STUDIES ON THE SYNTHESIS OF BISINDOLE ALKALOIDS. THE SYNTHESIS, STRUCTURE AND ABSOLUTE CONFIGURATION OF 18'-EPI-4'-DEOXO-4'-EPI VINBLASTINE, 18'-DECARBOMETHOXY-18'-EPI-4'-EPIVINBLASTINE AND 18'-EPI-3',4'-DEHYDROVINBLASTINE.

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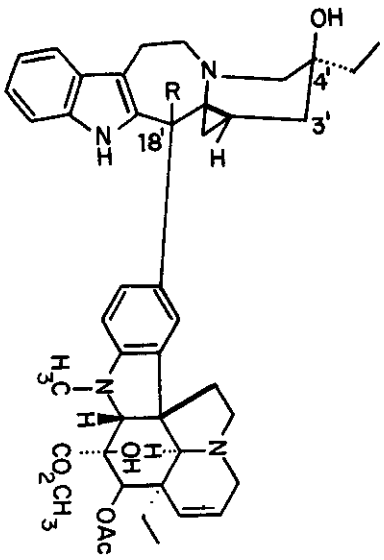
Studies on the syntheses of 18'-epi-4'-deoxo-4'-epivinblastine (III), 18'-epi-3',4'-dehydrovinblastine (III, 3',4'-double bond) and 18'-decarbomethoxy-18'-epi-4'-deoxo-4'-epivinblastine (V) are described. Particular emphasis is placed on the stereochemistry of the coupling reaction involving chloroindolenine derivatives of the cleavamine series with vindoline under acidic conditions. X-ray analyses on III and V provide the complete structures, including absolute configuration. These studies reveal that the chloroindolenine approach provides products bearing the unnatural configuration at $C_{18'}$.

The bisindole or "dimeric" alkaloids, vinblastine (I) and vincristine (I, N-formyl instead of N-methyl) are presently regarded as amongst the most active clinical agents for the treatment of various cancers in humans.[2] In spite of their clinical importance studies on structure-activity relationships are sadly lacking and it is not known whether the "dimeric" system portrayed by I and which of the functional groups or chiral centers in these complex natural products are required for anti-tumor activity. The extremely low concentration of the alkaloids in the plant, Catharanthus roseus G. Don, and the rather labile bond linking the indole and dihydroindole units has effectively prevented any detailed degradative studies to provide simplified synthetic analogues for biological screening.[3] It is clear that the development of a general and versatile laboratory synthesis of such systems would provide the ultimate solution. This communication describes some of our most recent studies to fulfill this objective.

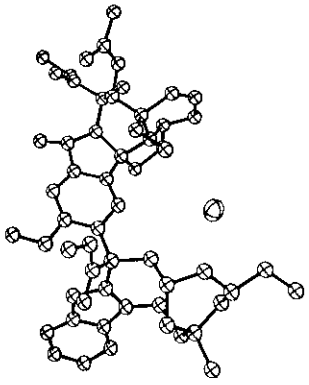


I, R = $COOCH_3$

In a previous publication[4] we had described a general approach to the synthesis of such dimeric systems. The formation of the bond linking the two units involved, in effect, the nucleophilic attack of the dihydroindole unit, vindoline, onto the double bond of the appropriate chloroindolenine derivatives derived from the 4β-dihydrocleavamine system. Although chemical and spectroscopic evidence was provided to establish the resultant dimeric products it was not possible to obtain any conclusive data for the stereochemistry at $C_{18'}$, the newly created chiral center linking the indole and dihydroindole units. The obvious importance of this center in terms of the synthetic problem and in determining biological activity in this series demanded further investigation and X-ray analysis was selected for this purpose.

Crystals of the methiodide of the synthetic dimer[4] derived from 18-carbomethoxy-4β-dihydrocleavamine (II) and vindoline (Figure 1) were grown from methanol. Since the crystals rapidly crumbled to a powder on removal from solvent, they were sealed in Lindemann capillaries with a generous portion of mother liquor. Preliminary X-ray photographs displayed mmm Laue symmetry with systematic absences of the type h00 (h = 2n + 1), 0k0 (k = 2n + 1) and 00ℓ (ℓ = 2n + 1). A pseudoextinction of the type 0kℓ (k + ℓ = 2n + 1) was also observed. Diffractometer measured cell constants were $\underset{\sim}{a}$ = 25.66(4), $\underset{\sim}{b}$ = 11.79(2) and $\underset{\sim}{c}$ = 17.26(3) Å. A calculated density of 1.15 g/cc was interpreted to mean that there were four molecules of dimer per unit cell of space group $P_{2_12_12_1}$. A total of 3693 unique reflections with $\theta \leq 55°$ was collected on a fully-automated four-circle diffractometer using Ni-filtered Cu K_α (1.5418 A) radiation. After correction for background, Lorentz and polarization effects 3428 reflections were judged observed ($I \geq 3\sigma(I)$).

The three-dimensional Patterson synthesis clearly revealed the iodide ion at (.10, .25, .10). The resulting three-dimensional F synthesis, which had the symmetry appropriate for P_{nam}, revealed a chemically reasonable fragment containing 17 atoms. The remaining non-hydrogen atoms were located in subsequent syntheses. Four molecules of methanol were revealed in the final difference syntheses. Full-matrix, least-squares refinement of all sixty-eight atoms has currently converged to 13.2%. The final X-ray model (less solvent) is shown in IIIa[5]. The solvent molecules are clustered about the iodide ion in the large hole fomed by the two halves of the dimer.



IIIa

It was thus clear, as shown in Figure 1, that the resultant dimer obtained in this reaction possesses the unnatural stereochemistry at $C_{18'}$ and is therefore 18'-epi-4'-deoxo-4'-epi vinblastine (III).

It is pertinent to note here that further studies to be reported later employing the above chloroindolenine approach have provided other synthetic dimers for purposes of biological evaluation. The above X-ray study

allowed unambiguous assignment of structure and absolute configuration to these products since direct correlation could be made with III or V (see later). It is of interest to mention briefly the synthesis of the dimer resulting from the reaction of the chloroindolenine derivative of 18-carbomethoxycleavamine (II, 3,4-double bond) with vindoline. The resultant product is the expected 18'-epi-3',4'-dehydrovinblastine (III, 3',4'-double bond). This compound could be correlated with III by catalytic reduction (Pt catalyst) methods, and thus its structural assignment is secure. It is mentioned here since Atta-ur-Rahman[6] has claimed the synthesis of "$\Delta^{15,20}$-anhydrovinblastine" by also coupling 18-carbomethoxycleavamine with vindoline in acidic methanol in a manner similar to that published from our laboratory several years earlier.[4] It is clear that his compound is also 18'-epi-3',4'-dehydrovinblastine and does not possess the natural vinblastine stereochemistry at $C_{18'}$ as claimed in that publication.

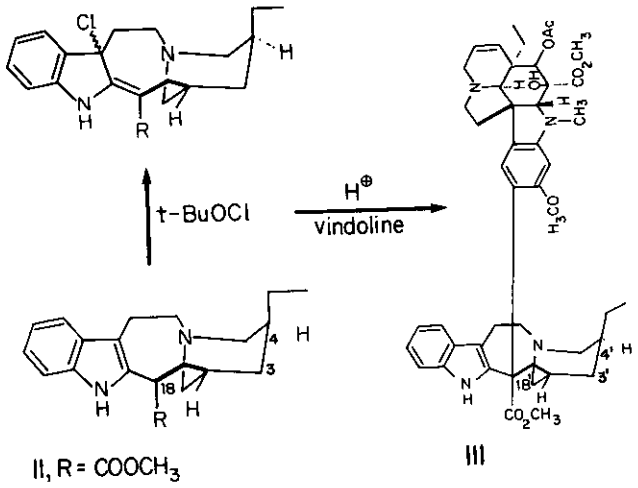
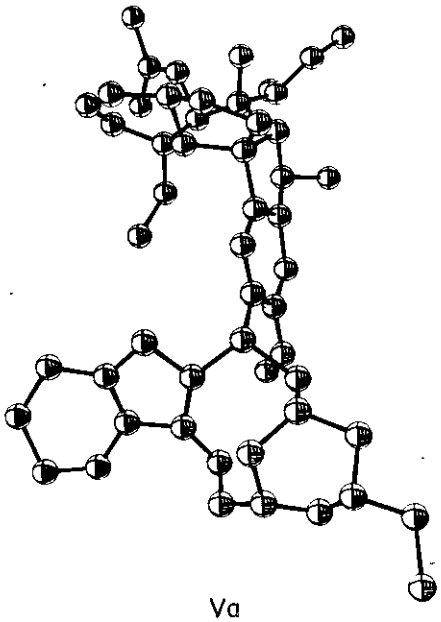


Figure 1. Synthesis of 18'-epi-4'-deoxo-4'-epivinblastine (III).

Finally it was of interest to consider the stereochemistry of dimers resulting from the 4β-dihydrocleavamine series (IV, Figure 2). In this instance the resultant product possessing an epimerizable hydrogen atom at $C_{18'}$ (see V) may convert to the natural stereochemistry at this center under the influence of the acidic medium. In addition some biological activity was recognized in this series and it became crucially important to establish the correct absolute configuration.

Crystals of the dihydrobromide of the synthetic dimer derived from 4β-dihydrocleavamine and vindoline (Figure 2) deposited from hot ethanol. Elemental analysis, which was subsequently confirmed by X-ray diffraction indicated two waters of crystallization per molecule of dimeric alkaloid. The crystals belonged to the tetragonal space group $P4_12_12$ with $\underset{\sim}{a} = \underset{\sim}{b} = 12.72(1)$ and $\underset{\sim}{c} = 57.62(5)$ Å *and one molecule of dimer dihydrobromide dihydrate per asymmetric unit.* All unique diffraction maxima with $\theta \leq 110°$ were collected but only 1170 were judged observed because of the small crystal size available. Solution of the structure proceeded as above and least squares refinement with isotropic temperature factors only converged to 0.112. A drawing of the final X-ray model is given in Va and the isolated product is therefore 18'-decarbomethoxy-18'-epi-4'-deoxo-4'-epivinblastine (V).

In conclusion the above studies reveal that although the chloroindolenine approach provides a general and mild synthetic method for the preparation of dimeric substances closely related to the clinical drugs, it yields the epimeric series at $C_{18'}$ and therefore alterations in the approach must be considered. Experiments in this direction are presently underway in our laboratory.



Va

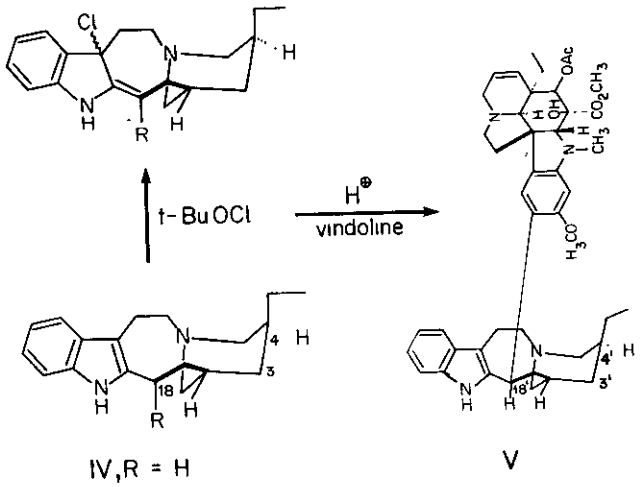


Figure 2. Synthesis of 18'-decarbomethoxy-18'-epi-4'-deoxo-4'-epi vinblastine (V).

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