186. Total Synthesis of Indole and Dihydroindole Alkaloids. VIII[1][2]. Studies on the Synthesis of Bisindole Alkaloids in the Vinblastine-Vincristine Series. The Chloroindolenine Approach.

by **James P. Kutney, John Beck, Feike Bylsma, James Cook, Walter J. Cretney, Kaoru Fuji, René Imhof** and **Adi M. Treasurywala**

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

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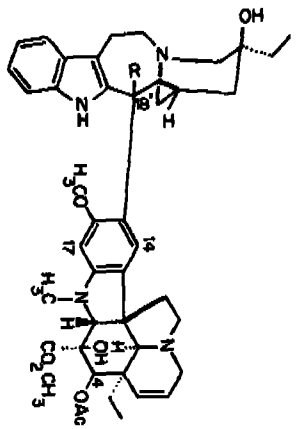
Summary. Studies on the syntheses of 18′-epi-4′-deoxo-4′-epivinblastine (IX, R = CO_2CH_3; R_1 = H), 18′-decarbomethoxy-18′-epi-4′-deoxo-4′-epivinblastine (IX, R = R_1 = H) and related analogues are described. The synthetic method employs a coupling reaction involving chloroindolenine derivatives of the cleavamine series (for example, III) with vindoline (V) under acidic conditions. The complete structures, including absolute configuration, of the resulting dimers are established by a combination of chemical and spectroscopic techniques, including X-ray analysis.

Catharanthus roseus G. Don (*Vinca rosea* L.), the common periwinkle plant, is an unusually rich source of indole and dihydroindole alkaloids[3]. In addition to the various members of the *Aspidosperma* and *Iboga* alkaloid families, some of which were already studied in the accompanying publications [2] [4], one finds a family of bisindole or 'dimeric' alkaloids which has attracted the interests of numerous research groups [3]. In addition to the chemical challenges provided by these complex natural systems, some of these compounds, particularly vinblastine (I) and vincristine (I, N-formyl instead of N-methyl) are presently regarded as important clinical agents for the treatment of various cancers in humans[4]. In spite of their established clinical value very little data is available on such aspects as structure-activity relationships

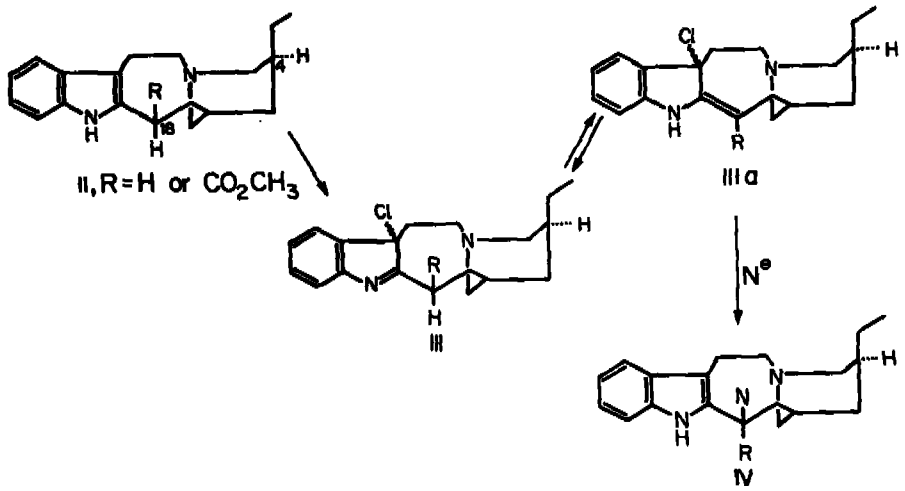


I, R = $COOCH_3$

[1] For preliminary reports on a portion of this work, see [1].
[2] Part VII, s. [2].
[3] For a recent general review see [3].
[4] For a general review of the biological activity see [5].

and it is not known whether the 'dimeric' system portrayed by I or which of the functional groups or chiral centers present are required for anti-tumor activity. Therefore in extending our synthetic interests to this area it was clear that the development of a general and versatile laboratory synthesis of such systems would be highly desirable. The results presented herein describe one of the approaches which has been developed for this purpose.

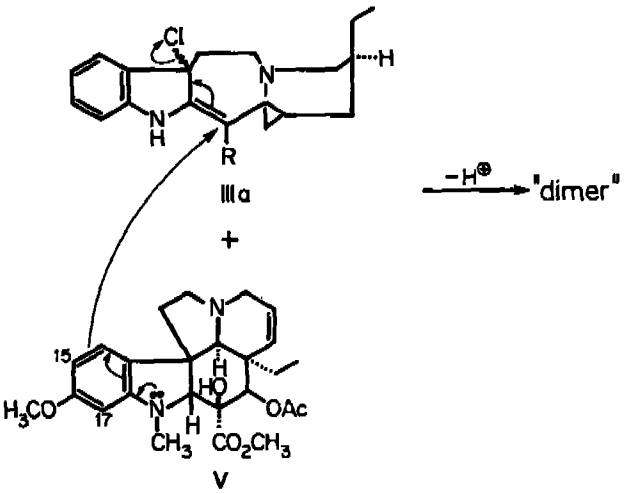
In previous studies [6] [2] [4] we have already described the utilization of chloroindolenine intermediates of the cleavamine series for the synthesis of C(18)-substituted cleavamine analogues (see II → III → IV). In this study the chloroindolenine III, perhaps *via* its enamine tautomer IIIa, is reacted with an appropriate nucleophile (for example, N = CN, OAc, H_2O etc.) under basic or acidic media to provide the



product IV. An extension of this reaction to the bisindole series was now considered. Vindoline (V), the dihydroindole unit present in vinblastine and vincristine, can be seen as a possible nucleophile in view of its electron density at C(15) and/or C(17) generated in any resonance structures involving the basic nitrogen atom of the dihydroindole system. Of the two possible sites of reaction, C(15) is clearly preferable in view of steric factors which would tend to disfavor C(17) (*Scheme 1*). The actual realization of this approach to provide a series of dimeric products is now described.

In our initial considerations of the spectral data which would provide strong evidence in support of the anticipated dimers, it was clear that mass spectrometry would play a dominant role. It had been shown previously that dimers such as vinblastine (I) which possess carbomethoxyl groups and have high molecular weight and low volatility tend to undergo thermal reactions in the inlet system of a mass spectrometer which results in 'molecular ion' peaks being observed which are multiples of 14 mass units larger than the true molecular weight [7] [8]. These spurious peaks have been attributed to thermal decomposition products which arise through intermolecular methyl transfer from a carbomethoxyl group to nitrogen followed by *Hofmann* elimination. However such processes do not occur in the hydrazide derivative of vinblastine in which the carbomethoxyl and acetate functions have been removed. In this instance a more normal mass spectrum is observed. In thus seemed logical to

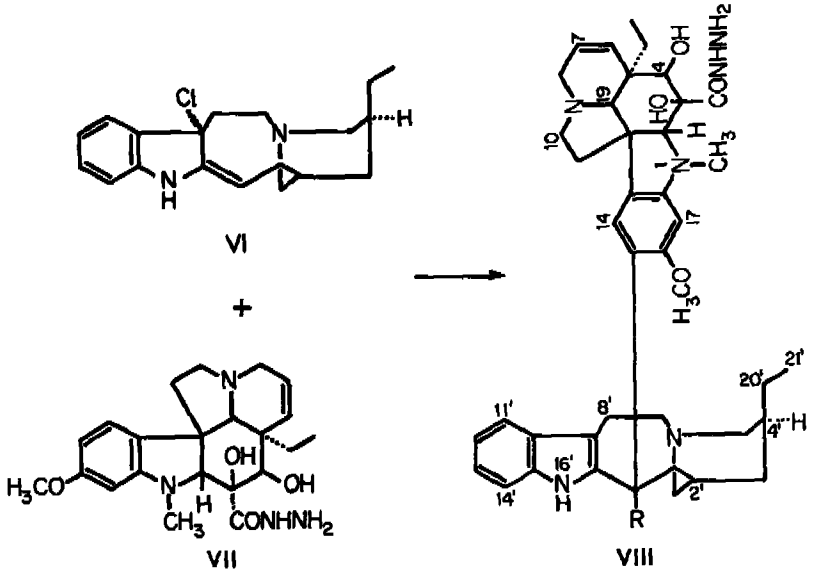
Scheme 1. *A proposal outlining the possible coupling of IIIa with vindoline* (V) *to provide a dimeric product*



initiate our investigations with the appropriate indole and dihydroindole components which would provide a synthetic dimer functionally similar with that of vinblastine hydrazide.

Deacetylvindoline hydrazide (VII) [9] was prepared from vindoline (V) by refluxing it with anhydrous hydrazine for 3 hours. The coupling reaction of the deacetylvindoline hydrazide with the chloroindolenine of 4β-dihydrocleavamine (VI) was carried out in refluxing anhydrous methanolic hydrogen chloride under a nitrogen atmosphere for 3 hours. In this manner a 77% yield of the dimer VIII was obtained (*Scheme 2*). This dimer proved to be virtually insoluble in methanol and was obtained

Scheme 2. *Coupling of deacetylvindoline hydrazide* (VII) *with the chloroindolenine derivative of 4β-dihydrocleavamine* (VI) *to provide dimer* VIII (R = H)



in crystalline form from the crude reaction mixture simply by washing with hot methanol. Recrystallization from ethanol provided an analytical sample of the compound, m.p. 190–192°. All the evidence was in accord with this compound being a dimer. The UV. spectrum displayed in superimposition the characteristic absorptions of both an indole and a dihydroindole system, while the mass spectrum of this compound established beyond doubt its dimeric nature. High resolution mass analysis provided the molecular formula $C_{41}H_{54}N_6O_6$ (Mol.-Wt.: Calc. 694.421, Found 694.421). Cleavage of the dimer in refluxing 2N aqueous hydrochloric acid in the presence of reducing agents (tin and stannous chloride) provided 4β-dihydrocleavamine (II, R = H) and deacetylvindoline hydrazide, the identities of which were established in the usual manner (m.p., TLC., IR). It was thus clear that the dimer contained deacetylvindoline hydrazide and 4β-dihydrocleavamine as intact units. The site of attachment in each unit thus remained to be determined. The NMR. spectrum of the dimer provided the remaining information that was required to establish the sites of attachment. In fact the important resonances arising from both 'halves' of the molecule were easily distinguishable from each other and the NMR. spectrum in itself, virtually constituted a proof of structure. The important signals in the NMR. spectra of deacetylvindoline hydrazide, 4β-dihydrocleavamine and the dimer are compared in Table 1. Deuterium exchange caused the signals attributed to the N(16'), C(4)

Table 1. *Comparison of NMR. data of deacetylvindoline hydrazide* (VII), *4β-dihydrocleavamine* (II, R = H) *and dimer* VIII (R = H)

Compound	II (R = H)	VIII (R = H)	VII
Proton(s)	NMR. signals[a]) Chemical shift, shape, no. of protons, coupling constant		
C(21)		0.72, *t*, 3	0.66, *t*, 3
C(21')	0.84, *t*, 3	0.82, *t*, 3	
C(19)		2.66, *s*, 1	2.64, *s*, 1
N_b—CH_3		2.70, *s*, 3	2.72, *s*, 3
C(2)		3.34, *s*, 1	3.42, *s*, 1
C(16)—OCH_3		3.83, *s*, 3	3.72, *s*, 3
C(4)		4.00, *s*, 1	4.10, *s*, 1
C(18')		4.40, '*d*', 1 J = 10 Hz	
C(6)		5.65, *d*, 1 $J_{6,7}$ = 10 Hz	5.76, *m*, 2
C(7)		5.83, pair of *m*' s, 1 $J_{7,6}$ = 10 Hz	
C(17)		6.03, *s*, 1	6.02, *d*, 1 $J_{17,15}$ = 2.3 Hz
C(15)			6.21, pair of *d*' s, 1 $J_{15,17}$ = 2.3 Hz $J_{14,15}$ = 8.5 Hz
C(14)		6.65, *s*, 1	6.81, *d*, 1 $J_{14,15}$ = 8.5 Hz
C(11')—C(14')	6.93–7.55, *m*, 4	6.93–7.55, *m*, 4	
N(16')	7.76, *s*, 1	8.25 ?, *s*, 1	
C(4)—O*H*		7.95 ?, *s*, 1	9.2, br. '*s*', 2
C(3)—O*H*		9.43 ?, *s*, 1	

[a]) NMR. data are recorded in δ values.

hydroxyl group and C(3) hydroxyl protons in the NMR. spectrum of the dimer to disappear and the integral showed that the three hydrazide protons must occur in the region above δ 4. Several important features of the dimer were seen from a comparison of the NMR. signals listed in Table 1. The signals attributed to the C(15) proton in deacetylvindoline hydrazide were absent in the spectrum of the dimer. In addition, the signals attributed to the C(14) and C(17) protons, which in deacetylvindoline hydrazide occurred as doublets, were found as sharp singlets in the dimer. The absence of signals attributable to the C(15) proton and the singlet nature of the C(14) and C(17) proton resonances could only be rationalized if the deacetylvindoline hydrazide portion of the dimer was coupled at the C(15) site. The appearance of an 'apparent doublet' at δ 4.40 in the dimer is consistent with the 4β-dihydrocleavamine portion of the dimer being coupled at the C(18) position. In conclusion, the above chemical and spectroscopic data were consistent with the structure VIII (R = H) although it must be emphasized that the chirality at C(18') shown in VIII was not known with certainty at this time. However more recent studies, involving X-ray analyses on two related dimers (see later), established beyond doubt the correctness of this assignment.

In view of the above success, it became of immediate interest to see if the same reaction conditions would bring about coupling between the chloroindolenine of a carbomethoxydihydrocleavamine (III, R = CO_2CH_3) and deacetylvindoline hydrazide. Obviously such a reaction would provide a dimer possessing a carbomethoxy function at C(18'), a feature present in the biologically active vinblastine-vincristine series.

The required starting material, 18β-carbomethoxy-4β-dihydrocleavamine (II, R = CO_2CH_3) was available from the high-yielding acid-catalyzed fragmentation of catharanthine developed earlier [10][5]). This compound upon reaction with *t*-butyl hypochlorite yielded a chloroindolenine in almost quantitative yield. This material, obtained as an amorphous powder, revealed spectral characteristics in accord with structure III (R = CO_2CH_3). Thus the NMR. spectrum showed a 'doublet' at δ 4.47 (H–C(18)) and *no* NH signal while the IR. portrayed saturated ester (1727 cm^{-1}) and typical indolenine (1612, 1575 cm^{-1}) absorptions. The mass spectrum of this compound was in accord with it being a monochloro derivative of II (R = CO_2CH_3) with molecular ion peaks at *m/e* 374 (^{35}Cl) and 376 (^{37}Cl). Finally regeneration of II (R = CO_2CH_3) on reduction established that chlorination had not brought about any unusual skeletal rearrangements.

The chloroindolenine III (R = CO_2CH_3) and deacetylvindoline hydrazide were allowed to react under the conditions mentioned above. The desired dimer VIII (R = CO_2CH_3) could be isolated (36.5% yield) as an amorphous powder by chromatographic purification of the product mixture. Apart from the spectral characteristics already detailed for the above dimer VIII (R = H) several significant differences should be noted in the spectral data for this dimer. The IR. spectrum showed an additional absorption attributable to an ester group at 1726 cm^{-1}, while in the mass spectrum of this compound, a series of peaks spaced by multiples of 14 mass units above the expected molecular ion peak at *m/e* 752 were observed. These data were

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[5]) Recent unpublished refinements have raised the yield in this conversion to 90%.

in accord with those observed earlier by *Biemann* [7] [8] in the vinblastine series and already noted in the earlier discussion. High resolution mass analysis provided the molecular formula $C_{43}H_{56}N_6O_6$ (requires: 752.426; found: 752.427). In the NMR. spectrum it was significant to note a complete absence of the H–C(18') signal noted in VIII (R = H) and the appearance of an additional three-proton singlet at δ 3.70 (C(18')–COOCH_3).

Chemical evidence in support of VIII (R = CO_2CH_3) was obtained when the dimer was reacted with hot 2N aqueous hydrochloric acid in the presence of tin and stannous chloride. The isolated products II (R = CO_2CH_3) and VII were identified by comparison with authentic samples.

The above results revealed that the chloroindolenine approach may provide a rather general and versatile synthetic entry into a series of novel vinblastine analogues which would be desirable for structure-activity studies in the cancer area. Consequently an extension of this approach to the coupling with vindoline (V), the natural unit in vinblastine, was now considered.

The chloroindolenine of 4β-dihydrocleavamine (VI) was reacted with vindoline in the manner described above and the major product obtained in crystalline form (m.p. 205–206°) exhibited the spectral characteristics for the dimer IX (R = R_1 = H). The UV. spectrum appeared as a simple superimposition of the indole and indoline absorption and this agreed qualitatively with the spectrum obtained for vinblastine. The mass spectrum showed a number of fragment ions corresponding to the fragmentation pattern normally exhibited by 4β-dihydrocleavamine and vindoline (see experimental part). High resolution mass determination of the parent ion established a molecular formula in agreement with the structure of the desired compound. As in the case of the previous hydrazide dimers, NMR. data was highly informative and virtually by itself established the assigned structure. The most crucial evidence concerning the point of attachment of the two units (C(15)–C(18')) is readily available from the spectrum (δ 6.68, *singlet*, H–C(14); 6.08, *singlet*, H–C(17), and 4.43, broad doublet, H–C(18')). Again it should be emphasized that the chirality at C(18') portrayed in IX (R = R_1 = H) was not available from the data presented but became known from a subsequent X-ray analysis (see later).

Cleavage of dimer IX (R = R_1 = H) under reductive acidic conditions described above allowed the isolation of 4β-dihydrocleavamine, vindoline and deacetylvindoline (V, C(4)–OH instead of C(4)–OAc), thereby confirming the presence of these units in the dimeric structure.

In similar fashion the chloroindolenine approach could be extended to the synthesis of various other dimers and a summary of the dimers isolated is given in Table 2. A comparison of the most pertinent NMR. data for the synthetic dimers and vinblastine is given in Table 3 while the remaining data in support of these structures are provided in the experimental part.

Although the data as presented provided strong evidence in favour of the proposed structures, they could not be considered definitive in terms of the chirality at C(18'). The NMR. data as shown in Table 3 provided suggestive evidence that the configuration at this center may be different from that in the natural vinblastine series. Thus an analysis of these data reveals that the chemical shift of one of the proton signals attributed to C(14) in the vindoline portion of the dimers is significantly shifted

Table 2. *Summary of dimers obtained via coupling of various chloroindolenine intermediates with vindoline and dihydrovindoline*

Dihydroindole unit	Chloroindolenine Intermediate	Dimer isolated
vindoline	VI	IX, R = R_1 = H
vindoline	III, R = CO_2CH_3	IX, R = CO_2CH_3; R_1 = H
vindoline	III, R = CN	IX, R = CN; R_1 = H
dihydrovindoline (V, 6,7-dihydro)	III, R = CO_2CH_3	IX, R = CO_2CH_3; R_1 = H; 6,7-dihydro
vindoline	XI, R = CO_2CH_3	IX, R = CO_2CH_3; R_1 = OH
vindoline	XII, R = CO_2CH_3	X, R = CO_2CH_3

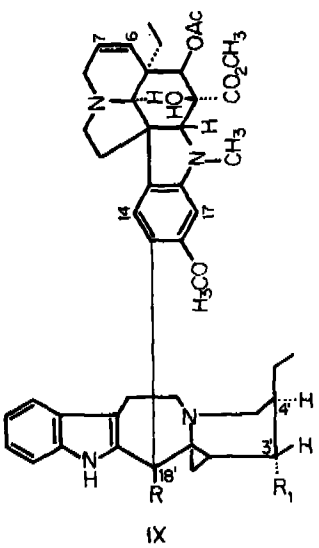
Table 3. *Comparison of important NMR. data of synthetic dimers and vinblastine*[a])

Dimer	NMR. signals		
	H–C(14)	H–C(17)	H–C(18′)
IX, R = R_1 = H	6.68	6.08	4.43
IX, R = CO_2CH_3; R_1 = H	6.95	5.95	
IX, R = CN; R_1 = H	6.21	6.06	
IX, R = CO_2CH_3; R_1 = H; 6,7-dihydro	6.92	5.96	
IX, R = CO_2CH_3; R_1 = OH	6.89	6.00	
X, R = CO_2CH_3	6.98	6.00	
vinblastine (I)	6.58	6.06	

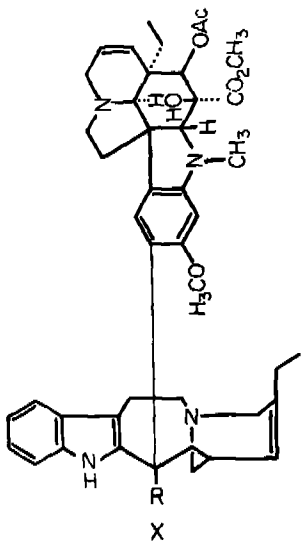
[a]) Only the NMR. data relating to the important questions concerning the point of attachment of the two units and the configuration at C(18′) are presented here.

relative to the corresponding signal in vinblastine. Clearly this proton being in close proximity to the indole system would be affected by the manner in which the two units are linked together. The other aromatic portion (C(17)) should not be so dependent and the difference in its chemical shift relative to that in vinblastine is insignificant. Obviously unambigous evidence to clarify this important question was mandatory and therefore an X-ray study was undertaken. Dimer IX (R = CO_2CH_3; R_1 = H) was converted to its crystalline methiodide and subjected to the X-ray investigation. The data published recently [1] established the correctness of the previous proposals and established the absolute configuration as portrayed in IX. On this basis dimer IX (R = CO_2CH_3; R_1 = H) could be given the name, 18′-epi-4′-deoxo-4′-epivinblastine since it differs from the natural products in terms of chirality at C(4′) and C(18′) and lacks a hydroxyl group at C(4′).

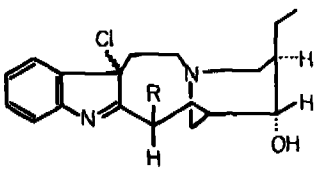
A second X-ray analysis was conducted on the hydrobromide salt derivative of the dimer possessing a hydrogen atom at C(18′) (IX, R = R_1 = H) since in this series this centre may undergo epimerization during the acidic conditions employed in the coupling reaction. However as already described [1] this dimer *also* possesses the unnatural configuration at C(18′) and may be named as 18′-decarbomethoxy-18′-epi-



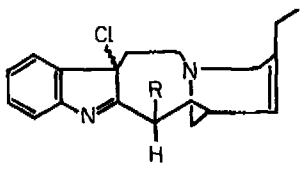
IX



X



XI



XII

4'-deoxo-4'-epivinblastine. In summary the series represented by VIII, IX and X are now correct in every detail.

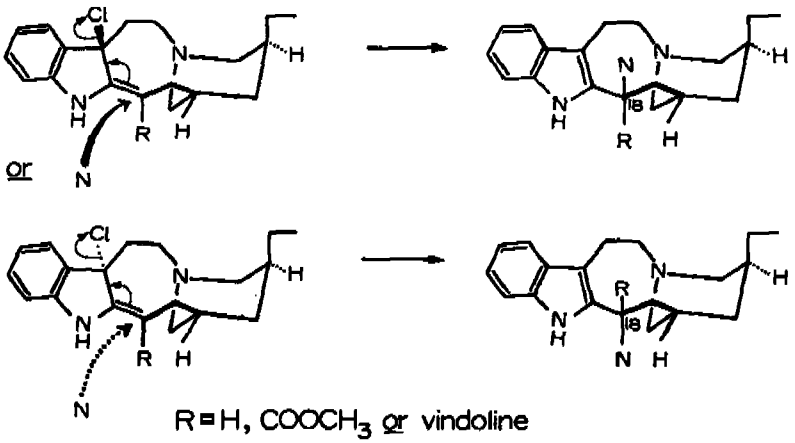
It should be noted here that in recent studies involving circular dichroism measurements on the synthetic dimers described above and the natural vinblastine-vincristine members, there is excellent correlation between the shapes of the CD. curves and chirality at C(18'). These results [11] reveal that the CD. method is now ideally suitable for evaluating chirality at this center in any future studies.

It is appropriate at this point in the discussion to comment on the possible mechanism involved in the coupling reaction. Although mechanistic studies had not been undertaken it appears reasonable to speculate that the coupling could proceed *via* a concerted process or one involving ionic intermediates (*Scheme 3*). The concerted mechanism is similar to the S_N2' reaction and clearly would involve a definite stereochemical orientation between the attacking nucleophile (vindoline, for example) and the departing chloride ion. Unfortunately the configuration with respect to the C-Cl bond in the chloroindolenine intermediates is uncertain since their instability prevents rigorous purification and characterization.

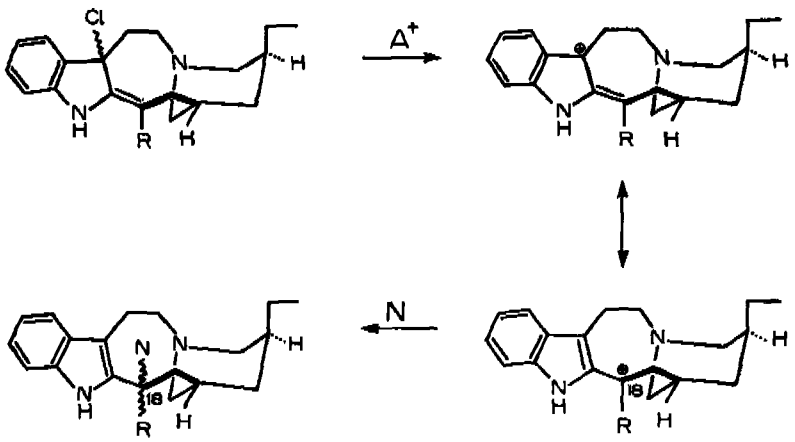
The ionic mechanism presented in *Scheme 3* is at best an approximation since any planarity associated with the reacting centre (C(18)) would allow the isolation of

Scheme 3. *Two possible mechanistic pathways to explain the coupling reaction*

A. Concerted Process



B. Carbonium Ion Process

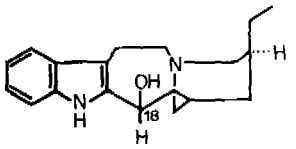


(epimers at C(18))

both possible isomeric series with respect to C(18') in the dimeric products. This situation is clearly contrary to the obtained results even though extensive analyses of the product mixtures have been performed in order to recognize the isomeric dimers.

Some evidence in support of the ionic mechanism became available from other studies in our laboratory. In order to evaluate another approach to the dimeric series we have studied a coupling reaction based on a method developed by *Buchi* for the synthesis of voacamine [12]. In these investigations, 18β-hydroxy-4β-dihydrocleavamine (XIII), available from another study in our laboratory, was reacted with vindoline in refluxing anhydrous methanolic 1% hydrochloric acid solution. The

product, isolated in 65% yield, proved to be identical with dimer IX (R = R_1 = H) obtained from the chloroindolenine approach. The only other dimeric material obtained from this reaction was a small amount (5%) of the deacetyl derivative of dimer IX (R = R_1 = H; HO–C(4) in place of AcO C(4)).



XIII

The coupling of XIII with vindoline at a much lower temperature (24 hours at −5°) led to a product mixture containing much of the starting materials, some dimer IX (R = R_1 = H) and one other product. The simple indole chromophore obtained for this latter product in the UV. spectrum excluded the possibility of this material being a dimer. Although complete characterization of this substance was not obtained the spectral properties suggested that it was an 18-methoxy-4β-dihydrocleavamine arising from reaction of XIII with the solvent.

These experiments therefore, confirmed that this dimerization followed the same stereochemical course as the chloroindolenine coupling reaction. It is tempting to postulate that the coupling of XIII with vindoline under acidic media would proceed through a carbonium ion process to yield an intermediate similar to that portrayed in *Scheme 3*. Again the isolation of only one stereochemical series with respect to C(18′) in the dimers is interesting.

Harley-Mason [13] has also reported the coupling of a 18-hydroxy-dihydrocleavamine with vindoline to a dimer but these workers make no mention of the configuration of their product.

The coupling reactions described had thus far provided a general synthetic pathway to a series of vinblastine analogues but unfortunately these substances possessed the incorrect configuration at C(18′). It was hoped that the stereochemical course of this reaction could be altered by appropriate changes in the reaction conditions at various stages of the coupling process. Thus changes in the conditions for formation of the chloroindolenine intermediates may lead to generation of different ratios of the two possible isomeric chloroindolenines (C-Cl bond α- *or* β- oriented). If the coupling reaction were to proceed in some manner similar to the concerted process shown in *Scheme 3* the orientation of the C-Cl bond is obviously a factor in determining the chirality at C(18′) in the resultant dimers. Alternatively, the second stage of this reaction, the coupling of vindoline with the chloroindolenine system is acid-catalyzed and it was of some importance to determine whether different acidic reagents and/or various solvents could play a role in allowing isolation of the isomeric C(18′) dimers. In order to provide some information pertinent to these considerations a series of experiments were undertaken. For this purpose one 'standard' coupling reaction was selected so as to provide an internally consis-

tent comparison between the various experiments. Thus the coupling of 18β-carbomethoxy-4β-dihydrocleavamine (II, R = CO_2CH_3) and vindoline (V) to provide the dimer IX (R = CO_2CH_3; R_1 = H) which proceeds in good good yield and for which all data, including X-ray analysis, are available on the product was chosen for the study. The series of experiments described below used, as a basis, the 'standard' coupling reaction conditions. Each experiment was repeated twice to check its reproducibility. In every case, the overall yield of dimer obtained in these duplicate runs agreed within $\pm$ 5%. In those cases where no dimeric material was isolated, reproducibly, the standard conditions were repeated using the same reagents and the yield of this reaction was used as a means to determine the validity of such results. The reactions were all performed on the same scale (50 mg of each of the reactants II, R = CO_2CH_3, and vindoline, V). Since the standard conditions play such a pivotal role in this study it is perhaps appropriate to describe them in some detail here.

The conventional reaction may be divided into two parts: (a) chloroindolenine formation and (b) coupling reaction.

(a) Chloroindolenines were formed by dissolving 18β-carbomethoxy-4β-dihydrocleavamine in a fixed volume of dry methylene chloride containing one equivalent of triethylamine, cooling it to 0° and adding one equivalent of an ice-cold solution of 0.05 M *t*-butyl hypochlorite in carbon tetrachloride over a period of twenty minutes. After this time thin-layer chromatography (TLC.) was applied and the reaction was routinely found to have proceeded to completion. Evaporation of the solvent at 0° under high vacuum usually resulted in a foam.

(b) To this foam was added an equal weight of vindoline followed by a solution of 1.5% hydrogen chloride in methanol under a stream of nitrogen, and the whole was plunged into a preheated oil bath at 70°, and allowed to reflux for three hours. Chromatographic purification of the reaction mixture according to a rigidly standardized procedure resulted in the isolation of dimer IX (R = CO_2CH_3; R_1 = H) in 65% yield. The NMR. spectrum of this compound was taken to be the 'standard' spectrum to which all subsequent experimental results were compared.

To evaluate the significance of altering conditions in the first stage of the process, that is, chloroindolenine formation, the above 'standard' conditions were used on several chloroindolenine reaction mixtures prepared by the use of different chlorinating agents. The most important results are summarized in Table 4. It is clear from this table that these alterations failed to produce any trace of a new dimer when the products were coupled in the standard way with vindoline.

The temperature of chloroindolenine formation was the next variable examined. Reaction temperatures between 0° and 77° were used by refluxing the substrate (II, R = CO_2CH_3) in the appropriate mixture of methylene chloride and carbon tetrachloride, followed by the addition of neat tert-butyl hypochlorite. The results are summarized in Table 5. It is obvious from this table that the yield of dimer resulting from coupling chloroindolenines so derived with vindoline is sensitive to the temperature of the chlorination step but that the stereochemical outcome is completely unaltered by changes in the temperature at which the chlorination is performed.

It was thus clear from the data in Tables 4 and 5 that efforts to change either the configuration or the yield of the dimeric product must concentrate on the area of the coupling reaction itself and not on the chloroindolenine-forming step. Attention was thus turned to this part of the coupling process.

Table 4. *Effect of different conditions for chloroindolenine formation on the overall yield and configuration of dimers produced*

Reagent	Solvent	Time to completion (min)	Temperature °C	Yield of dimer (%)	Dimer isolated
1-chlorobenzotriazole	benzene	20	20	60	IX (R = CO_2CH_3; R_1 = H)
1-chlorobenzotriazole	methylene chloride	20	20	60	IX (R = CO_2CH_3; R_1 = H)
0.05 M *t*-BuOCl in CCl_4	methylene chloride	15	49	50	IX (R = CO_2CH_3; R_1 = H)
N-Chlorosuccinimide	methylene chloride	20	25	50	IX (R = CO_2CH_3; R_1 = H)
N-Chloroacetamide	methylene chloride	20	25	50	IX (R = CO_2CH_3; R_1 = H)
NaOCl (H_2O) (household bleach)	methylene chloride (two phase)	60	25	30	IX (R = CO_2CH_3; R_1 = H)

Table 5. *Temperature of chloroindolenine formation* vs. *yield and type of dimer produced*

Solvent CH_2Cl_2	CCl_4	Temperature °C	Yield of dimer (%)	Dimer isolated
1	1	0	65	IX (R = CO_2CH_3; R_1 = H)
1	0	50	45	IX (R = CO_2CH_3; R_1 = H)
3	2	50	10	IX (R = CO_2CH_3; R_1 = H)
1	1	55	5	IX (R = CO_2CH_3; R_1 = H)
0	1	77	No dimer	
1	4	72	No dimer	

Several factors were involved at this stage. In order to reach a clear understanding about the role that they played, a systematic study of each of these factors was necessary. The temperature of the coupling reaction was the first variable to be examined. It was conceivable that, regardless of the exact nature of the transition

state, the stereoselectivity of this coupling step was a reflection of the steric interactions experienced by vindoline when it approached the α-face of the molecule in order to lead to the natural configuration at C(18'). If these were considerably greater than those on the β-face, then at lower temperatures when conformational mobility was diminished in the intermediate, attack would occur exclusively on the less hindered β-face to produce only the unnatural configuration at C(18'). If this line of reasoning was correct, the attack upon the α-face of the molecule may be favoured by increasing the temperature of this step so as to supply greater conformational mobility to the system.

One disadvantage of this approach was that the chloroindolenine could be expected, on the basis of the work mentioned above, to be heat-sensitive. Thus, the yield of dimeric material resulting from dimerizations at higher temperatures may be expected to be substantially diminished. Coupling reactions were performed at several temperatures ranging from room temperature to 140°. These reactions were monitored by TLC. until a maximum amount of dimer had been formed and then worked-up in the usual way. This time of optimum reaction provided a measure of the relative rate of the reaction. The pertinent data derived from this sequence of experiments are summarized in Table 6. It can be seen that the 'rate' of this reaction increased as the temperature was increased.

Table 6. *Effect of the temperature of the coupling step on the yield and configuration of dimer*

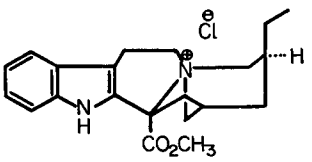
Temperature (°C)	Time (h)	Yield of dimer (%)	Dimer isolated
140	0.5	0	
100	0.25	30	IX ($R = CO_2CH_3$; $R_1 = H$)
68	2.5	65	IX ($R = CO_2CH_3$; $R_1 = H$)
25	18	75	IX ($R = CO_2CH_3$; $R_1 = H$)

The yield, as had been expected, was inversely correlated with the temperature, being optimal at the relatively lower temperatures. Unfortunately, the configuration at C(18') of the resulting product was completely independent of the temperature of the coupling step. Thus, again the hope of altering the stereochemical outcome of this reaction, based on the above arguments, had been frustrated.

Vindoline itself had previously demonstrated a remarkable inertness to 1.5% methanolic hydrogen chloride (*vide supra*). The fate of the chloroindolenine during the coupling step may have been critical in determining the overall yield of dimeric material isolated. It was possible that when this compound was mixed with vindoline and then treated with the acidic methanol, the chloroindolenine decomposed partially before it had an opportunity to couple. Vindoline was dissolved in a small amount of the solvent in a separate flask and added slowly to the chloroindolenine. When this addition was complete at room temperature, the remaining amount of the solvent was added and the normal coupling was performed. A slight improvement in the overall yield of dimer IX ($R = CO_2CH_3$; $R_1 = H$) was realized by this approach

(70%) but it was not sufficiently significant to be classified as a real advance particularly in view of the added inconvenience associated with this modification.

An interesting discovery was made, however, upon inverting the direction of this addition. When the chloroindolenine was mixed with 1.5% methanolic hydrogen chloride and then added to solid vindoline, the yield of dimer isolated after the standard coupling appeared to be remarkably dependent upon the length of time for which the chloroindolenine had remained in this solution prior to the addition, and the temperature at which this solution had been maintained. In fact, if the chloroindolenine solution was allowed to reflux for five minutes, or stand at room temperature for an hour, or at 0° overnight, the yield of dimeric material isolated after the standard coupling step was reduced to zero. Such experiments with the chloroindolenine under the above conditions revealed by TLC. that it had completely disappeared and had been replaced by a polar, salt-like material which had the characteristic UV. absorptions of an indole. On the basis of other studies in our laboratories [6] [2], the structure XIV was tentatively assigned to this compound on the basis of its NMR. and UV. spectra. All attempts to couple this so-called quaternary salt in a separate step failed completely under the standard conditions. It should be pointed



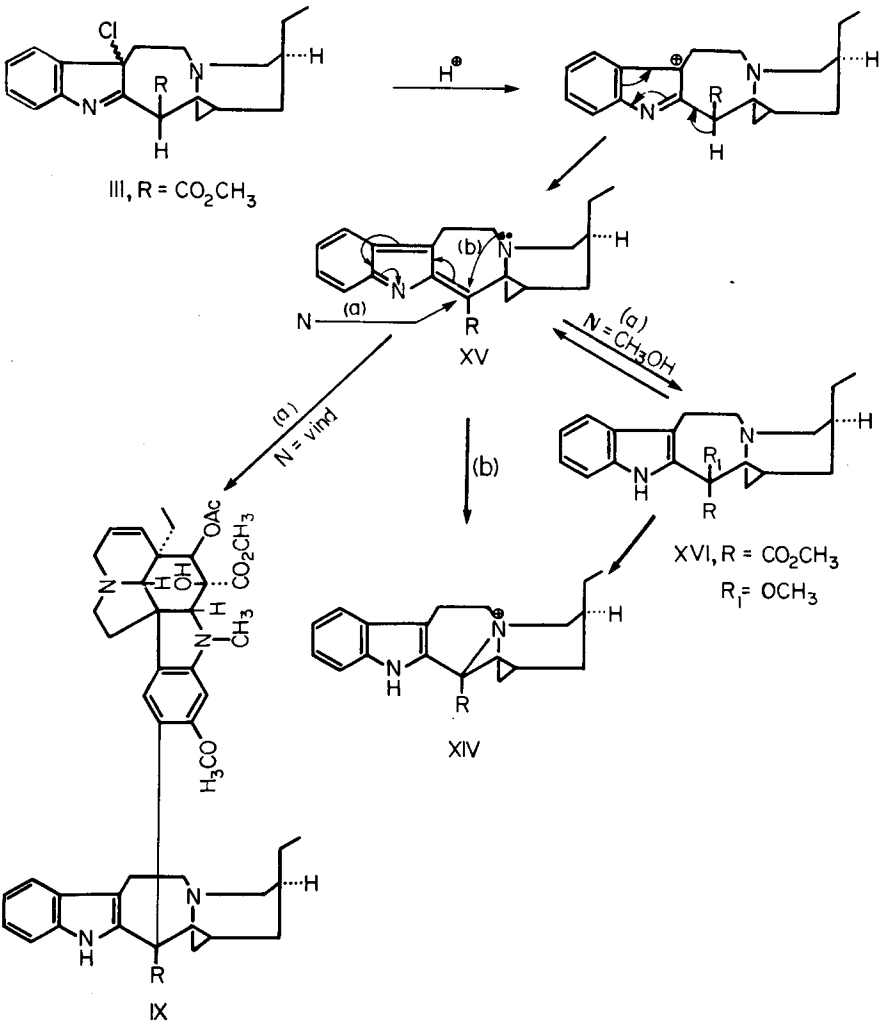
XIV

out that the chloroindolenine was quite unreactive in the absence of acidic or basic catalysts, and could be recovered unchanged when refluxed in anhydrous acetone or anhydrous methanol for periods in excess of six hours.

Clearly then, the acid catalyst reacted with the chloroindolenine to produce an intermediate which could react with vindoline or any other nucleophile that may be present or else undergo the intramolecular condensation with N_b to yield XIV. To explain these various observations an ionic mechanism is proposed (*Scheme 4*) and the reactive intermediate is assigned the structure XV.

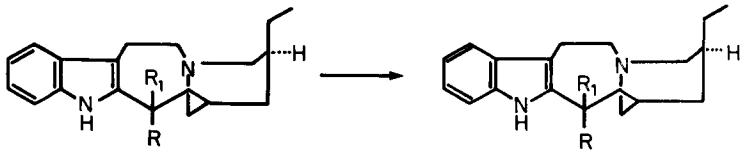
Additional experiments to provide some support for this mechanistic proposal were undertaken. It was hoped that intermediate XV could be captured in the form of a stable compound which could be isolated and then used in an independent reaction to regenerate XV in the presence of vindoline so as to allow normal coupling to proceed. If the yield and stereochemical outcome of such an experiment were the same as the standard one, some support for the postulate would be available. Fortunately, such an experiment was indeed possible. Treatment of the chloroindolenine III (R = CO_2CH_3) with 1% methanolic hydrogen chloride under milder conditions (0° for ten minutes) yielded a rapid and visible reaction with the initially clear light yellow solution becoming a deep wine-red colour. The product isolated from this reaction was quite unstable and decomposed, upon standing for several hours, to the salt XIV. Its structure XVI could be inferred from the UV. spectrum (normal indole) and NMR. spectrum which showed the complete disappearance of the C(18) proton and the

Scheme 4. *Proposed mechanism of the standard coupling reaction*



appearance of a new three-proton singlet due to the C(18) methoxyl group. Conclusive proof for the structure became available when XVI was reduced with lithium aluminum hydride to the stable, crystalline alcohol-ether XVII. This latter substance was completely characterized (see experimental part). An additional byproduct in this reduction was a dihydrocleavaminol (XVIII) arising from a hydrogenolysis of the methoxyl group.

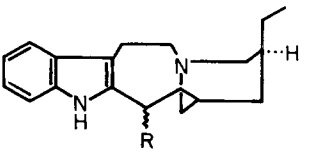
In a subsequent experiment compound XVI was dissolved in 1.5% methanolic hydrogen chloride containing vindoline. Coupling in the standard manner afforded a 55% yield of dimer IX (R = CO_2CH_3; R_1 = H) thereby providing support for the mechanism proposed in *Scheme 4*.



XVI, R = CO_2CH_3
R_1 = OCH_3

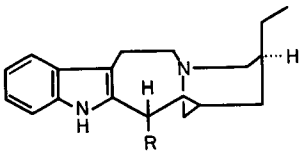
XVII, R = CH_2OH
R_1 = OCH_3

+



XVIII, R = CH_2OH

It was expected that if an ionic mechanism prevailed in the coupling process the configuration of the resulting dimer should be independent of the configuration at C(18) in the cleavamine system. Thus 18α-carbomethoxy-4β-dihydrocleavamine (XIX, R = CO_2CH_3) [10], the C(18) epimer of the cleavamine system normally employed, was coupled with vindoline in the normal manner. The product isolated in 45% yield was dimer IX (R = CO_2CH_3; R_1 = H).



XIX, R = CO_2CH_3

Now that some additional insight into the possible mechanism of the coupling process was available the coupling of vindoline with the chloroindolenine was investigated under a variety of acidic conditions and in various solvents. Table 7 provides a summary of the results obtained. The reactions were run at three temperatures: room temperature, 70° and the reflux temperature of the solvent employed. In each case the progress of the reaction was monitored by TLC. and the reaction interrupted when optimal concentration of dimeric material was observed. Again, apart from considerable differences in the yields of dimer, there was no indication of any C(18') isomeric material. Finally a study of the yield and stereochemical outcome of the conventional coupling reaction as a function of acid concentration was undertaken (Table 8). Concentrations of acid below 1.5% were used to see whether or not the same dimer was formed under these conditions. At very low acid concentrations, however, it appears that the rate of formation of the reactive intermediate was sufficiently slow to permit the decomposition of the chloroindolenine irreversibly to other products and thus the yield of dimeric material obtained was dramatically reduced. In the limiting case, when the coupling was attempted in absolute methanol

Table 7. *Effect of solvents and various acids on the coupling reaction*

Solvent	Acid	Temp. (°C)	Time (h)	Yield (%)	Dimer isolated
Trifluoroacetic acid	–	25	48	5	IX ($R = CO_2CH_3$; $R_1 = H$)
N,N-Dimethyl-formamide	HCl	25	19	60	IX ($R = CO_2CH_3$; $R_1 = H$)
Tetrahydrofuran	HCl	66	2.5	25	IX ($R = CO_2CH_3$; $R_1 = H$)
Dioxane	$HClO_4$	25	48	5	IX ($R = CO_2CH_3$; $R_1 = H$)
Methanol	HCl	70	3	65	IX ($R = CO_2CH_3$; $R_1 = H$)
Methanol	HBr	25	48	50	IX ($R = CO_2CH_3$; $R_1 = H$)
Methanol	$ZnCl_2$	25	48	0	
Methanol	SiO_2	25	48	0	
Benzene	$AlCl_3$	25	48	0	
Benzene	BF_3	80	1.75	5	IX ($R = CO_2CH_3$; $R_1 = H$)

Table 8. *Effect of acid concentration on the coupling reaction*

% HCl in methanol	Time (h)	Yield (%)	Dimer Isolated
1.5	3.5	30	IX ($R = CO_2CH_3$; $R_1 = H$)
1.6	3.5	36	IX ($R = CO_2CH_3$; $R_1 = H$)
2.0	3.0	45	IX ($R = CO_2CH_3$; $R_1 = H$)
5.0	2.0	56	IX ($R = CO_2CH_3$; $R_1 = H$)
7.0	3.5	50	IX ($R = CO_2CH_3$; $R_1 = H$)
15	3.5	40	IX ($R = CO_2CH_3$; $R_1 = H$)
30	3.0	30	IX ($R = CO_2CH_3$; $R_1 = H$)
60	3.0	25	IX ($R = CO_2CH_3$; $R_1 = H$)

it was found that the chloroindolenine and vindoline could be recovered unchanged as virtually the only products after several hours of refluxing. Gradual increase in acid concentration up to 5% revealed no detectable change in the configuration of the dimeric product. Further increases in acid concentration ($> 7\%$) brought about an overall decrease in dimeric material and an increasingly complex product mixture presumably arising from decomposition of the reactants.

Although these various studies failed to provide any indication of dimeric products possessing the same configuration at C(18′) as vinblastine they did add considerable information about the possible mechanism of coupling. In addition, a very mild method for coupling was also fortuitously realized. During these investigations, the methanolic hydrogen chloride had been prepared by adding the calculated volume of purified acetyl chloride to anhydrous methanol which had been cooled to 0°. These two compounds reacted instantly to yield methyl acetate and hydrogen chloride. Thus, during the study of the effects of acid concentration mentioned above, successively larger aliquots of acetyl chloride had been added. For the sake of completeness, a conventional coupling in acetyl chloride as solvent at its reflux temperature (51–52°) was attempted. The chloroindolenine and vindoline readily dissolved in this solvent. However, as the reaction proceeded, dimer IX ($R = CO_2CH_3$; $R_1 = H$)

precipitated. Removal of the solvent under vacuum provided a white solid which upon subsequent extraction into methylene chloride provided a near quantitative yield of this dimer. We believe this technique to be the best method for preparing dimeric substances *via* the chloroindolenine approach.

In summary this detailed study of the chloroindolenine approach has provided a general synthetic method for the preparation of 18-epi analogues of the vinblastine series. These compounds provide the opportunity for further structure-activity studies in the cancer area and it is hoped will allow some assessment of the importance of the configuration at C(18') in terms of the anti-tumor activity exhibited by this family. Other studies directed at the synthesis of dimers possessing the natural configuration at this crucial centre are now underway.

Experimental Part

All details concerning spectral measurements, chromatographic separations, etc. are described in the accompanying publication [2].

Synthesis of dimer VIII ($R = H$). A solution of the chloroindolenine VI prepared in the manner previously described [6] (601 mg) and deacetylvindoline hydrazide (521.5 mg) in an anhydrous methanolic 1.5% hydrochloric acid solution (52 ml) was refluxed under a dry nitrogen atmosphere for 3 h. After this period of time had passed and the solution had been allowed to cool to room temperature (RT.), it was diluted with water (78.5 ml) and made slightly basic by the addition of sodium carbonate. The basic solution was extracted with methylene chloride (3× 130 ml). The methylene chloride extract was dried and the solvent was removed to yield 985.5 mg of a powder. Some of this powder (894.5 mg) was washed several times with hot methanol to provide 543.6 mg of small white crystals. Concentration of the methanol washings provided a further 18.2 mg of the same crystalline material. This material displayed rather unusual melting properties. When the temperature was raised slowly (*ca.* 2°/min) no melting point could be observed but rather a progressive darkening beginning at about 190°. When the temperature was raised rapidly (*ca.* 10°/min) the sample appeared to melt and immediately solidify in the range 189–194°. Recrystallization from 95% ethanol provided a sample which melted and solidified in the same manner in the range 190–192°. – IR.: 3400 and 3280 (NH and OH), 1668 and 1616 cm^{-1} ($CONHNH_2$). – UV.: 309 (sh, 3.85), 294 (4.04), 285 (4.05), 263 (4.17), 224 (sh, 4.60), 215 (4.64) nm. – NMR.: 0.60 (*t*, 3 H, CH_2CH_3 of the vindoline portion); 0.82 (*t*, 3 H, CH_2CH_3 of cleavamine portion); 4.40 (*d*, 1 H, H–C(18'), $J = 10$); 6.03 (*s*, 1 H, H–C(17)); 6.65 (*s*, 1 H, H–C(14)); 6.93–7.55 (diffuse, 4 H, aromatic protons of cleavamine portion). – MS.: Mol.-Wt.: 694.420 (Calc. for $C_{41}H_{54}N_6O_4$: 694.421, 692.403 (Calc. for $C_{41}H_{52}N_6O_4$ ($M^+ - 2$):692.405).

$C_{41}H_{54}N_6O_4$ (694.421) Calc. C 70.86 H 7.83 N 12.10% Found C 70.53 H 7.72 N 12.24%

Cleavage of the dimer VIII ($R = H$). A mixture of the dimer VIII (R = H) (51 mg), tin (205 mg), stannous chloride dihydrate (205 mg) and 2N hydrochloric acid (10 ml) was refluxed for 2 h under a nitrogen atmosphere. After this period of time had passed and the mixture had been cooled to RT., a saturated aqueous solution of potassium carbonate was added until the mixture was basic. The mixture was extracted with methylene chloride (5× 5 ml). The methylene chloride extract contained a fine white suspension which was effectively removed by centrifugation. The methylene chloride centrifugate was dried and rotary evaporated to give 41 mg of a brown coloured residue which was chromatographed on alumina (4 g). Elution with petroleum ether (b.p. 30–60°)/benzene 1:1 provided a material (8 mg) which on crystallization from methanol gave 4β-dihydrocleavamine (II, R = H), m.p. 135–138°, identical with an authentic sample as shown by m.p., mixed m.p., comparison IR. and TLC. (alumina, benzene/chloroform 3:1 and silica gel, chloroform). Elution with methanol provided a material (22 mg) which on crystallization from ethanol/water and recrystallization from 95% ethanol provided a sample that was shown to be identical with deacetylvindoline hydrazide (VII) by comparison IR. and TLC. (silica gel, 95° ethanol and alumina, 95% ethanol).

Chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (III). To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (II, R = CO_2CH_3) (400 mg) in methylene chloride (40 ml) and triethylamine (0.2 ml) cooled in an ice-water bath, was added a solution of *t*-butyl hypochlorite in carbontetrachloride (25 ml, 0.05 M) over a period of 45 min. The solution was washed with ice-water (2 × 30 ml), dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give the chloroindolenine as an amorphous solid (440 mg). – IR.: 2775 (*Bohlmann* bonds), 1727 (C=O), 1612 and 1575 cm^{-1} (indolenine, C=N). – UV.: 292 (3.44), 275 (3.44), 227 (4.30) nm. – NMR.: 0.86 (*t*, 3 H, $-CH_2CH_3$); 3.59 (*s*, 3 H, $-COOCH_3$); 4.47 (*d*, 1 H, H—C(18)); 7.02–7.60 (4 H, aromatic). – MS.: 376, 374, 138, 124. Mol.-Wt.: 374.174 (Calc. for $C_{21}H_{27}ClN_2O_2$: 374.176).

Catalytic reduction of the chloroindolenine III (R = $COOCH_3$). The chloroindolenine III (R = $COOCH_3$) (29 mg) was hydrogenated over *Adam's* catalyst (37 mg) in ethyl acetate (10 ml) at RT and atmospheric pressure for 1 h. Then, the reaction mixture was filtered through a celite pad and the pad was washed with methanol. The methanol and ethyl acetate solutions were combined and evaporated. The material obtained was dissolved in methylene chloride and washed with a saturated aqueous potassium carbonate solution. The methylene chloride solution was then dried with anhydrous sodium sulfate and evaporated to give 19 mg of a material, which on recrystallization from methanol gave 18β-carbomethoxy-4β-dihydrocleavamine, (II, R = CO_2CH_3), m.p. 146–148° identical with an authentic sample as shown by m.p. and mixed m.p., comparison IR. and TLC. (alumina, benzene/chloroform 3:1 and silica gel, chloroform/ethyl acetate 1:1).

Synthesis of dimer VIII (R = CO_2CH_3). A solution of deacetylvindoline hydrazide (294 mg) and the chloroindolenine III (R = $COOCH_3$) prepared in the manner previously described (386 mg) in an anhydrous methanolic 1.5% hydrochloric acid solution (2.9 ml) was refluxed for 3 h under a dry nitrogen atmosphere. After this period of time had passed and the solution had been allowed to cool to RT., the solution was diluted with water (44 ml) and made slightly basic by the addition of sodium carbonate. The basic solution was extracted with methylene chloride (3 × 75 ml). The methylene chloride extract was dried and the solvent removed to give a powdery material (621 mg). Some of this material (409.5 mg) was subjected to separation by preparative TLC. Silica gel (*Woelm*) plates (20 × 20 cm, 0.5 mm thickness) were used, with about 60 mg of material being applied to each plate. After elution with methanol/water 3:1, the desired band was scraped off each plate and extracted, first with methanol at RT. and then with boiling methanol. The solvent was then removed and the residue was extracted with methylene chloride. Filtration of the methylene chloride extracts and removal of the solvent provided 118 mg of the dimer VIII (R = CO_2CH_3) as an amorphous solid. – IR.: 3410 and 3290 (NH and OH), 1726 (ester C=O), 1663 and 1615 cm^{-1} ($CONHNH_2$). – UV.: 313 (sh, 3.88), 296 (4.05), 290 (4.06), 269 (4.11), 218 (4.69) nm. – NMR.: 0.80 (*t*, 3 H, CH_2CH_3 of vindoline portion); 0.92 (*t*, 3 H, CH_2CH_3 of cleavamine portion); 3.70 (*s*, 3 H, C(18')—$COOCH_3$); 5.93 (*s*, 1 H, H—C(17)); 6.94 (*s*, 1 H, H—C(14)); 6.90–7.52 (diffuse, 4 H, aromatic protons on cleavamine portion). – MS.: Mol.-Wt.: 752.427.

$C_{43}H_{56}N_6O_6$ (752.426) Calc. C 68.58 H 7.44 N 11.16% Found C 68.23 H 7.31 N 10.98%

Cleavage of the dimer VIII (R = CO_2CH_3). A mixture of the dimer VIII (R = CO_2CH_3) (13 mg), tin (750 mg), stannous chloride dihydrate (750 mg) and 2 N hydrochloric acid (25 ml) was refluxed for 1 h. The mixture was then diluted with water, cooled and neutralized with sodium hydrogencarbonate. The solid material was separated by filtration. Both the solid and filtrate were extracted with chloroform. The chloroform extract was dried and the solvent removed to give a residue (9 mg). This residue was subjected to separation by preparative TLC. A silica gel (*Woelm*) plate (5 × 20 cm, 0.5 mm) was used. After transport with ethyl acetate/acetone 1:1, the desired bands were scraped off and extracted with methanol. In this manner 4 mg of a material was obtained which was shown to be 18β-carbomethoxy-4β-dihydrocleavamine (II, R = CO_2CH_3) by comparison (TLC. and IR.) with an authentic sample. Another 3 mg of a material was obtained which displayed the TLC. properties of deacetylvindoline hydrazide (VII).

Synthesis of the dimer IX (R = R_1 = H). The chloroindolenine of 4β-dihydrocleavamine (VI) (557 mg) and vindoline (523 mg) were dissolved in anhydrous methanolic 1.5% hydrochloric acid solution. This solution was reflexed for 2.5 h under a dry nitrogen atmosphere. The reaction mixture was diluted with water (87 ml) and the resulting solution was made just basic with potassium carbonate. Extraction with methylene chloride (5 × 50 ml) and removal of the solvent

after drying the extracts over anhydrous sodium sulfate gave the crude dimer as a yellow glass-like material (1.006 g). Chromatography on alumina (100 g) gave the pure dimer IX (R = R_1 = H) on elution with benzene/ethylether 1:1 as a colourless glass (656 mg). Crystallization from methanol gave an analytical sample, m.p. 205–206°. – IR. 3430 (N—H), 1733 (C=O for —OAc, —CO_2CH_3), 1630 cm^{-1} (C=C for vindoline). – UV.: 214 (4.63), 257 (4.18), 287 (4.06), 293 (4.07), 310 (sh, 3.88) nm. – NMR.: 0.07 (*t*, 3 H, $C_5CH_2CH_3$); 0.83 (*t*, 3 H, C(4') CH_2CH_3); 2.03 (*s*, 3 H, OAc); 2.65 (*s*, 3 H, N—CH_3); 3.64 (*s*, 1 H, H—C(2)); 3.72 (*s*, 3 H, —$COCH_3$); 3.86 (*s*, 3 H, C(16)—OCH_3); 4.43 (br. *d*, 1 H, H—C(18')); 5.24 (br. *d*, 1 H, C(6)=C(7)*H*R); 5.34 (*s*, 1 H, *H*—C(4)—OAc); 5.84 (br. *d* × *d*, 1 H, C(6)=C(7)HR); 6.08 (*s*, 1 H, indoline H—C(17)); 6.68 (*s*, 1 H, indoline, H—C(14)); 7.0–7.5 (diffuse, 4 H, indole aromatic); 7.87 (br. *s*, 1 H, indole N—H); 9.62 (br. *s*, 1 H, O—H). – MS.: 58, 60, 74, 91, 92, 106, 107, 121, 122, 135, 138, 149. Mol.-Wt.: 736.420 (Calc. for $C_{44}H_{56}N_4O_6$: 736,420; 737.425. Calc. for $C_{44}H_{57}N_4O_6$ (M^+ + 1): 737.428; 735.408. Calc. for $C_{44}H_{55}N_4O_6$ (M^+ – 1): 735.412).

$C_{44}H_{56}N_4O_6$ (736.955) Calc. C 71.71 H 7.66 N 7.60% Found C 71.52 H 7.49 N 7.35%

Cleavage of the dimer IX (*R* = R_1 = *H*). The dimer IX (R = R_1 = H) as the hydrochloride (30 mg) was dissolved in anhydrous methanolic 7% hydrochloric acid solution (5 ml). To this solution was added tin (50 mg) and stannous chloride (50 mg) and the reaction mixture was refluxed for 2 h under a nitrogen atmosphere. After this time, acetyl chloride (1 ml) and an additional amount of tin (50 mg) was added and the mixture refluxed for another 1 h. This solution was then made basic with ammonium hydroxide solution and extracted with methylene chloride (2 × 25 ml). The crude reaction product obtained on taking the organic extract to dryness was separated into its components using preparative alumina TLC. eluting with ethyl acetate/chloroform 1:1. The products isolated were 4β-dihydrocleavamine (II, R = H) (5.9 mg), vindoline (V) (2.3 mg), starting dimer (3.4 mg) and deacetylvindoline (V, HO—C(4) in place of AcO—C(4)) (10.2 mg). Each of these materials were identified by TLC. and IR. comparison with samples of authentic material.

Synthesis of the dimer IX (*R* = CO_2CH_3; R^1 = *H*). The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (III, R = CO_2CH_3) (400 mg) and vindoline (336 mg) were dissolved in anhydrous methanolic 1.5% hydrochloric acid solution and the resulting solution was refluxed under a nitrogen atmosphere for 3 h. The solvent was removed under reduced pressure and the residue was partitioned between dichloromethane (100 ml) and aqueous potassium hydrogencarbonate solution (100 ml, 1%). The aqueous phase was extracted with a further amount of methylene chloride (2 × 30 ml) and the combined extracts were dried over anhydrous sodium sulfate. The solvent was removed to give a yellow glass-like residue (823 mg). This material was chromatographed on alumina (100 g); benzene/diethylether 1:1 elution gave the desired dimer as a colourless glass. Crystallization from methanol gave an analytical sample, m.p. 221–225°. – IR.: 3430 (N—H), 1730 (C=O), 1630 cm^{-1} (C=C). – UV.: 217 (4.48), 265 (3.93), 287 (3.92), 296 (3.92), 313 (sh, 3.78) nm. – NMR.: 0.66 (*t*, 3 H, $C_5CH_2CH_3$); 0.91 (*t*, 3 H, C(4')—CH_2CH_3); 2.04 (*s*, 3 H, —OAc); 2.60 (*s*, 3 H, N—CH_3); 3.64 (*s*, 1 H, H—C(2)); 3.71 (*s*, 6 H, 2 $COOCH_3$); 3.84 (*s*, 3 H, —OCH_3); 5.28 (br. *d*, 1 H, C(7)=C(6)*H*); 5.33 (*s*, 1 H, H—C(4)); 5.88 (*d* × *d*, 1 H, C(6)=C(7)HR); 6.96 (*s*, 1 H, H—C(17)); 6.95 (*s*, 1 H, H—C(14)); 7.5–7.0 (diffuse, 4 H, indole aromatic); 9.00 (br. *s*, 1 H, N—H); 9.51 (br. *s*, 1 H, OH). – MS.: 58, 74, 91, 106, 107, 121, 122, 135, 138. Mol.-Wt.: 794.419 (Calc. for $C_{46}H_{58}N_4O_8$ (M^+): 794.425; 795.428. Calc. for $C_{46}H_{59}N_4O_8$ (M^+ + 1): 795.433; 796.437. Calc. for $C_{46}H_{60}N_4O_8$ (M^+ + 2): 796.441; 793.410. Calc. for $C_{46}H_{57}N_4O_8$ (M^+ – 1): 793.417; 792.403. Calc. for $C_{46}H_{56}N_4O_8$ (M^+ – 2): 792.409).

$C_{46}H_{58}N_4O_8$ (794.425) Calc. C 69.48 H 7.30 N 7.05% Found C 69.42 H 7.19 N 7.21%

Cleavage of the dimer IX (*R* = CO_2CH_3; R_1 = *H*). The dimer IX (R = CO_2CH_3; R^1 = H) as the hydrochloride (50 mg) was dissolved in anhydrous methanolic 7% hydrochloric acid solution (5 ml), and tin (100 mg) and stannous chloride (100 mg) were added. This mixture was refluxed for 1 h under a nitrogen atmosphere, and was then basified using ammonium hydroxide solution. To this suspension was added methylene chloride (50 ml) and water (30 ml) and the emulsion formed on shaking was filtered under reduced pressure. The aqueous phase was extracted with a further amount of methylene dichloride and combined organic extracts were dried over anhydrous sodium sulfate and taken to dryness. The crude product (53 mg) was separated into its components by preparative alumina TLC. using ethyl acetate/chloroform 1:1 as eluting solvent.

The products obtained were vindoline (12.6 mg), deacetylvindoline (3.2 mg), 18α-carbomethoxy-4β-dihydrocleavamine (8.1 mg) and 18β-carbomethoxy-4β-dihydrocleavamine (8.0 mg). The identity of these products was established by a comparison with authentic materials on TLC.; superimposable IR. spectra were obtained for all except 18α-carbomethoxy-4β-dihydrocleavamine. The identity of this compound was established by NMR. comparison with an authentic sample.

Synthesis of the dimer IX ($R = CN$; $R_1 = H$). 18β-Cyano-β-dihydrocleavamine prepared according to a previously described procedure [6] (147 mg) was dissolved in a solution of methylene chloride (17 ml) and triethylamine (0.079 ml) and this solution was cooled to iceacetone bath temperature. A solution of *t*-butyl hypochlorite in carbon tetrachloride (12 ml, 0.042 M) was added dropwise to the cooled solution, over a 0.5 h period. The solvent was removed under reduced pressure and an aliquot of benzene (20 ml) was added to the residue and distilled off in order to azeotrope off any water which might be present. To this crude chloroindolenine was added vindoline (217 mg) and this mixture was dissolved in an anhydrous methanolic hydrochloric acid solution (15 ml, 1%). The solution was refluxed under a nitrogen atmosphere for 2 h. After this time, the solvent was removed, the residue was taken up in water (50 ml) and made slightly basic. This mixture was extracted with methylene chloride (3 × 30 ml) and the combined extracts taken to dryness. The residue was chromatographed on alumina (100 g). Benzene/ethyl acetate 95:5 elution gave unconsumed vindoline (119 mg) and benzene/ethyl acetate 9:1 elution gave the dimer IX (R = CN; R_1 = H) (22 mg). This material crystallized from diethylether, m.p. 204–224°. – IR.: 3320 (N—H, O—H), 1740 cm^{-1} (C=O). – UV.: 215 (4.68), 265 (4.19), 285 (4.02), 294 (4.01), 312 (sh, 3.78) nm. – NMR.: 0.35 (*t*, 3 H, C(5)—CH_2CH_3); 0.84 (*t*, 3 H, C(4′)—CH_2CH_3); 2.01 (*s*, 3 H, —OAc); 2.65 (*s*, 3 H, N—CH_3); 3.64 (*s*, 1 H, H—C(2)); 3.71 (*s*, 3 H, —CO_2CH_3); 3.88 (*s*, 3 H, —OCH_3); 5.20 (br. *d*, 1 H, C(7)=C(6)HR); 5.30 (*s*, 1 H, H—C(4)); 5.80 (*d* × *d*, 1 H, C(6)=C(7)*H*R); 6.06 (*s*, 1 H, H—C(17)); 6.21 (*s*, 1 H, H—C(14)); 7.5–7.0 (diffuse, 4 H, indole aromatic); 8.10 (br., 1 H, N—H); 9.3 (very br., 1 H, O—H). – MS.: 50, 51, 55, 57, 67, 69, 77, 78, 79, 107, 122, 124, 128, 135, 138. Mol.-Wt.: 761.413.

$C_{45}H_{55}N_5O_6$ (761.415) Calc. C 70.92 H 7.22 N 9.19% Found C 70.72 H 7.04 N 9.31%

Synthesis of the dimer IX ($R = CO_2CH_3$; $R_1 = H$; *6,7-dihydro*). A solution of 6,7-dihydrovindoline (V, 6,7-dihydro) and the chloroindolenine III (R = $COOCH_3$) (440 mg) in anhydrous methanolic 1.5% hydrochloric acid (57 ml) was refluxed for 3 h under a dry nitrogen atmosphere. The solvent was then removed in a rotary evaporator and the residue was dissolved in methylene chloride (100 ml). Water (100 ml) was added and then potassium carbonate with mixing until the mixture was basic. The organic phase was then separated from the aqueous phase and the aqueous phase was extracted with an additional quantity of methylene chloride (2 × 30 ml). After the methylene chloride extracts had been combined and dried, the solvent was removed to give a glassy residue (694.4 mg). The residue was chromatographed on alumina (70 g). Elution with benzene/diethyl ether 4:1 provided 248.4 mg of the dimer IX (R = CO_2CH_3; R_1 = H; 6,7-dihydro) as an amorphous solid. – IR.: 3430 (NH and OH); 1735 cm^{-1} (ester C=O). – UV.: 307 (sh, 4.01), 295 (4.14), 287 (4.15), 263 (4.19), 217 (4.64) nm. – NMR.: 0.53 (*t*, 3 H, CH_2CH_3 of vindoline portion); 0.96 (*t*, 3 H, $CHCH_3$ of cleavamine portion); 3.72 (*s*, 3 H, C(18′)—$COOCH_3$); 5.96 (*s*, 1 H, H—C(17)); 6.92 (*s*, 1 H, H—C(14)); 6.90–7.46 (diffuse, 4 H, aromatic protons of cleavamine portion). – MS.: Mol.-Wt.: 796.441.

$C_{46}H_{60}N_4O_8$ (796.441) Calc. C 69.31 H 7.53 N 7.03% Found C 69.17 H 7.36 N 6.99%

Cleavage of the dimer IX ($R = CO_2CH_3$; $R_1 = H$; *6,7-dihydro*). A mixture of the dimer IX (R = CO_2CH_3; R_1 = H; 6,7-dihydro) (50.1 mg), tin (100 mg), stannous chloride dihydrate (100 mg) and anhydrous 1.5 N (6.5%) methanolic hydrochloric acid (10 ml) was refluxed for 1 h under a nitrogen atmosphere. Then, after the mixture had been cooled to RT., an aqueous 10% potassium carbonate solution (15 ml) was added and the resulting mixture was extracted with methylene chloride (5 × 10 ml). The extract was centrifuged to precipitate the suspended white solid and the clear centrifugate was dried. Removal of the solvent provided a gummy residue (55 mg). The residue was subjected to chromatography on alumina (5 g). Elution with petroleum ether (b.p. 30–60°)/benzene 4:1 provided 4.2 mg of a crystalline compound. Recrystallization of this compound provided a sample with m.p. 146–148° which was identical with an authentic sample of 18β-carbomethoxy-4β-dihydrocleavamine (II, R = CO_2CH_3) as shown by m.p. and

mixed m.p., comparison IR. and TLC. (alumina, benzene/chloroform 3:1 and silica gel, chloroform/ethyl acetate 1:1).

Further elution in the above chromatography with petroleum ether (b.p. 30–60°)/benzene 4:1 provided 8.0 mg of a material which could not be induced to crystallize but was shown to be identical with 18α-carbomethoxy-4β-dihydrocleavamine by comparison IR. and TLC. (above systems).

Further elution in the above chromatography with benzene/chloroform 4:1 provided 25.6 mg of a foam which was shown to be identical with an authentic sample of 6,7-dihydrovindoline by comparison IR. and TLC. (silica gel, ethyl acetate and the above systems).

Synthesis of dimer IX (R = R₁ = H) from 18β-hydroxy-4β-dihydrocleavamine and vindoline. 18β-Hydroxy-4β-dihydrocleavamine (XIII) (106 mg) and vindoline (157 mg) were dissolved in an anhydrous methanolic hydrochloric acid solution (1%, 10 ml) and the solution was refluxed under a nitrogen atmosphere for 4 h. The reaction mixture was then poured into ice-cold aqueous ammonium hydroxide solution (10 ml, 5 N) and the resulting suspension was extracted with methylene chloride (3 × 30 ml). The combined organic extract was taken to dryness and the residue was chromatographed on alumina (100 g). Dichloromethane elution gave vindoline (10 mg) in the early fraction and further elution gave the dimer IX (R = R₁ = H) (173 mg). Dichloromethane/methanol 99:1 elution gave deacetylvindoline (3 mg) which was followed by deacetyl dimer IX (R = R₁ = H; HO—C(4) in place of AcO—C(4)) (15 mg). The isolated dimer IX (R = R₁ = H), had identical TLC. properties and gave the same NMR. spectrum as that obtained for the product from the dimerization of the chloroindolenine of 4β-dihydrocleavamine (II) with vindoline.

Chloroindolenine of 18β-carbomethoxycleavamine (XII). To a solution of 18β-carbomethoxycleavamine (102 mg) in abs. benzene (2 ml) was added, at RT., with stirring 1-chlorobenzotriazole (94.6 mg) in benzene (2 ml) over a period of 20 min. After this addition was complete the remaining 1-chlorobenzotriazole was rinsed in with a further portion of benzene (0.5 ml) over a period of 5 min. The reaction mixture was then partitioned between benzene and ice-cold water (10 ml of each). The benzene layer was separated, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield a yellowish-brown foam (115 mg). This material was quickly columned through a plug of alumina (neutral *Woelm* III) with benzene elution followed by methylene chloride and then finally methanol. The pure chloroindolenine was eluted first from the column (29 mg, ~30%). – IR.: 2990, 1790 (sh), 1749, 1675, 1620, 1575, 1455, 1425 cm^{-1}. – UV.: 335 (sh), 291 (sh), 285, 260 (sh), 222 nm. – NMR.: 3.62 (*s*, 3 H, CO_2CH_3); 4.35 (br. *d*, $J \sim 10$, 1 H, H—C(18)); 5.4 (br. *d*, $J \sim 8$, 1 H, C(3), olefinic); 6.60–7.65 (*m*, 4 H, aromatic).

The remainder of the material was unidentifiable and corresponded to decomposition on the column. Prior to chromatography a TLC. check of the reaction mixture revealed that it contained > 90% of the desired material.

Synthesis of the dimer X (R = CO_2CH_3). Vindoline (400 mg) was dissolved in anhydrous 1.5% methanolic hydrogen chloride (75 ml) prepared by the addition of purified acetyl chloride (9.25 ml) to anhydrous methanol (400 ml). This solution was added quickly, under a stream of nitrogen, to the chloroindolenine of 18β-carbomethoxycleavamine (XII) (400 mg). The reaction mixture rapidly turned to a deep wine-red colour when refluxed for 2.5 h under a positive pressure of nitrogen. After this time it was cooled to RT., diluted with water and cautiously basified with potassium hydrogencarbonate. When it was weakly basic, it was extracted (4 × 40 ml) with methylene chloride and the combined organic layer was dried over anhydrous sodium sulfate and filtered. Evaporation of the solvent under reduced pressure afforded a brown foam (800 mg). This material was introduced onto a column of Sephadex LH-20 prepared in the following way. The gel-beads were allowed to swell by stirring them in a beaker containing methanol for 4 h at RT. The resulting slurry was packed into a column 36″ × 1″ diameter. This column was allowed to settle for 1 h and then stabilized under run conditions overnight. The flow rate at this stage was 2 ml/min. Several 10 ml fractions were collected after the sample was introduced onto the column. Fractions 7 to 10 inclusive contained the desired dimer X (R = CO_2CH_3) (60 mg). – UV.: 310 (sh, 3.8), 292 (3.9), 286 (sh, 3.9), 259 (3.9), 221 (sh, 4.4), 212 (4.5) nm. – NMR.: 0.60 (*t*, $J \sim 8$, 3 H, C(5)—CH_2CH_3); 1.00 (*t*, $J \sim 8$, 3 H, C(4′)—CH_2CH_3); 2.05 (*s*, 3 H, C(4)—$COCH_3$); 2.61 (*s*, 3 H, N_1—CH_3); 3.73 (*s*, 6 H, C(18′)—CO_2CH_3 and C(3)—CO_2CH_3); 3.86 (*s*, 3 H, C(16)—OCH_3); 5.30 (*d*, $J = 10$, 1 H, H—C(6)); 5.36 (*s*, 1 H, H—C(4)); 5.50 (*m*, 1 H, H—C(3′)); 5.90 (*d* × *d*, $J \sim 10$, 4,

1 H, H—C(7)); 6.00 (*s*, 1 H, H—C(17)); 6.98 (*s*, 1 H, H—C(14)); 7.00–7.45 (*m*, 4 H, aromatic); 9.09 (br. *s*, 1 H, O*H*); 9.30 (br. *s*, 1 H, N—*H*). – MS.: 106, 107, 108, 121, 122, 135, 136, 149, 188, 282, 339, 335, 669, 791, 792. Mol.-Wt.: 792.4103 (Calc. for $C_{45}H_{56}N_4O_8$: 792.4098).

Further fractions contained this same dimer contaminated with small amounts of blue spot (25 mg). Total yield of dimer could be estimated at 10% from this reaction. The major new product of the reaction was the socalled blue spot (obtained in fraction 12–20). *Blue spot.* IR.: 3000, 2960, 2840, 1740, 1620, 1500, 1468, 1440, 1380, 1260, 1150 (br.), 900, 880 cm^{-1}. – UV.: 307 (3.8), 295 (sh, 3.8), 257 (4.0), 212 (4.3) nm. – NMR.: 0.28 (*t*, *J* = 8, 3 H); 1.98 (*s*, 3 H); 2.58 (*s*, 3 H); 3.72 (*s*, 7 H); 5.20 (diffuse *m*, 2 H); 5.41 (*s*, 1 H); 5.80 (diffuse *m*, 2 H); 6.01 (*s*, 1 H); 6.73 (*s*, 1 H); 6.90–8.00 (*m*, 4 H). – MS.: 370, 356, 355, 290, 289, 69, 57, 55, 50, 44, 43, 41.

Found C 66.16 H 7.05 N 6.31%.

Synthesis of the dimer X (R = CO_2CH_3) by modified conditions. The chloroindolenine of 18β-carbomethoxycleavamine was made by injecting neat *t*-butyl hypochlorite (0.07 ml) into a solution of 18β-carbomethoxycleavamine (200 mg) in refluxing methylene chloride (6.5 ml) containing triethylamine (0.1 ml). The resulting yellowish-orange solution was stirred for 30 min at reflux, then cooled to RT. and evaporated to dryness under reduced pressure to yield a light brown foam.

This compound was dissolved in methylene chloride and divided into two equal portions which were again taken to dryness. To each of these portions was added a solution of vindoline (100 mg) in 1.5% methanolic hydrogen chloride (20 ml). One portion was heated to reflux for 3.5 h. After this period it was cooled to RT. and cautiously added to an ice-cold aqueous solution of sodium hydrogencarbonate (10%, 50 ml), and extracted with methylene chloride (3 × 25 ml). The combined organic phase was dried over anhydrous sodium sulfate filtered and evaporated to dryness to yield a brown foam (200 mg) from which dimer X (R = CO_2CH_3) (16.4 mg) was purifiable by column chromatography on alumina (neutral *Woelm* III) by elution with benzene/ethyl acetate 85:15 (8% yield).

The other portion was allowed to stir at RT. for 10 days. After this period, the solvent was removed under reduced pressure to yield a green gum which was taken up in 0.1M citric acid (25 ml, resulting pH = 1.8) and extracted with benzene (25 ml). The benzene layer was isolated and evaporated to dryness to yield 27 mg of monomeric materials identified by TLC. and UV. Then, the pH was adjusted stepwise to 10.7 and a benzene extraction performed at each step. The dimer enriched fractions were found to be between pH values of 4.5 and 5.4 (74 mg). Total weight recovery was 192.3 mg from 200 mg or 96.5%. The dimer enriched fractions were combined and purified by preparative TLC. on alumina (ethyl acetate) to yield pure dimer X (R = CO_2CH_3) (40 mg) identical in every respect to that obtained previously (20% yield).

Cleavage of dimer X (R = CO_2CH_3). Dimer X (R = CO_2CH_3) (9.0 mg) was dissolved in anhydrous 7% methanolic hydrogen chloride (4 ml) and tin (39 mg) and stannous chloride (36 mg) were added. The reaction mixture was refluxed for 2 h, and then cooled, diluted with water, and basified with ammonium hydroxide. This basic solution was extracted with methylene chloride (4 × 20 ml). Drying of the combined organic phase over anhydrous sodium sulfate, filtration, and evaporation of the solvent under reduced pressure afforded a brown foam. This material could be separated by preparative TLC. on alumina using chloroform as the developer to yield cleavamine (3 mg), vindoline (3 mg), deacetylvindoline (1 mg) and starting dimer (1.1 mg) as the only isolable materials. These could all be identified by IR. and m.p. comparisons with the authentic materials.

Synthesis of the dimer IX (R = CO_2CH_3; R_1 = OH). To a solution of 18β-carbomethoxy-4β-dihydrocleavamin-3α-ol (250 mg) in methylene chloride (25 ml) containing triethylamine (1 ml) was added a solution of *t*-butyl hypochlorite (40 ml of a 0.02M solution) in carbon tetrachloride at 0° with stirring over 1.5 h. Evaporation of the solvent at 0° under high vacuum afforded the chloroindolenine XI as a reddishbrown foam.

Vindoline (150 mg) was dissolved in 1.5% methanolic hydrogen chloride (50 ml) and added, under a stream of nitrogen, to the above chloroindolenine and the whole refluxed for 2.5 h. After this time it was cooled to RT., diluted with water, basified with solid potassium hydrogencarbonate and extracted with methylene chloride (4 × 25 ml). The combined organic phase was dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a foam (402 mg).

This material was chromatographed on alumina (neutral *Woelm* III) to yield, upon elution with benzene/ethyl acetate 75:25, the desired dimer IX (R = CO_2CH_3; R_1 = OH) as a whitish glass (40 mg, 8% yield). – UV.: 315 (3.8), 295 (3.9), 286 (3.9), 262 (3.9), 225 (sh, 4.4), 216 (4.5) nm. – NMR.: 0.60 (*t*, *J* ~ 7, 3 H, $-CH_2CH_3$); 0.98 (*t*, *J* ~ 7, 3 H, CH_2CH_3); 2.25 (*s*, 3 H, C_4—OAc); 2.61 (*s*, 3 H, N_1—CH_3); 3.75 (*s*, 6 H, 2 × CO_2CH_3); 3.86 (*s*, 3 H, C_{16}—OCH_3); 5.37 (*s*, 1 H, C_7—*H*); 5.40 (*m*, 1 H, C_6—*H*), 5.90 (*m*, 1 H, C_7—*H*); 6.00 (*s*, 1 H, C_{17}—*H*); 6.89 (*s*, 1 H, C_{14}—*H*); 7.0–7.5 (*m*, 4 H, aromatic); 8.98 (*s*, 1 H, C_3—*OH*); 9.42 (br. *s*, 1 H, N_a'—*H*). – MS.: 810, 765, 469, 352' 323, 282, 202, 200, 188, 156, 154, 144, 138, 136, 135, 124, 122, 121, 107.

Separation of dimer IX (*R* = CO_2CH_3; R_1 = *OH*) by Sephadex chromatography. 18β-Carbomethoxy-4β-dihydrocleavamin-3α-ol (250 mg) was converted to its chloroindolenine according to the procedure above and dimerized with vindoline (200 mg) in 1.5% methanolic hydrogen chloride (100 ml) by refluxing the reaction mixture for 2.75 h. After this time it was worked-up as above to afford the crude reaction mixture as a light yellowish-brown foam (500 mg). This material was introduced onto the Sephadex LH-20 column mentioned previously. A 2 ml/min flow rate was maintained and 10 ml fractions were collected. Fractions 12, 13 and 14 afforded the desired dimer slightly contaminated with blue spot material. This crude dimer could be purified by preparative TLC. to afford the pure dimer (92.3 mg, 18.4% yield).

Cleavage of dimer IX (*R* = CO_2CH_3; R_1 = *OH*). Dimer IX (R = CO_2CH_3; R_1 = OH) (13 mg) was dissolved in anhydrous 7% methanolic hydrogen chloride and tin (35 mg) and stannous chloride (50 mg) were added. The reaction mixture was refluxed for 1.5 h and then cooled, diluted with water and basified with ammonium hydroxide. The resulting basic solution was extracted with methylene chloride (4 × 20 ml) and the combined organic phase dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a foam. From this material, vindoline, deacetylvindoline, 4β-dihydrocleavamin-3α-ol, and dimer could be isolated by preparative TLC., and compared with authentic samples.

18-α-Methoxy-18β-carbomethoxy-4β-dihydrocleavamine (*XVI*) (*as hydrochloride salt*). To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (250 mg) in methylene chloride (25 ml) in a 100 ml round-bottomed flask was added, at 0° with stirring, *t*-butyl hypochlorite (200 micro/t.). TLC. evaluation (alumina, benzene, ceric sulfate spray, then alumina, benzene, antimony pentachloride in carbon tetrachloride spray) revealed that the starting material had been completely replaced by the chloroindolenine. The solvent was removed under reduced pressure at RT. to yield a whitish foam.

To this material was added 1.5% methanolic hydrogen chloride (8 ml) at 0° with stirring. The reaction mixture was a deep wine-red colour. After stirring for 0.5 h at 0° a TLC. examination (alumina, benzene, UV., then ceric sulfate spray) revealed that the chloroindolenine had completely disappeared, and had been replaced by a slightly more polar major spot. Evaporation of the solvent under high vacuum at 0° afforded a reddish-brown powder identified as the desired compound (XVI) chiefly on the basis of its NMR.: 2.35–3.00 (*m*, 4 H, aromatic); 4.85 (br. *s*, 1 H, N_b^+—*H*); 5.70 (*t*, *J* ~ 7 Hz, 1 H); 6.18 (*s*, 3 H, C_{18}—CO_2CH_3); 7.00 (*s*, 3 H, C_{18}—OCH_3); 9.20 (br. unresolved triplet, 3 H, C_4—CH_2CH_3).

This material was quite stable and could be recrystallized from non-hydroxylic solvents such as purified ethyl acetate. It was however quite unstable as the free base.

18α-Methoxy-18β-hydromethylene-4β-dihydrocleavamine (*XVII*). Compound (XVI) (255 mg) was dissolved in freshly distilled dry tetrahydrofuran (30 ml) and cooled to RT. Lithium aluminum hydride (250 mg, 27 mol-equiv.) was added in four equal portions with stirring. After 1.75 h, the excess lithium aluminum hydride was destroyed by the dropwise addition of a saturated aqueous solution of sodium sulfate at 0° (an improvement in the recovery of material was realized by using solid $Na_2SO_4 \cdot 10H_2O$ instead). When no further effervescence was detectable upon the addition of additional drops of the aqueous solution, the reaction mixture was filtered under vacuum through a bed of celite. A white, almost crystalline, residue was collected and returned to the flask. Some more tetrahydrofuran was added and the whole refluxed gently on a steam bath and then filtered again. The combined filtrate was evaporated to dryness under reduced pressure to afford a white foam (232.7 mg).

This material was chromatographed on alumina (neutral *Woelm* III). Elution with gradually increasingly polar solvent combinations yielded the desired compound (XVII) (115 mg) in semi-pure form by eluting with ethyl acetate. This material was re-chromatographed in a similar manner

to yield pure XVII (recrystallized twice from ethyl acetate, 100 mg, 40% yield), m.p. 139–141°. – IR.: 3560, 3410, 2920, 1490, 1465, 1440, 1375, 1340, 1305, 1155, 1140, 1080, 1040 cm^{-1}. – UV.: 292 (3.8), 285 (3.9), 279 (sh, 3.8), 225 (4.5) nm. – NMR.: 0.92 (*t*, $J \sim 7$, 3 H, C_4—CH_2CH_3); 3.12 (*s*, 3 H, C_{18}—OCH_3); 7.0–7.60 (*m*, 4 H, aromatic); 9.86 (br. *s*, 1 H, N—*H*). – MS.: main peaks: *m/e* 342, 311, 310, 271, 185, 181, 180, 168, 156, 144, 139, 138, 137, 126, 125, 124, 122, 110. Mol.-Wt.: 342.2282. Calc. for $C_{21}H_{30}N_2O_2$: 342.2306; 341.2203. Calc. for $C_{21}H_{29}N_2O_2$ ($M^+ - 1$): 341.2228; 340.2122. Calc. for $C_{21}H_{28}N_2O_2$ ($M^+ - 2$): 340.2150; 138.1276. Calc. for $C_9H_{16}N$: 138.1282).

$C_{21}H_{30}N_2O_2 \cdot 1/2$ EtOAc Calc. C 71.47 H 8.87 N 7.25%
Found ,, 71.33 ,, 8.81 ,, 7.06%

Detailed Study of the 'Standard' Coupling Reaction. Each of the experiments below has been checked for reproducibility and where no dimers could be observed, the quality of the reagents has been tested by repetition of the standard reaction with yield of isolated dimer obtained as 60% ± 5%.

Experiment 1: 'Standard' Coupling Procedure. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (40.4 mg) in methylene chloride (4 ml) and triethylamine (0.02 ml; one drop) cooled in an ice-water bath, was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (3 ml of a 0.05 M solution) over a period of 45 min. The solution was washed with ice-water (2 × 3.0 ml) dried over anhydrous Na_2SO_4 and the solvent removed *in vacuo* to give the chloroindolenine as a foam (44.0 mg). The chloroindolenine and vindoline (31.1 mg) were dissolved in anhydrous methanolic 1.5% hydrogen chloride (2 ml) and the resulting solution was stirred at RT. under a nitrogen atmosphere and then refluxed for a further 2 h. The solvent was removed *in vacuo* and the residue partitioned between methylene chloride and aqueous 10% sodium hydrogencarbonate solution. The aqueous phase was extracted with further portions of methylene chloride (2 × 10 ml) and the combined organic extracts were dried over sodium sulfate. The solvent was removed to give a light yellow foam (~70 mg). This material was chromatographed on *Woelm* III alumina (~10 g). Benzene/ethyl acetate 4:1 elution gave the desired dimer IX (R = CO_2CH_3; R_1 = H) in 65 yield (45.0 mg). This material was analyzed by NMR. spectroscopy and compared with the data of previous studies.

Experiment 2: Coupling of 18α-Carbomethoxy-4β-dihydrocleavamine. To a solution of 18α-carbomethoxy-4β-dihydrocleavamine (XIX) (38.6 mg) in methylene chloride (4 ml) and triethylamine (one drop) cooled in an icewater bath was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (2.5 ml of a 0.05 M solution) over a period of 1 h. The solution was washed with ice-water (2 × 3 ml) dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to give the chloroindolenine as a whitish foam (40.0 mg). The chloroindolenine and vindoline (31.2 mg) were dissolved in anhydrous methanolic 1.5% hydrogen chloride (2 ml) and the resulting solution was stirred at RT. for 30 min under a nitrogen atmosphere and then refluxed for a further 2.5 h.

The solvent was removed *in vacuo* and the residue partitioned between methylene chloride and aqueous 10% sodium hydrogencarbonate solution. The aqueous phase was extracted with further portions of methylene chloride (3 × 10 ml) and the combined extracts were dried over sodium sulfate. The solvent was removed *in vacuo* to give a light brownish foam (~70 mg). This material was chromatographed on *Woelm* III alumina (~10 g). Benzene/ethyl acetate 4:1 elution afforded dimeric material in 45% yield. This material was found to be identical to dimer IX (R = CO_2CH_3; R_1 = H) by NMR. and TLC. comparison.

Experiment 3: Coupling with the Chloroindolenine Formation in Refluxing Methylene Chloride. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (43.5 mg) in methylene chloride (5 ml) maintained at reflux temperature by immersion in an oil bath kept at 50°, was added neat *t*-butyl hypochlorite (16.8 mg) with a microsyringe all at once under a nitrogen atmosphere. The yellow coloured solution was refluxed for a further 5 min, then cooled to RT. washed with ice-water (2 × 15 ml) dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield a foam. The chloroindolenine and vindoline (33.0 mg) were dissolved in anhydrous methanolic 1.5% hydrogen chloride (2.3 ml) and the resulting solution was stirred at RT. for 30 min under a nitrogen atmosphere and then refluxed for a further 2 h. The solvent was removed *in vacuo* and the residue partitioned between methylene chloride and aqueous 10% sodium hydrogencarbonate. The aqueous phase was then further extracted with portions of methylene chloride (2 × 10 ml). The combined organic phase was dried over anhydrous sodium sulfate and the solvent remover under reduced pressure to yield a foam (64 mg). This material was chromatographed on 8 g of *Woelm* III

alumina. Benzene/ethyl acetate 4:1 elution gave dimeric material in roughly 35% yield. This was found to be identical to dimer IX (R = CO_2CH_3; R_1 = H).

Experiment 4: As in Experiment 3 but with Triethylamine. A solution of 18β-carbomethoxy-4β-dihydrocleavamine (40.8 mg) in methylene chloride (4.5 ml) containing triethylamine (one drop) was maintained at reflux temperature by immersion in an oil bath kept at 50°. To this solution was added *t*-butyl hypochlorite (14.0 mg) all at once under a nitrogen atmosphere and the reflux continued for a further 5 min. The yellow solution was then cooled, washed with ice-water (2 × 15 ml) dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield a light brown foam (71.6 mg). This material was columned on 9.5 g of *Woelm* III alumina in the usual way to yield ~40% dimeric material. This was identified as dimer IX (R = CO_2CH_3; R_1 = H).

Experiment 5: As in Experiment 4, but using a Solution of Chlorinating Agent in Carbon Tetrachloride. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (40 mg) in methylene chloride (4 ml) containing triethylamine (one drop) heated to reflux in an oil bath, was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (3 ml of a 0.05 M solution; bath temperature 49°) under nitrogen over a period of 15 min. The reaction mixture was cooled, washed with ice-water (3 × 2 ml) dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield a light foam. The standard coupling with 32 mg of vindoline and 2 ml of anhydrous methanolic 1.5% hydrogen chloride followed by the usual column resulted in an isolation of a 50% yield of dimer which was identified as dimer IX (R = CO_2CH_3; R_1 = H).

Experiment 6: As in Experiment 3, but using a Mixture of Methylene Chloride and Carbon Tetrachloride as Solvent. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (20.7 mg) in 3.5 ml of carbon tetrachloride and 1 ml of methylene chloride heated to reflux in an oil bath at 72°, was added *t*-butyl hypochlorite (20 mg) all at once under a nitrogen atmosphere. After 5 min the reaction mixture was cooled to RT. and washed (2 × 10 ml) with icewater. The organic phase was dried over sodium sulfate, filtered and the solvent evaporated to yield a greenish-brown foam. The above chloroindolenine and vindoline (15 mg) were dissolved in 1 ml of anhydrous methanolic 1.5% hydrogen chloride and coupled in the usual way. No dimer could be isolated by subsequent column chromatography. This experiment was checked in the usual way for reproducibility and then the standard coupling was repeated to yield dimer IX (R = CO_2CH_3; R_1 = H) in 60% yield.

Experiment 7: As in Experiment 6, but with 1:1 Mixture of Methylene Chloride, Carbon Tetrachloride. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (~30%, 18α, 70%, 18β) (41 mg) in methylene chloride/carbon tetrachloride 1:1 (7 ml) maintained at a reflux temperature of 53° by immersion in an oil bath at 55° was added *t*-butyl hypochlorite (20 mg) all at once under nitrogen. The reflux was allowed to continue for a further 5 min. The reaction mixture was then cooled to RT., washed with ice-water (2 × 10 ml), dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield a golden coloured foam. This material was coupled under standard conditions with vindoline (28.7 mg) in methanolic 1.5% hydrogen chloride to yield a negligible amount of dimeric material (*i.e.* not visible either under UV. light or upon spraying with antimony pentachloride on an alumina plate).

Experiment 8: As in Experiment 7, but with Mixture of Methylene Chloride to Carbon Tetrachloride 2:1. Experiment 7 above was repeated exactly using 43.4 mg of 18β-carbomethoxy-4β-dihydrocleavamine and 7 ml of methylene chloride/carbon tetrachloride 2:1. Reflux temperature here was 50°. The coupling was performed under standard conditions using 29.0 mg of vindoline and 2.0 ml of methanolic 1.5% hydrogen chloride. The yield of dimer was estimated as above to be 10.0%.

Experiment 9: 'Standard' Coupling with Prior Reaction of the Chloroindolenine with Methanol. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (41.9 mg) in methylene chloride (4 ml) cooled in an ice-water bath, was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (2.6 ml of a 0.05 M solution) over a period of 30 min. The solution was washed with ice-cold saturated brine (2 × 20 ml), dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield a light foam. This was treated with chloroform and methanol at 0° followed by evaporation of the solvent at RT. No reaction was apparent by TLC. Coupling in methanolic 1.5% hydrogen chloride (2 ml) with 26.5 mg of vindoline resulted in a 75% yield of dimer isolated after column chromatography in the usual way on 10 g of *Woelm* III alumina (isolated 49.8 mg from 66.5 mg of crude product). This material was found to be dimer IX (R = CO_2CH_3; R_1 = H).

Experiment 10: 'Standard' Coupling with Prior Reaction of the Chloroindolenine with 1.5% Methanolic Hydrogen Chloride. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (42.2 mg) in methylene chloride (4 ml) cooled in an ice-water bath, was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (2 ml of a 0.05 M solution) over a period of 30 min. The usual workup as above yielded a light yellow foam. To this foam was added, at RT., methanolic 1.5% hydrogen chloride and the resulting solution stirred for 15 min. A check by TLC. at this point revealed the complete disappearance of starting chloroindolenine and the appearance of new more polar spots, one of which was clearly the major one and could be ascribed to an CH_3O—C(18) compound by correlation with some other work (*vide supra*). At this point solid vindoline was added (32.1 mg) and the solution stirred at RT. for a further 15 min and then plunged into a preheated oil bath at 65°. Within 30 min of heating, the reaction was complete and normal work-up and column chromatography yielded 55% (32.8 mg from 72.8 mg crude product) of a dimeric compound which was identified as dimer IX (R = CO_2CH_3; R_1 = H).

Experiment 11: As in Experiment 10 but with an NMR. of the Crude Intermediate after Methanolic HCl Reaction on the Chloroindolenine. Experiment 10 was repeated exactly as above with the single exception that the reaction product from the reaction of the chloroindolenine with methanolic hydrogen chloride was isolated by basification with dilute ammonium hydroxide and extraction with methylene chloride. The resulting organic phase was dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to yield a glass (82 mg from 87 mg of starting chloroindolenine). This material was analyzed by NMR. and showed a clear incorporation of a methoxyl group into the molecule. Subsequent coupling followed by isolation of the dimeric material yielded 40.5% yield (34 mg from 84 mg of crude reaction mixture obtained from 44 mg of the above methoxyl-containing compound with 40 mg of vindoline) of dimer IX (R = CO_2CH_3; R_1 = H).

Experiment 12: Coupling at 140°. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (55 mg) in methylene chloride (4.5 ml) cooled in an ice-water bath, was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (2.5 ml of a 0.05 M solution) over 30 min. Evaporation of the solvent *in vacuo* yielded the desired chloroindolenine (60 mg) as a light brown foam. The above material was combined with vindoline (110 mg) in methylene chloride (1 ml) and the resulting reddish oily solution was transferred to a tube which was then evacuated to yield a white foam. The tube was flushed with dry nitrogen and methanolic 1.5% hydrogen chloride (30 ml) was added at 0°. The tube was sealed and plunged in an oil bath which had been preheated to 140°. After 30 min the reaction mixture was cooled to RT. and worked up in the usual way to yield no dimer at all.

Experiment 13: As Experiment 12, but at 100°. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (160 mg) was made as above in experiment 12. This material was combined with 200 mg of vindoline in methylene chloride (1 ml) and transferred to a tube which was then evacuated to yield a white foam. To this was added, at 0°, methanolic 1.5% hydrogen chloride (2 ml). The tube was sealed and immersed in an oil bath which had been preheated to 100°. After 15 min the reaction mixture was cooled to RT. and worked-up in the usual way to yield a dimeric product which was identified as dimer IX (R = CO_2CH_3; R_1 = H) ($\sim$30% yield).

Experiment 14: Coupling using Benzene as the Solvent and BF_3 Etherate as the Catalyst. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (31.2 mg) was made as in experiment 13 above. This material was combined with vindoline (32.5 mg) in benzene (4 ml) and the solution was treated with BF_3 etherate (five drops). The mixture was stirred at RT. overnight and then worked-up in the usual way to yield no dimeric material at all.

Experiment 15: Coupling in N,N-Dimethylformamide with HCl Catalyst. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (47.5 mg) in methylene chloride (5 ml) cooled in an ice-water bath, was added a solution of *t*-butyl hypochlorite in carbon tetrachloride (2.5 ml of a 0.05 M solution) over 30 min. Evaporation of the solvent *in vacuo* resulted in a light yellow foam. To this foam was added vindoline (103.2 mg) and methylene chloride (1 ml). The resulting solution was evaporated to yield an intimate mixture of vindoline and the chloroindolenine as a crisp white foam. This material was dissolved in 3 ml of N,N-dimethylformamide and dry HCl gas was bubbled in for 15 seconds at a rapid rate, care being taken to ensure that the reaction vessel remained at RT. After stirring for 19 h under nitrogen at RT. the reaction mixture was poured

onto ice-cold aqueous 10% sodium hydrogencarbonate and extracted several times with methylene chloride (3 × 25 ml). The combined reddish-brown organic phase was dried over anhydrous sodium sulfate and the solvent was evaporated to yield a reddish oil which could be converted to a foam by extended evacuation. The crude reaction mixture (~150 mg) was subjected to column chromatography on 30 g of *Woelm* III alumina and yielded 60 mg of dimeric material (~60% based on the fact that only 50 mg of vindoline are expected to react) which was identified as dimer IX ($R = CO_2CH_3$; $R_1 = H$).

Experiment 16: Coupling in Tetrahydrofuran (THF) with HCl Catalyst. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was formed under standard conditions. This material was dissolved with vindoline (50 mg) in anhydrous THF containing 1.5% hydrogen chloride. The resulting solution was refluxed for 3 h under nitrogen. The solvent was removed under reduced pressure and the residue partitioned between methylene chloride and aqueous 10% sodium hydrogencarbonate. The aqueous phase was washed with further portions of methylene chloride and the combined organic phase was dried over anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* resulted in a gum (102 mg) which upon chromatography yielded dimeric material (26 mg or roughly 25%) which was identified as dimer IX ($R = CO_2CH_3$; $R_1 = H$).

Experiment 17: Coupling in Methanolic 1.5% Hydrogen Chloride Prepared from Wet Methanol. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (50 mg) in methylene chloride (5 ml) was added *t*-butyl hypochlorite (2.5 ml of a 0.05 M solution) over 30 min at 0°. Evaporation of the solvent *in vacuo* resulted in a light brown foam (52 mg). Reagent grade methanol (400 ml) was cooled in an ice bath and treated dropwise with dry acetyl chloride (9.25 ml). This solution (3 ml) was used to dissolve the above chloroindolenine and vindoline (100 mg) together. The resulting solution was stirred at RT. for 4 h and then briefly heated (45 min) at reflux. The usual workup procedure yielded crude material (171.9 mg) as a foam. This was chromatographed on *Woelm* III alumina (20 g) to yield dimeric material in roughly 30% yield which was identified as dimer IX ($R = CO_2CH_3$; $R_1 = H$).

Experiment 18: Standard Coupling with Acetic Acid added as a Co-catalyst. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (40 mg) in methylene chloride (4 ml) was added *t*-butyl hypochlorite (2 ml of a 0.05 M solution in carbon tetrachloride) at 0° over 30 min. Evaporation of the solvent *in vacuo* yielded a brownish foam (43 mg). To a solution of the above chloroindolenine and vindoline (40 mg) in methanolic 1.5% hydrogen chloride (5 ml) was added glacial acetic acid (2 ml). The whole solution was refluxed under nitrogen for 3 h and then worked-up in the usual way to yield some small quantity of dimeric material (less than 5%). The reaction was repeated two more times with the quantity of acetic acid being 3.0 and 4.0 ml respectively. It was found that the yield of dimeric material decreased sharply being just detectable in the former and completely absent in the latter case. The combined dimeric fractions from the first two attempts were analyzed by NMR. and found to be dimer IX ($R = CO_2CH_3$; $R_1 = H$).

Experiment 19: Coupling in Trifluoroacetic Acid as Solvent and Catalyst. To a solution of 18β-carbomethoxy-4β-dihydrocleavamine (50 mg) in benzene (10 ml) was added a solution of 1-chlorobenzotriazole (31.2 mg) in benzene (10 ml) at RT. over 30 min. After an additional 30 min the solvent was removed *in vacuo* and the resulting material partitioned between ice-cold brine and methylene chloride. The aqueous phase was washed with further portions of methylene chloride (2 × 10 ml) and the combined organic phase was dried over anhydrous sodium sulfate. Filtration and removal of the solvent *in vacuo* yielded a light brown foam. A solution of the above material and vindoline (10 mg) in trifluoroacetic acid (10 ml) was stirred at RT. for 50 h and then worked-up as follows. The contents of the reaction flask was poured gradually onto ice-cold ammonium hydroxide (dilute) and the resulting milky suspension was extracted with methylene chloride (3 × 50 ml). The combined organic phase was dried over sodium sulfate and filtered. Evaporation of the solvent *in vacuo* yielded crude material (110 mg) from which dimeric material could be isolated in minute quantities by column chromatography on *Woelm* III alumina. This dimeric material was analyzed by NMR. spectroscopy and found to be dimer IX ($R = CO_2CH_3$; $R_1 = H$).

Experiment 20: Coupling in Dioxane with Perchloric Acid as Catalyst. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was formed under standard conditions. This material was dissolved with vindoline (50 mg) in dioxane (10 ml) and a few drops of perchloric

acid were added. The resulting bluish solution was stirred under nitrogen at RT. for 48 h. At the end of this period, it was poured into an aqueous solution of 10% sodium hydrogencarbonate and extracted several times with ethyl acetate (4 × 30 ml). The combined organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent removed under reduced pressure to yield a brownish-red foam. Column chromatography yielded dimer IX (R = CO_2CH_3; R_1 = H) (5 mg).

Experiment 21: Coupling in Methanol containing HBr as Catalyst. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was formed in the standard way. To this material, vindoline (50.0 mg) was added and the whole was dissolved in methanol (10 ml) which had been freshly distilled from magnesium turnings into the reaction flask. Hydrogen bromide was bubbled into this reaction mixture, after passage through a drying tower, for a short period (15 seconds). After this the reaction vessel was connected to a positive pressure of nitrogen and stirred at RT. for 48 h. Work-up and chromatography in the usual way afforded dimer IX (R = CO_2CH_3; R_1 = H) as the only dimeric product (50 mg, 50% yield).

Experiment 22: An Investigation of the Effect of Hydrogen Chloride Concentration on the Coupling Reaction. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (200 mg) was made in the standard way and was then divided into 4 equal parts and treated as follows: A) 50 mg of vindoline with 5 ml of methanolic 1.5% HCl; B) 50 mg of vindoline with 5 ml of methanolic 1.6% HCl; C) 50 mg of vindoline with 5 ml of methanolic 2.0% HCl; D) 50 mg of vindoline with 5 ml of methanolic 5.0% HCl.

These were all simultaneously lowered into a preheated oil bath at 60°. Reaction rates as evidenced by colour changes and TLC. monitoring could be clearly compared and were found to roughly vary in linear fashion with acid concentration. The reactions were all worked-up in 2 h and after column chromatography the following results were obtained by NMR. analysis: A) 30 mg dimer IX (R = CO_2CH_3; R_1 = H) (30% yield); B) 36.4 mg dimer IX (R = CO_2CH_3; R_1 = H) (36% yield); C) 45.0 mg dimer IX (R = CO_2CH_3; R_1 = H) (45% yield); D) 56.4 mg dimer IX (R = CO_2CH_3; R_1 = H) (56% yield).

Experiment 23: An Investigation into the Effects of HCl Concentration at Higher Concentrations of Acid. The chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine (200 mg) was made in the standard way and was then divided into 4 equal parts and treated as follows: A) 50 mg of vindoline with 5 ml of methanolic 15% HCl; B) 50 mg of vindoline with 5 ml of methanolic 30% HCl; C) 50 mg of vindoline with 5 ml of methanolic 60% HCl; D) 50 mg of vindoline with 5 ml of acetyl chloride.

These were all simultaneously lowered into a preheated oil bath at 67°. After 3.0 h these were all worked-up. Column chromatography of each reaction mixture in the usual way afforded the following results by NMR. analysis: A) 40 mg of dimeric material (IX, R = CO_2CH_3; R_1 = H) containing a trace of some unidentified material, yield 40%; B) 26.8 mg of dimeric material containing mostly dimer IX (R = CO_2CH_3; R_1 = H), yield 27%; C) 25 mg of dimeric material containing mostly dimer IX (R = CO_2CH_3; R_1 = H), yield 25%; D) 97 mg of dimer IX (R = CO_2CH_3; R_1 = H), yield 97%.

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REFERENCES

[1] *J. P. Kutney, J. Beck, F. Bylsma & W. J. Cretney,* J. Amer. chem. Soc. *90,* 4504 (1968); *J. P. Kutney, J. Cook, K. Fuji, A. M. Treasurywala, J. Clardy, J. Fayos & H. Wright,* Heterocycles *3,* 205 (1975).

[2] *J. P. Kutney & F. Bylsma,* Helv. *58,* 602 (1975).

[3] *A. A. Gorman, M. Hesse & H. Schmid* in 'The Chemical Society, Specialist Periodical Reports, The Alkaloids', Vol. 1, Chap. 14, 1970.

[4] *J. P. Kutney, Ka Kong Chan, Amedeo Failli, John M. Fromson, Constantine Gletsos, Albert Leutwiler, Vern R. Nelson & Joao Pedro de Souza,* Helv. *58,* 1648 (1975).

[5] *R. B. Livingstone & S. K. Carter,* 'Single Agents in Cancer Chemotherapy', IFT/Plenum, New York 1970, pp. 279–317.

[6] *J. P. Kutney, W. J. Cretney, P. Le Quesne, B. McKague & E. Piers*, J. Amer. chem. Soc. *92*, 1712 (1970).
[7] *P. Bommer, W. McMurray & K. Biemann*, J. Amer. chem. Soc. *86*, 1439 (1964).
[8] *K. Biemann*, Lloydia *27*, 397 (1964).
[9] *N. Neuss, M. Gorman & N. J. Cone*, Lloydia *27*, 389 (1964).
[10] *J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall & V. R. Nelson*, J. Amer. chem. Soc. *92*, 1704 (1970).
[11] *J. P. Kutney, D. E. Gregoris, R. Imhof, I. Itoh, E. Jahngen, A. I. Scott & W. K. Chan*, to be published elsewhere.
[12] *G. Buchi, R. E. Manning & S. A. Monti*, J. Amer. chem. Soc. *86*, 4631 (1964).
[13] *J. Harley-Mason & A. Rahman*, Chem. Commun. *1967*, 1048.

187. Darstellung und Charakterisierung von (±)-Coralydin und (±)-O-Methylcorytenchirin und ihrer optischen Antipoden

(Vorläufige Mitteilung)

von **Hans Bruderer, Jean Metzger** und **Arnold Brossi**
Chemische Forschungsabteilung der F. Hoffmann-La Roche & Co. AG, Basel, Schweiz

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Summary. The synthesis and characterization of (±)-coralydine and (±)-O-methylcorytenchirine and their optical isomers are described.

Bei der säurekatalysierten Cyclisierung von (±)-Tetrahydropapaverin (I) mit Acetaldehyddiäthylacetal wurde eine als (±)-Coralydin bezeichnete Berbin-Base erhalten, die nach dem Umlösen aus Methanol Lösungsmittel enthält und je nach dessen Anteil zwischen 93–148° schmilzt [1] [2]. Das gleiche Verhalten zeigen auch Coralydine, die bei der chemischen Reduktion von Coralyn (IV) erhalten werden [3] [4][1]). Die Base, die als (±)-β-Coralydin bezeichnet wurde, verliert beim Trocknen Methanol und geht in die lösungsmittelfreie, als (±)-α-Coralydin bezeichnete Form vom Smp. 150–151° über. *Hahn et al.* haben gezeigt, dass es sich bei diesen Präparaten immer um das gleiche Diastereomere handelt [2]. Das zweite Diastereomere wurde bis anhin nicht beschrieben.

Im folgenden berichten wir summarisch über die Darstellung des zweiten Diastereomeren und über die gelungene optische Spaltung der beiden Racemate: Die Kondensation von rac. Tetrahydropapaverin (I) mit Acetaldehyd in 5N Salzsäure ergibt nach üblicher Aufarbeitung ein Basengemisch, das nach Filtration durch Alox, Ansäuern mit äthanolischer Salzsäure und Kochen in Äthanol, nach Stehenlassen ein Hydrochlorid vom Smp. 249–250° liefert. Daraus erhielten wir, nach Umlösen aus Methanol, eine Base vom Smp. 93–94°. Diese enthält, wie aus Mikroanalysenresultat und Kernresonanzspektrum ersichtlich ist, Methanol. Letzteres wird beim Trocknen

[1]) *Kametani et al.* haben in einer Fussnote berichtet [4], dass es sich bei (±)-α- und β-Coralydin wahrscheinlich um dimorphe Formen handelt. Die Untersuchungen von *Hahn et al.* [2] und unsere eigenen Befunde sprechen jedoch dafür, dass es sich bei den Coralydinen um Verbindungen handelt, deren Smp. vom Methanolgehalt abhängt.