 67.15	H 7.81	N 8.90%
(787.42)	Found	,, 67.29	,, 7.30	,, 8.93%

Table 6. *Effect of temperature and concentration on the reaction of decarbomethoxycatharanthine N-oxide (XXVIII) with vindoline-N-methyl-amide (XXVII)*[a]

Expt.	Reactants in mmol				Temp. °C	CH_2Cl_2 ml[b]	Dimers (yield %)		Recovered %
	XXVIII	MCPBA	XXVII[a]	TFA			XXVIII	XXIX	XVII[a]
29[c]	0.18	0.198	0.18	0.9	−30	45	0	0	88
30	0.5	0.53	0.5	2.5	−10	45	21	18	–
31	0.1	0.105	0.1	0.5	+10	45	10	20	–
32	0.33	0.364	0.33	1.65	−10	17	6	7	–
33	0.4	0.42	0.4	2.0	−10	28	15	19	–
34	0.1	0.105	0.1	0.55	−10	80	17	12	–
35	0.1	0.105	0.1	0.5	−10	180	–	–	72

a) XXVII ($R^1 = CH_3$; $R^2 = COCH_3$).
b) ml/mmol of decarbomethoxycatharanthine.
c) Reaction for 5 h; all others overnight.

Reaction of Catharanthine N-oxide (XII) with Vindoline-N-methyl-amide (XXVII) in presence of TFA. – To a solution of III (0.1 mmol) in CH_2Cl_2 (1.7 ml) cooled to −30° a solution of MCPBA (0.105 mmol) in CH_2Cl_2 (0.6 ml) was added and the mixture stirred for a few min. A solution of XXVII (0.1 mmol) in CH_2Cl_2 (1 ml) and TFA (0.5 mmol) were added and the mixture stirred under nitrogen at −30° for 5 h. It was then treated with a solution of sodium borohydride in methanol at 0°, diluted with water (20 ml), extracted with CH_2Cl_2 and the organic extract dried over anhydrous sodium sulfate, the solvent removed *in vacuo* and the crude product mixture separated by TkLC. (silica gel, ethyl acetate/methanol, 2:1).

The more polar product (Rf 0.4, 25%) was the desired amide XXVIII ($R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_2$) *viz.*, 3′,4′-dehydrovinblastine-N-methyl-amide. – IR.: 3680, 3440, 1725, 1680 and 1610 cm^{-1}. – UV.: 302 (3.88), 292 (4.00), 285 (4.03), 264 (4.16) nm. – CD.: 262 (+6.3), 243 (−3.5), 226 (+11.2), 211 (−43.9) nm. – NMR.: 8.10 (br. *s*, 1H, NH); 7.54–6.92 (*m*, 4H, aromatic); 6.58 (*s*, 1H, H–C(14)); 6.14 (*s*, 1H, H–C(17)); 5.86 (*d* × *d*, *J* = 10 and 4, 1H, olefinic), 5.58 (*s*, 1H, *H*COCOCH$_3$); 5.48 (*m*, 1H, olefinic); 5.34 (br. *d*, *J* = 10, 1H, olefinic); 3.80 (*s*, 3H, OCH_3); 3.62 (*s*, 3H, CO_2CH_3); 2.80 (*d*, *J* = 5, 3H, $CONCH_3$); 2.74 (*s*, 3H, NCH_3); 2.04 (*s*, 3H, $OCOCH_3$); 1.00 (*t*, *J* = 8, 3H, CH_2CH_3). – MS.: 188, 221, 283 (base peak), 331, 333, 345, 451, 453, 463, 467, 524, 552, 555, 610, 612, 670, 731, 733, 791. Mol. wt.: 791.425. Calc. for $C_{46}H_{57}N_5O_7$: 791.424.

$C_{46}H_{57}N_5O_7 \cdot 4H_2O$	Calc.	C 64.38	H 7.63	N 8.16%
(863.42)	Found	,, 64.31	,, 7.30	,, 8.05%

The less polar product (Rf 0.75, 7.5%) was assigned the structure XXX ($R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_3$) *viz.*, 19′-hydroxy-3′,4′-dehydrovinblastine-N-methyl-amide, on the following basis. – IR.: 3620, 3440, 1735, 1675 and 1610 cm^{-1}. – UV.: 310 (3.64), 294 (3.87), 285 (3.93), 266 (4.05) nm. – CD.: 260 (+12.55), 243 (+2.8), 227 (+24.4), 210 (−40.46) nm. – NMR.: 8.12 (br. *s*, 1H, NH); 7.68–7.08 (*m*, 4H, aromatic); 6.44 (*s*, 1H, H–C(14)); 6.12 (*s*, 1H, H–C(17)); 5.88 (*d* × *d*, *J* = 10 and 4, 1H); 5.58 (*m*, 1H, olefinic); 5.52 (*s*, 1H, *H*COCOCH$_3$); 5.32 (br. *d*, *J* = 10, 1H, olefinic); 3.82 (*s*, 3H, OCH_3); 3.64 (*s*, 3H, CO_2CH_3); 2.82 (*d*, *J* = 5, 3H, $CONCH_3$); 2.76 (*s*, 3H, NCH_3); 2.04 (*s*, 3H, $OCOCH_3$); 1.03 (*t*, *J* = 8, 3H, CH_2CH_3); 0.78 (*s*, *J* = 8, 3H, CH_2CH_3).

In a related study employing a 2:1 molar ratio catharanthine: vindoline-N-methyl-amide, dimer XXVIII ($R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_3$) was obtained consistently in about 50% yield.

Conversion of 19′-hydroxy-3′,4′-dehydrovinblastine-N-methyl-amide (XXX, $R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_3$) to 3′,4-dehydrovinblastine-N-methyl-amide (XXVIII, $R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_3$). A mixture of the above dimer XXX, tin (62 mg) and stannous chloride (63 mg) in 7% aqueous hydrochloric acid (5 ml) was heated at 60° for 2 h, cooled and the reaction mixture diluted with water (30 ml), made basic with ammonium hydroxide and extracted with CH_2Cl_2. The organic extract was dried over anhydrous sodium sulfate, the solvent removed *in vacuo* and the crude product purified by TLC. (silica gel, ethyl acetate/methanol 2:1) to provide dimer XXVIII ($R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_3$, 6 mg).

Alternatively this conversion was accomplished as follows: a suspension of the same dimer XXX (0.048 mmol), and zinc powder (1 mmol) in glacial acetic acid (1.7 ml) was stirred at RT. for 24 h. The reaction mixture was then diluted with water (50 ml), made basic with ammonium hydroxide and extracted with CH_2Cl_2, the organic extract washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. TkLC. (silica gel, ethyl acetate/methanol 2:1) provided 3′,4′-dehydrovinblastine-N-methyl-amide (6 mg, 15%) and unreacted starting material (12 mg).

Cleavage of 3′,4′-dehydrovinblastine-N-methyl-amide (*XXVIII*, $R = CO_2CH_3$; $R^1 = CH_3$; $R^2 = COCH_3$). A mixture of the dimer (0.048 mmol), tin (0.505 mmol), stannous chloride (0.339 mmol) and 7% aqueous hydrochloric acid (5 ml) was refluxed for 3 h. The mixture was then cooled, diluted with water (50 ml), made basic with ammonium hydroxide and extracted with CH_2Cl_2 and, after drying the extract over anhydrous sodium sulfate, the solvent was removed *in vacuo* to provide a crude product, which by TkLC. (silica gel, ethyl acetate/methanol 4:1) afforded cleavamine (7 mg) and deacetylvindoline-N-methyl-amide (10 mg), both identified by comparison with authentic samples.

Financial aid from the *National Research Council of Canada*, and under Contract N01-CM-23223 from the *National Cancer Institute, National Institutes of Health*, Bethesda, Maryland, is gratefully acknowledged. The authors are also very grateful to the *Lilly* Research Laboratories for supplies of catharanthine and vindoline.

REFERENCES

[1] *J. P. Kutney, A. H. Ratcliffe, A. M. Treasurywala & S. Wunderly*, Heterocycles *3*, 639 (1975).
[2] *J. P. Kutney, J. Beck, F. Bylsma, J. Cook, W, J, Cretney, K. Fuji, R. Imhof & A. M. Treasurywala*, Helv. *58*, 1690 (1975).
[3] a) *G. H. Svoboda & D. A. Blake* in 'The Catharanthus Alkaloids', W. I. Taylor & N. R. Farnsworth, ed., Marcel Dekker, New York, 1975, p. 45; b) *R. C. DeConti & W. A. Creasy*, ibid., p. 237.
[4] *R. B. Livingstone & S. K. Carter*, 'Single Agents in Cancer Chemotherapy', IFT/Plenum, New York 1970, p. 279.
[5] *M. Gorman, N. Neuss & N. J. Cone*, J. Amer. chem. Soc. *87*, 93 (1965).
[6] *J. P. Kutney, R. T. Brown & E. Piers*, Canad. J. Chemistry *43*, 1545 (1965).
[7] *J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall & V. R. Nelson*, J. Amer. chem. Soc. 92, 1704 (1970).
[8] *P. D. Bartlett*, 'Non-Classical Ions', W. A. Benjamin Inc., New York, 1965.
[9] *A. Ahond, A, Cave, C. Kan-Fan, Y. Langlois & P. Potier*, Chem. Commun. *1970*, 517 and references cited therein.
[10] *J. P. Kutney, D. E. Gregonis, R. Imhof, I. Itoh, E. Jahngen, A. I. Scott & W. K. Chan*, J. Amer. chem. Soc. *97*, 5013 (1975).
[11] *J. P. Kutney, J. Cook, K. Fuji, A. M. Tresurywala, J. Clardy, J. Fayos & H. Wright*, Heterocycles 3, 205 (1975).
[12] *A. J. Jones, J. P. Kuthey, E. Jahngen & V. E. Ridaura*, to be published elsewhere.
[13] *E. Wenkert, E. W. Hagaman, B. Lal, G. E. Gutowski, A. S. Katner, J. C. Miller & N. Neuss*, Helv. *58*, 1560 (1975).
[14] *G. A. Russel & G. J. Mikol*, 'Mechanisms of Molecular Migrations', Vol. 1, *B. S. Thyagarajan*, ed., Interscience, New York, N.Y. 1968, p. 176 (general review); *R. N. Renaud & L. C. Leitch*, Canad. J. Chemistry *46*, 387 (1968); *R. Michelot*, Bull. Soc. chim. France *1969*, 4377; *R. T. LaLonde, E. Auer, C. F. Wong & V. P. Muralidharan*, J. Amer. chem Soc. *93*, 2501 (1971); *C. A. Scherer, C. A. Dorschel, J. M. Cook & P. W. Le Quesne*, J. org. Chemistry *37*, 1083 (1972); *Y. Hayashi, Y. Nagano, S. Hongyo & K. Teramura*, Tetrahedron Letters *1974*, 1299.
[15] *N. Neuss, M. Gorman, N. J. Cone & L. L. Huckstep*, Tetrahedron Letters *1968*, 783.
[16] *J. P. Kutney, R. T. Brown, E. Piers & J. R. Hadfield*, J. Amer. chem. Soc. *92*, 1708 (1970).
[17] *M. J. Sweeney, G. J. Cullinan, G. A. Poore & K. Gerzon*, Proc. Tenth Annual Meeting, Amer. Soc. Clin. Oncology *15*, March 1974.
[18] *P. Potier, N. Langlois, Y. Langlois & F. Gueritte*, Chem. Commun. *1975*, 670.