*Tetrahedron* Vol 47, No 7, pp 1265-1274, 1991
Printed in Great Britain

0040-4020/91 $3 00+ 00


# SYNTHESIS OF VINCA ALKALOIDS AND RELATED COMPOUNDS. PART LVI.[1] 15',20'-ANHYDROVINBLASTINE BORANE COMPLEX. STRUCTURAL INVESTIGATIONS USING NMR METHODS.

Csaba Szántay Jr,[a,b*] Mihály Balázs,[c] Hedvig Bölcskei,[a] and Csaba Szántay[c]

[a] Chemical Works of Gedeon Richter, ([b] NMR Laboratory of the Research Dept of Physical Chemistry), H-1475, Budapest, POB 27, Hungary

[c] Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1025 Budapest, POB 17, Hungary

(*Received in UK 8 October* 1990)

*Abstract In the course of coupling catharanthine* **1** *to vindoline* **2**, *the* **3** *borane complex of anhydrovinblastine, a new diindole derivative was isolated The structure of this compound was studied by detailed NMR investigations Full* $^1H$ *and* $^{13}C$ *assignments for both the* **3** *borane complex and anhydrovinblastine are given*

Vincristine-vinblastine type diindole alkaloids play an important role in cancer chemotherapy The plant Catharanthus roseus contains these naturally occurring alkaloids, but only in minute concentrations Many efforts have thus been made to synthesize diindole compounds with the right stereochemistry by coupling the appropriate units[2] Notably, the modified Polonovski reaction resulted in diindole derivatives with the natural stereochemistry in 40-50% yield[3] Recently Vukovic et al published a new and simple coupling method in aqueous medium in the presence of ferric chloride with even higher yield (77%)[4]

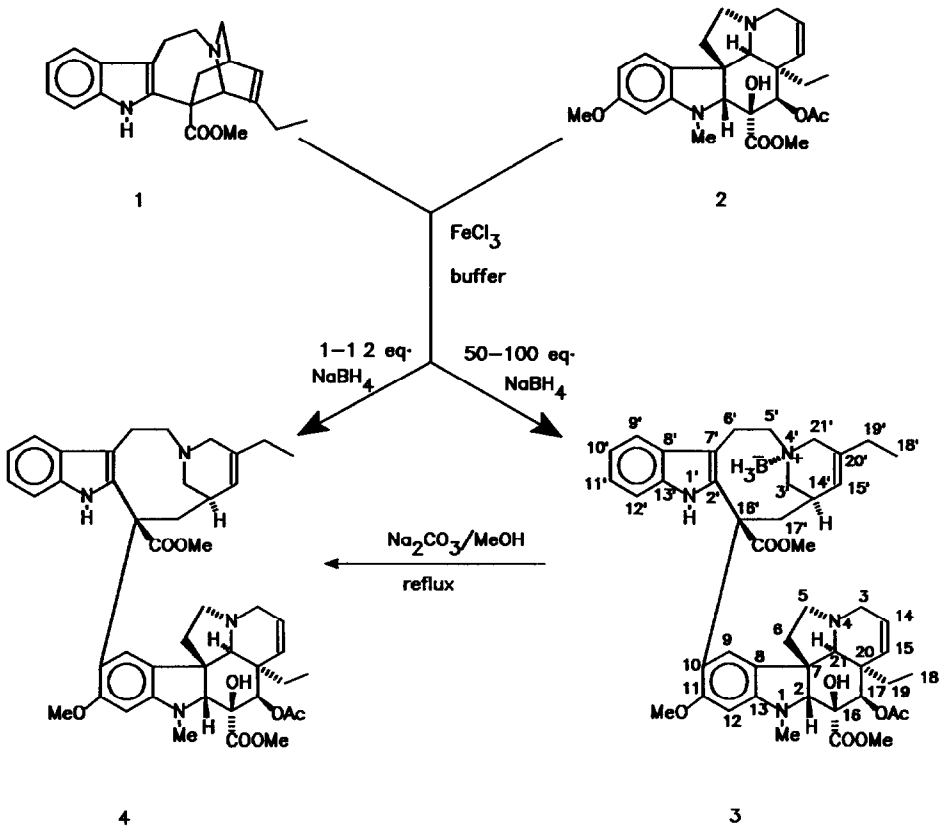
Here we report that under slightly modified conditions of the above reaction (room temperature, lower ferric ion concentration, 1-1 2 equiv of $NaBH_4$) anhydrovinblastine (AVLB) **4** is prepared with at least 80% yield when coupling catharanthine **1** to vindoline **2** (Scheme) More importantly, when using extreme excess of $NaBH_4$, the borane complex **3** of AVLB is obtained as the final product This compound has the advantage that it is highly stable as opposed to the AVLB base, which is known to be oxidized quickly and spontaneously by air oxygen into leurosine[5] However, **3** could be stored at room temperature for weeks without the formation of detectable amounts of leurosine or other sideproducts (see experimental section) The AVLB borane complex **3** may also be prepared under the usual conditions of the Polonovski reaction which, depending on the amount of excess $NaBH_4$ used, gave either the AVLB base **4**, or the borane complex **3** The latter can be easily transformed into **4** using the common procedure[6] Refluxing **3** in methanol in the presence of $Na_2CO_3$ gave the AVLB base **4** in quantitative yield without any measurable leurosine contamination All this points to an obvious synthetic utility of **3**, and possibly of similar analogs

Some previous examples of similar borane complexes of "monomeric" cleavamine derivatives were reported in ref 7 (see below) The formation of an analogous $BH_3$ complex of the diindol deoxyvinblastine, with a different synthetic aspect, was described in ref 8 This paper also gives a detailed $^1H$ NMR account of the latter $BH_3$ compound using decoupling methods and 2D J spectra, but leaving some ambiguities in the assignments (especially concerning assignments of $\alpha$ and $\beta$ geminal proton pairs), and does not give finer stereochemical details

Here we report full $^1H$ and $^{13}C$ assignments for both the AVLB $BH_3$ complex **3** and the AVLB base **4**, obtained by a concerted use of $^1H\{^{11}B\}$ selective decoupling, $^1H\{^1H\}$ NOE difference measurements, 2D $^1H$-$^1H$ (COSY) and $^{13}C$-$^1H$ (HETCOR) shift correlation methods

**Assignment of the $^1H$ and $^{13}C$ NMR spectra**

Considering the expected relative basicity of the three $sp^3$ nitrogens in **4** (relevant $pK_a$ values for some closely related "dimers" were reported in ref 9) it seems plausible to assume that the $BH_3$ complexation site involves N-4' in the cleavamine unit rather than N-4 or N-1 in the vindoline molety However, it is noted that a) steric influences which may

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differ considerably in different vinblastine-type derivatives may also play an important role in determining the complexa tion site, b) in the present case the high stability of the borane complex **3** is unexpected in view of the observed be havior of related "monomeric" borane derivatives, as reported by Kuehne and Zebovitz [7] (for details see below) Thus, s as not to make any *a priori* conclusions regarding the structure of compound **3**, and also to gain more insight into som of its stereostructural details, we have undertaken thorough NMR investigations on this compound Furthermore, seemed desirable to obtain complete $^{13}C$ and $^{1}H$ assignments on **3** in order to aid future structure determinatio problems of similar derivatives Naturally, the AVLB base **4** lends itself as an obvious analog for comparing it with **3** i terms of their NMR parameters However, we ran into the problem that although $^{13}C$ data for AVLB are known[10], onl incomplete $^{1}H$ NMR assignments have up to now been reported[3,10] which were inadequate for our purposes We hav therefore extended our investigations to the AVLB base **4**, as detailed below

The complete $^{1}H$ assignments, together with the detected scalar (COSY) and NOE connectivities for **3** and **4** ar collected in Tables 1 and 2 (In the absence of an adequate plane of reference, for some protons in the cleavamin half "$\alpha$" and "$\beta$" refer to the sites simply as depicted in Figure 2) $^{13}C$ chemical shifts are listed in Table 4, some $^{1}H$-$^{1}$ coupling constants are given in Table 3

Compound **3**

In order to maintain an approach as rigorous as possible during the structure determination, the $^{1}H$ assignments an most of the $^{13}C$ assignments were carried out within **3** itself (using $CDCl_3$ as solvent at first), without exploiting any pre viously published chemical shift data of relatively close analogs, such as those of vinblastine[11] or vindoline[12] (As will b seen below, this "precaution" was well justified)

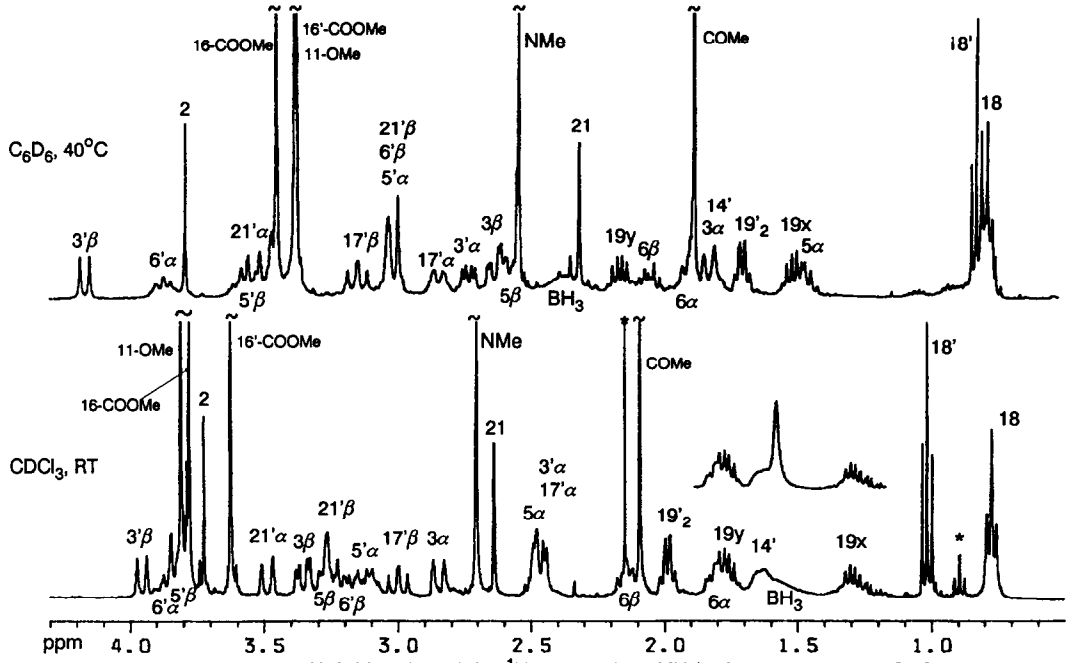


Figure 1 Upfield region of the $^1H$ spectra (400 MHz) of compound **3**, in $CDCl_3$ and $C_6D_6$ solutions The inset shows the effect of selective $^{11}B$ decoupling

**3** Gave a resonance at $\delta$ -8 8 in the $^{11}B$ NMR spectrum Selective decoupling of this peak resulted in the sharpening of the broad hump at $\delta$ 1 59 In the $^1H$ spectrum in $CDCl_3$ (see inset in Fig 1) This signal integrates to 3H, which readily verifies the presence of the $BH_3$ group on N-4', or possibly on N-4 or N-1

The $^1H$ assignments were arrived at by the concerted use of the observed J- and NOE connectivities Scalar coupling connectivities within the different subsystems could be easily established from the COSY spectrum (The HETCOR map provides a direct preliminary knowledge of the location of the geminal $^1H$-$^1H$ coupling partners, which gives a con-

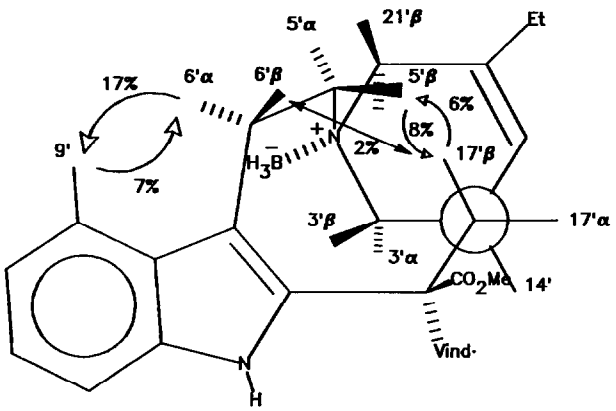


Figure 2 Proposed geometry (approximate) of the cleavamine half of the AVLB borane complex **3** Some of the observed NOE enhancements are also indicated For clarity, unlabeled bonds denote H

Table 1 $^1$H chemical shifts in $CDCl_3$ (25°C) and $C_6D_6$ (40°C) together with the through-bond and through-space connectivities established from the COSY and NOE difference spectra, respectively, in the vindoline half of **3** and **4**

| proton | **3** δ (CDCl3) | **3** δ (C6D6) | **4** δ (CDCl3) | **3, 4** scalar connectivities (in CDCl3) | **3,4** NOE connectivities (in CDCl3) |
|---|---|---|---|---|---|
| 2 | 3 72 | 3 79 | 3 72 | | NMe, 6β, 17, OH |
| 3β | 3 35 | 2 63 | 3 37 | 3α, 15, 14 | 3α, 14 |
| 3α | 2 85 | 1 82 | 2 83 | 3β, 15, 14 | 21, 3β, 14, 5α |
| 5β | 3 27 | 2 57 | 3 28 | 5α, 6β, 6α | 5α, 6β , OH |
| 5α | 2 50 | 1 44 | 2 45 | 5β, 6α, 6β | 5β, 3α , 6α, 21 |
| 6β | 2 13 | 2 04 | 2 15 | 6α, 5β, 5α | 6α, 5β, 2, 16-COOMe, **9** |
| 6α | 1 79 | 1 90 | 1 80 | 6β, 5β, 5α | 6β, 5α, 9 |
| 9 | 6 45 | 6 58 | 6 62 | | NH, 3'β, 21, 6α, 18, 14' |
| 11-OMe | 3 81 | 3 37 | 3 82 | 12 | 16'-COOMe, 12 |
| 12 | 6 10 | 5 86 | 6 13 | 11-OMe | 11-OMe, NMe |
| 14 | 5 86 | 5 45 | 5 86 | 15, 3β, 3α | 15, 3β, 3α |
| 15 | 5 29 | 5 23 | 5 31 | 3β, 3α, 14 | 14, 19y, 18 |
| 17 | 5 42 | 5 93 | 5 47 | | NMe, COMe, 2, 19y, 19x |
| 18 | 0 78 | 0 77 | 0 81 | 19x,y | 19x, 19y, 21, 15, 14, 9, 12', NH, **17** |
| 19x | 1 30 | 1 50 | 1 35 | 19y, 18 | |
| 19y | 1 76 | 2 15 | 1 79 | 19x, 18 | 17, 15 |
| 21 | 2 65 | 2 30 | 2 68 | | 19x, 18, 5α, 3α, 9, 15, OH |
| OH | 9 90 | 8 65 | 9 88 | | 17, 2, 15, 14, 16-COOMe, COMe, 21, 3β, 5β |
| COOMe | 3 78 | 3 45 | 3 80 | | NMe, COMe, 17, 12, OH |
| COMe | 2 09 | 1 87 | 2 12 | | 15, 17 |
| NMe | 2 70 | 2 53 | 2 71 | | 2, 16-COOMe, 12, 17 |

Small characters denote long-range (allylic or "W") J connectivities or weak (<1%) NOE enhancements Underlined characters indicate NOE interactions between the vindoline and cleavamine units The absence of NOE connections in an entry indicates that the irradiation of the proton in question was not carried out Bold characters indicate small negative enhancements

venient aid in the course of tracing the coupling networks in the COSY spectrum) On the other hand, the NOE measurements gave abundant information on through-space linkages among the various spin systems, allowing the completion of the assignments (Absolute magnitudes of the NOEs are not given in Tables 1 and 2 because of reasons similar to those mentioned in ref 11, the magnitudes of some especially informative enhancements were, however, depicted in Fig 2, it is also noted that the majority of the inter-spin-system NOEs was in the 3-10% range, and enhancements between geminal protons were typically 14-20%)

Several peaks are readily identified in the $^1$H spectrum due to their characteristic chemical shifts and couplings These, then, can be used as convenient starting points for the assignments One possible route leading to the assignments given in Table 1 is the following In the vindoline half locating the NMe, COMe, H-14 and H-15 signals is straightforward, both H-14 and H-15 are in turn coupled to the $H_2$-3 protons The distinction between H-3α and H-3β stems from the observed H-3α--H-21 NOE connection, which also gives the assignment of H-21 Irradiation of the NMe identifies the H-12, 16-COOMe, H-2 and H-17 signals A differentiation between the latter two singlets is provided by the NOEs from H-17 to H-19$_y$ and H-19$_x$, these, in turn, are coupled to $H_3$-18 in the COSY spectrum Irradiation of H-2, on the other hand, identifies the OH signal and, most importantly, H-6β Considering the relevant J-connectivities, H-6α and the two geminal H-5 signals are then also located The latter are ascribed to the α and β sites in view of the NOEs found on H-5α and H-5β upon irradiating H-6α and H-6β, respectively Moreover, the assignment of H-5α is also confirmed by its NOE connection to H-21 Irradation of the latter, on the other hand, locates H-9 The 11-OMe is NOE connected to H-12 which completes the $^1$H assignments in the vindoline half At this point it is noted that the $^1$H chemical shifts of the vindoline unit in **3** show only insignificant deviations from those reported for vindoline[12] or the vindoline unit in VLB[11], indicating that the $BH_3$ must indeed be bound to N-4' in the cleavamine moiety

Table 2 $^1$H chemical shifts in $CDCl_3$ (25°C) and $C_6D_6$ (25°C) together with the through-bond and through-space connectivities established from the COSY and NOE difference spectra, respectively, in the cleavamine half of 3 and 4

| proton | 3 δ ($CDCl_3$) | 4 δ ($C_6D_6$) | 3,4 δ ($CDCl_3$) | 3 scalar connectivities (in $CDCl_3$) | 4 NOE connectivities (in $CDCl_3$ and in $C_6D_6$ where indicated) | NOE connectivities |
|---|---|---|---|---|---|---|
| 3'β | 3 96 | 4 17 | 3 31 | 3'α, 21'β, 15' | 3'α, 9 | |
| 3'α | 2 46 | 2 72 | 2 58 | 3'β, 14' | 14'(17'α), 21'α, 3'β,15'(17'α), 9 | 14', 21'α, 3'β, 9 |
| 5'β | 3 83 | 3 55 | ~3 4 | 5'α[b] | 17'β, 6'β(6'α), 5'α(6'α) $C_6D_6$ 17'β, [6'β, 5'α], 6'α | |
| 5'α | 3 10 | 3 02 | ~3 4 | 5'β[b] | [6'α, 5'β], 9' | |
| 6'β | 3 17 | 3 02 | 3 41 | 6'α[b] | [6'α, 5'β], 17'β, 9' | |
| 6'α | 3 85 | 3 87 | 3 05 | 6'β[b] | 6'β(5'β), 5'α(5'β), 9' $C_6D_6$ 9', 5'β, [6'β, 5'α] | 6'β(17'β), [5'α,5'β(17'β)], 9' |
| 9' | 7 65 | 7 59 | 7 53 | 10', 11'[a] | 6'α, 10' | 6'α, 10' |
| 10' | 7 12 | 7 08 | ~7 14 | 9', 11', 12'[a] | | |
| 11' | 7 16 | 7 15 | ~7 14 | 10', 12', 9'[a] | | |
| 12' | 7 09 | 7 15 | ~7 14 | 11', 10'[a] | | |
| 14' | 1 63 | 1 79 | 1 30 | 15', 17'α, 3'α, 17'β | [3'α, 17'α], 3'β, 15', 9 | 3'α, 17'α, 3'β, 15', 9, 21 |
| 15' | 5 48 | 5 24 | 5 47 | 3β, 21'α, 21'β, 19', 14' | [3'α, 17'α], $19_2$', 18', 14' | 17'α, $19_2$', 18', 14' |
| 17'β | 3 00 | 3 14 | 3 04 | 17'α, 14' | 17'α, 6'β, 5'β,14' $C_6D_6$ 17'α, 14', 6'β, 5'β | 17'α, 6'β(6'α)[+5'β?(6'α)],14' |
| 17'α | 2 46 | 2 84 | 2 40 | 17'β, 14' | 14'(3'α), 17'β, 15' (3'α) $C_6D_6$ 14', 17'β, 15' | 14', 17'β, 15' |
| 18' | 1 01 | 0 81 | 0 99 | 19' | | $19_2$', 15' |
| 19' | 1 98 | 1 64 | 1 93 | 18', 15' | 18', 21'α, 21'β, 15' | |
| 21'β | 3 23 | 3 01 | 3 52 | 21'α, 3'β, 15' | 21'α | |
| 21'α | 3 48 | 3 49 | 3 28 | 21'β, 15' | 21'β, 3'α | 21'β, 3'α, $19_2$', 18' |
| NH | 8 06 | 8 46 | 8 05 | | 12', 9, 16'-COOMe, 19x, 18 | 12', 9, 16'-COOMe, 19x, 18 |
| COOMe | 3 62 | 3 37 | 3 62 | | NH, 11-OMe | NH, 11-OMe |
| $BH_3$ | 1 59 | 2 36 | | | 21'β, 21'α, 5'α, 6'α, 3'α, 3'β, 9' | |

Small characters denote long-range (allylic or "W") J connectivities or weak (<1%) NOE enhancements Underlined characters indicate NOE interactions between the vindoline and cleavamine units The absence of NOE connections in an entry indicates that the irradiation of the proton in question was not carried out Signals in parentheses refer to protons which were also saturated to some extent together with the entry proton due to overlap, and may also cause the observed NOE Squared brackets denote overlapping signals that may all contribute to the observed enhancements but can not be clearly designated to specific protons Bold characters refer to small negative enhancements [a] From decoupling (in 3) [b] Due to the crowded signals in the $H_2$-5',$H_2$-6' subsystem, it is difficult to attribute the interconnecting crosspeaks to specific vicinal couplings

Table 3 Selected J(H,H) coupling constants (Hz) in the cleavamine half of 3 and 4

| proton pair | 3 J | 4 J |
|---|---|---|
| 15',14' | 5 5 | 5 5 |
| 17'α,17'β | 15 6 | 15 5 |
| 17'β,14' | 13 5 | 12 6 |
| 3'α,3'β | 14 | 13 2 |
| 21'α,21'β | 16 2 | 16 7 |
| 3'α,14' | 5 5 | 3 1 |
| 3'β,14' | ~1 | ~1 |
| 17'α,14' | ~4 | 2 2 |

In the cleavamine unit the NH signal and H-15' are readily located Irradiation of the NH enhances H-12', simple decoupling experiments then give the assignments of the rest of the aromatic signals Using H-15' as a starting point, the entire H-17', H-14', H-15', $H_2$-19', $H_3$-18', H-21', $H_2$-3' system can be traced in the COSY spectrum The remaining $H_2$-5' and $H_2$-6' protons are easily located in the HETCOR map on the basis of the characteristically different C-5' and C-6' chemical shifts (Table 4) The geminal proton-pairs were ascribed to the appropriate α and β sites by the following considerations Irradiation of one of the H-17' signals (δ 3 00) gave an enhancement on one of the H-6' signals (δ 3 17),

Table 4 $^{13}C$ chemical shifts for compound **3** and for **4**[a] in $CDCl_3$

| Vindoline half | | | Cleavamine half | | |
|---|---|---|---|---|---|
| Carbon | **3** | **4** | Carbon | **3** | **4** |
| C-2 | 83 1 | 83 3 | C-2' | 130 8[c] | 130 9 |
| C-3 | 50 1 | 50 2 | C-3' | 52 0 | 45 8 |
| C-5 | 49 9 | 50 2 | C-5' | 57 5 | 54 5 |
| C-6 | 44 5 | 44 6 | C-6' | 21 9 | 25 7 |
| C-7 | 53 1 | 53 3 | C-7' | 113 0 | 117 3 |
| C-8 | 123 0 | 122 8 | C-8' | 129 6[c] | 129 4 |
| C-9 | 123 1 | 123 6 | C-9' | 118 7 | 118 3 |
| C-10 | 119 8 | 121 2 | C-10' | 122 7 | 122 2 |
| C-11 | 157 8 | 158 0 | C-11' | 119 4 | 118 8 |
| C-12 | 94 0 | 94 2 | C-12' | 110 3 | 110 5 |
| C-13 | 152 9 | 152 7 | C-13' | 134 7 | 135 0 |
| C-14 | 124 6 | 124 5 | C-14' | 31 2 | 32 9 |
| C-15 | 129 7 | 130 0 | C-15' | 122 0 | 123 8 |
| C-16 | 79 5 | 79 7 | C-16' | 54 7 | 55 4 |
| C-17 | 76 3 | 76 4 | C-17' | 33 9 | 34 3 |
| C-18 | 8 9 | 8 3 | C-18' | 11 7 | 12 3 |
| C-19 | 30 7 | 30 8 | C-19' | 27 9 | 27 8 |
| C-20 | 42 6 | 42 6 | C-20' | 135 3 | 139 9 |
| C-21 | 65 2 | 65 4 | C-21' | 61 4 | 52 1 |
| C=O | 170 8[b] | 170 9[b] | C=O | 173 9[b] | 174 7[b] |
| COOMe | 52 1 | 52 2 | OMe | 52 5 | 53 3 |
| 11-OMe | 55 7 | 55 8 | | | |
| NMe | 38 2 | 38 4 | | | |
| Ac C=O | 171 5[b] | 171 7[b] | | | |

[a] The $^{13}C$ assignments given here for **4** agree within 0 2 ppm with those reported in ref 10 (The only excepti is C-17' and C-3' which were wrongly assigned in reverse order in reference 10) [b,c] Like superscripts denote ten tive assignments

which should therefore be ascribed to H-6'$\beta$, and the irradiated proton to H-17'$\beta$, as illustrated in Fig 2 (for more o stereochemical details see below) The H-17'$\beta$--H-6'$\beta$ NOE connection accords with that seen in VLB [11] Irradiation 17'$\beta$ also enhances the complex multiplet at ca $\delta$ 3 84 which contains the overlapping H-6'$\alpha$ and one of the H-5' sig This NOE can be interpreted as reflecting a short H-17'$\beta$--H-5'$\beta$ interproton distance, and gives the assignment of H The assignment of H-17'$\beta$, H-6'$\beta$ and H-5'$\beta$ automatically provides the location of the corresponding geminal part with reference to the C,H correlation map The observed H-9'--H-6'$\alpha$ NOE connection also locates H-6'$\alpha$ - in accord with the aforementioned assignments (Assignments of the $H_2$-5' and $H_2$-6' protons were further substantiated by n urements undertaken in $C_6D_6$ solution - as discussed below)

The $H_2$-3' and H-21' protons were assigned by considering both the H-3'$\alpha$--H-21'$\alpha$ NOE connection and the pres of the H-3'$\beta$--H-21'$\beta$ long-range "W" coupling

Irradiation of the $BH_3$ signal enhances most protons surrounding the N-4' nitrogen, readily proving the site of plexation (Enhancements into the $BH_3$ protons were not observed due to their fast relaxation) We note that adeq saturation of the $BH_3$ protons, with the required selectivity, could only be achieved by simultaneously also decouplin $^{11}B$ signal

All NOE connectivities included in Table 1 and 2 but not specifically mentioned above are consistent with the as ments given, and with the geometry as represented in Fig 2 (see also below)

With the $^1H$ assignments accomplished, most of the $^{13}C$ signals are readily assigned (Table 3) from the HET spectrum Assignments of the quaternary carbons were based on values reported for 15',20',-Anhydrodeacetyl-VLB [1]

A comparison of the $^1H$ chemical shifts with data published on vinblastine[11] shows deviations of the kind

below) that prompted us to further secure our assignments of the H-6'$\beta$, H-5'$\beta$, H-6'$\alpha$ and H-5'$\alpha$ protons For this reason, and also to confirm the presence of the H-17'$\beta$--H-5'$\beta$ NOE connection (which was not observed in VLB and has further stereochemical implications, as will be discussed below), we sought to find a condition in which the H-6'$\alpha$, and H-5'$\beta$ signals (overlapping in $CDCl_3$) are separated, but H-17'$\beta$ remains in reasonable isolation for 1D NOE work After several trial-and-error experiments, such a condition was achieved in pure $C_6D_6$ at 40°C - see Figure 1 (In this case, although the H-6'$\alpha$ and H-5'$\beta$ signals are conveniently dispersed, the previously well separated H-6'$\beta$ and H-5'$\alpha$ signals become overlapped, in a way the situation is the inverse of that found in $CDCl_3$) The $^1H$ assignments in $C_6D_6$ (Table 1 and 2) were readily arrived at with the aid of a HETCOR spectrum (Most of the $^{13}C$ shifts in $C_6D_6$ deviate by less than 1 ppm from those measured in $CDCl_3$, thus allowing $^1H$ assignments in a straightforward way)

In $C_6D_6$ irradiation of H-17'$\beta$ gave an enhancement on the downfield H-5' signal ($\delta$ 3 55), while no enhancement was seen on the downfield H-6' signal ($\delta$ 3 87) (Table 2) These signals were thus ascribed to H-5'$\beta$ and H-6'$\alpha$, respectively, in agreement with our previous arguments Moreover, irradiation of H-6'$\alpha$ gave a large NOE on H-9' while it did not enhance H-17'$\beta$ All this makes the assignments of the $H_2$-5' and $H_2$-6' protons given in Table 2 unambiguous

AVLB

The $^1H$ (and $^{13}C$) assignments of AVLB were accomplished by an entirely similar procedure to that outlined above for **3**, details therefore will not be discussed

As for the $^1H,^1H$ couplings in **3** and **4**, most coupling constants were directly extractable only for the vindoline halfs, these conform with data published earlier[11,12] and were therefore not included in Table 3 Unfortunately, in the conformationally more intriguing cleavamine units only few couplings were available (Table 3), mainly because of the non-first-order character of the $H_2$-5', $H_2$-6' system (the problem being accentuated by overlaps)

**Comparison of chemical shifts**

Both the $^1H$ and $^{13}C$ shifts of the vindoline halfs of **3** and **4** show only insignificant deviations from those of VLB (as measured in $CDCl_3$[11]) or vindoline[12,13] On the other hand, as compared to **4** the $^{13}C$ data of **3** shows pronounced differences on the carbons surrounding the N-4' borane complexation site $\delta$ C-3' and $\delta$ C-21' are shifted downfield from their values in **4** by 6 2 and 9 3 ppm, respectively Interesingly, as compared to C-3' and C-21' the $\beta$(SCS) (substituent-induced chemical shift) value of the $BH_3$ is much less on C-5' (3 0 ppm), presumably this stems from the $BH_3$-to-$H_2$-5' geometrical arrangement being different from the $BH_3$-to-$H_2$-3' and $BH_3$-to-$H_2$-21' relationships (Fig 2) (Similarly large deviations in the $\beta$(SCS) values involving N-oxides were observed earlier [14]) In **3** $\delta$ C-6' is shifted 3 8 ppm upfield relative to its value in **4** due to the $\gamma$-gauche steric effect of the $BH_3$

As seen in Table 2, the protons in the vicinity of the $BH_3$ group (notably the $H_2$-3', $H_2$-5', $H_2$-6' and $H_2$-21' protons) in **3** show pronounced differences relative to **4**, also, these $\Delta\delta$ values differ widely for the different protons, resulting in large upfield as well as downfield shifts in an apparently unpredictable manner

A comparison of **3** with vinblastine requires some comment In both **3** and VLB the $H_2$-6' as well as the $H_2$-5' geminal proton-pairs show relatively large chemical shift differences [11,15] (For VLB $\delta$ H-5'$\beta$=3 05, $\delta$ H-5'$\alpha$=3 46, $\delta$ H-6'$\alpha$=3 15, $\delta$ H-6'$\beta$=3 95 in a $C_6D_6$-$CDCl_3$ 3 1 mixture as reported in ref 11) In our case however, although these shift differences are practically retained in **3** ($CDCl_3$), both the $H_2$-6' and $H_2$-5' geminal protons are assigned to the respective $\alpha$ and $\beta$ sites in reverse order relative to VLB (Table 2) This seems to be somewhat surprising, even when noting the constitutional and possible slight geometrical differences between **3** and VLB Differences between the chemical shifts of the above protons are less conspicuous when **3** is compared to its direct analog AVLB **4** Still, it is noted that the $H_2$-21' geminal protons "swap" their chemical shifts in **3** relative to **4** while the $H_2$-3' protons do not, despite the fact that both geminal pairs are geometrically similarly situated with respect to the $BH_3$ group (Fig 2) All this reflects the complex anistropic, VdW, etc influences of the $BH_3$ group exerted on these protons, which is compounded by the effects of possible small alterations in geometry with respect to **4**, and illustrates the difficulties met when trying to base $^1H$ assignments simply on comparative chemical shift arguments

**Stereochemical considerations**

The majority of our NOE results in **3** and **4** (including those revealing the relative positions of the two units in both compounds) match those reported for VLB by Hunter et al [11], indicating a fair degree of similarity between the three derivatives in terms of conformational details In the vindoline moieties, one notable feature is that the OH proton gave clearly observable NOEs into the H-3$\beta$ and H-5$\beta$ protons (~3% each) in both **3** and **4** These NOEs indicate directly that

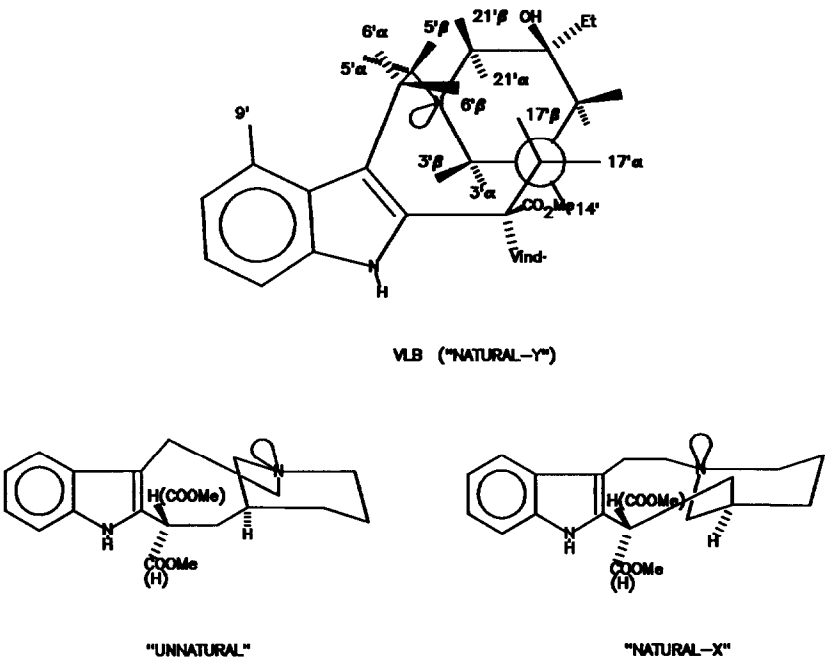


Figure 3 Proposed geometry of vinblastine (unlabeled bonds denote H), and the "natural" and "unnatural" conformations (idealized representations) of 20-Desethyldihydrocleavamines All structures have been redrawn by us with slight modifications from refs 11 and 7

the OH proton spends a considerable part of its lifetime being H-bonded to the N-4 nitrogen Although this feature of the OH, as being reflected in its large chemical shift, was pointed out by Hunter et al for VLB[11], in their case the above NOEs were not seen unambiguously

As for the cleavamine parts, the deduced stereochemistry of **3** is well secured by the available set of spatial connectivities (Fig 2 illustrates the approximate geometry of the cleavamine unit, together with some of the most informative NOE enhancements) In this regard an interesting aspect of the conformational possibilities is to be considered In the case of C16-C14 Pref 20-Desethyldihydrocleavamine and C16-C14 Parf 20-Desethyldihydrocleavamine Kuehne and Zebovitz described the so called "natural" and "unnatural" conformations of these compounds[7], as illustrated in Fig 3 These conformers are interconverted by the inversion of the piperidine ring and, for the above compounds, were separated by an exceptionally high energy barrier which permitted the isolation of the "unnatural" conformer at room temperature via a suitably designed synthetic route Upon heating, the "unnatural" conformation converted into the thermodynamically stable "natural" one Interestingly, for the above "monomers" it was the "unnatural" conformation which gave rise to a stable $N^b$ $BH_3$ complex, while the borane complex of the "natural" conformers proved to be highly unstable, quickly decomposing into the corresponding amine In our case, however, the cleavamine unit in **3** (as well as in **4**) is in the "natural" conformation which is, on the one hand, obvious from chemical reasons (treatment of **4** "directly" with $NaBH_4$ also gave **3**), and is also clearly reflected by the established NOE connectivities (see also below) Yet the borane complex **3** possesses high stability which is somewhat surprising in view of the behavior of the above mentioned "monomeric" derivatives (indeed, this presents an apparent contradiction which was one of the reasons that necessitated an elaborate structural investigation of **3**) All this shows that such diindoles may exhibit dramatically different chemical behavior from the corresponding "monomers"

It is further noted that in **3** (Fig 2)(as well as in VLB[11]- Fig 3) the N-4' nitrogen is inverted with respect to the way the stereostructure of the "natural" conformation of the above "monomeric" cleavamines was described in ref 7 (Fig 3) [For the latter, the $N^b$ nitrogen lone pair was inferred to be $\beta$ and axial (with respect to the piperidine ring) with reference

to the pronounced Wenkert-Bohlmann bands observed in the IR spectra of these compounds] We can refer to these stereostructures as "natural-y" and "natural-x", respectively (Fig 3) In the "natural-x" geometry, as compared to that shown in Fig 2 ("natural-y") the H-5'$\beta$--H-17'$\beta$ distance becomes too long for NOE to be observed between them, while the H-5'$\alpha$--H-3'$\alpha,\beta$ distances decrease so that they are likely to allow the detection of the relevant NOEs In **3** the NOE found on H-9' upon irradiating the $BH_3$ signal, *together* with the observed H-5'$\beta$--H-17'$\beta$ NOE connection, are perhaps the most conspicuously irreconcilable with either a "natural-x" conformer (and thus having a $\beta$ $BH_3$ group), or with an envisaged "unnatural" conformer (which, in this case, could result from a half-chair to half-chair pseudorotation of the anhydro-piperidine ring) In fact, by attributing either of the latter two stereostructures to compound **3** (as opposed to the geometry indicated in Fig 2), the internal consistency of our assignments and that of the observed set of NOE connections would break down As for the base analog **4**, the presence of the H-5'$\beta$--H-17'$\beta$ NOE connection could not be observed unambiguously due to the highly crammed character of the $H_2$-5', $H_2$-6' subsystem (As opposed to **3**, we could not improve the situation in this regard, despite using different solvents) Therefore, conclusions concerning the conformation of the nine-membered ring and the $N^b$ nitrogen are in this case less clear-cut Nevertheless, three pieces of evidence seem to support the stereostructure of **4** being analogous to that of **3** ("natural-y") a) the geminal J(H-21'$\alpha$,H-21'$\beta$) and J(H-3'$\alpha$,H-3'$\beta$) couplings are both relatively large, indicating the equatorial (i e in this case $\alpha$) position of the N-4' lone pair[16], b) In accordance with this geometry, no Wenkert-Bohlmann bands are observed in the IR spectrum of **4** (as opposed to the relevant "monomeric" derivatives with the "natural-x" conformation), c) in **4** H-9' gives a 5% NOE into H-6'$\alpha$ and no NOE into H-6'$\beta$, which complies well with the pertinent enhancements in **3**, and therefore accords with a similar conformation of the cleavamine part, also, no H-5'$\alpha$--H-3'$\alpha$ NOE connection is observed which could be associated with the "natural-x" geometry

For VLB, the preferential conformation of the nine-membered ring (Fig 3) was established from the analysis of the NOE experiments and $^1H$-$^1H$ coupling constants[11] In our case, the H-6'$\beta$--H-17'$\beta$ and H-5'$\beta$--H-17'$\beta$ NOE connections in **3** (the latter through-space connectivity was not present in VLB) indicate that there is a slight departure in the predominant conformation of the nine-membered ring relative to that in VLB (cf Fig 2 and Fig 3) [For precesion's sake, an alternative explanation could be that the conformational equilibrium of the mobile nine-membered ring is altered so that, in addition to the conformer involving a short H-6'$\beta$--H-17'$\beta$ distance, one associated with a short H-5'$\beta$--H-17'$\beta$ (but relatively long H-6'$\beta$--H-17'$\beta$) distance also becomes appreciably populated] In any case, the proposed geometry is further supported by the small negative enhancement (three-spin effect) on H-9' from both H-5'$\alpha$ and H-6'$\beta$ This shows the presence of the H-5'$\alpha$--H-6'$\alpha$--9' and H-6'$\beta$--H-6'$\alpha$--9' cross-relaxation pathways, neither of which must deviate significantly from linearity Such a conformer involving a short H-5'$\beta$--H-17'$\beta$ distance is clearly destabilized in VLB by the steric interaction between H-5'$\beta$ and the $\beta$ axial 20'-OH group, while for **3** a preferential conformation similar to that of VLB is unfavored because of the $NBH_3$<--> H-5'$\alpha$ repulsion Unfortunately, for both **3** and **4** the non-first-order character of the $H_2$-5', $H_2$-6' system prevented us from obtaining a reliable set of coupling constants, and thus from gaining more insight into the ring-geometry through the calculation of dihedral angles

As mentioned above, in the "natural" conformation of "monomeric" cleavamines, the $N^b$ lone pair was depicted as being $\beta$ and axial (Fig 3)[7] Also, the authors explain the differences in chemical behavior (such as the relative stability of the respective $BH_3$ complexes) between the two conformers in terms of the $N^b$ lone pair being equatorial in the "unnatural", while axial in the "natural" isomer It is interesting to note that in the light of the present study these observations indicate that the cleavamine unit may behave very differently in VLB-type diindoles and "monomeric" cleavamines, both conformationally and chemically

## Experimental

Synthesis

### $\Delta^{15'}$20'-Dehydroxyvinblastine (anhydrovinblastine) (4)

a) Catharanthine **1** hydrogensulfate (258 mg, 0 6 mmol) and ferric chloride hexahydrate (810 mg, 3 mmol) were combined in a mixture of glycine buffer (15 $cm^3$, containing 7 505 g glycine and 5 85 g sodium chloride in 1000 $cm^3$ water) and hydrochloride acid (15 $cm^3$, 0 1 n) under argon atmosphere After 10 min stirring at room temperature vindoline base **2** (273 mg, 0 6 mmol) was added After stirring for 2 hours at room temperature the suspension had changed into a clear, orange-coloured solution Sodium borohydride (25 mg, 0 65 mmol) in cc ammonium hydroxide (3 $cm^3$) was then added dropwise The reaction mixture was extracted with dichloromethane (2 x 30 $cm^3$), dried (sodium sulfate) and the solvent evaporated The crude oil containing anhydrovinblastine **4** (450 mg, 95 0 %) was crystallized from acetone (310 mg, colourless crystals, 66 0 %)

b) (From **3**) The borane complex **3** (100 mg, 0 124 mmol) was heated in the presence of sodium carbonate (30 mg,

0 3 mmol) in methanol (6 $cm^3$) at 65°C for 3 hours The reaction was controlled by TLC, after **3** had disappeared, **4** (98 mg) was prepared in 100 % yield

**$\Delta^{15'}$20'-Dehydroxyvinblastine (anhydrovinblastine) borane complex 3**

a) Excess of sodium borohydride (1 g, 26 mmol) was used Apart from this the reaction was carried out under the same conditions as used for the preparation of **4** when starting from **1** The crude product (410 mg) was purified on silica column (eluent = toluene acetone methanol cc ammonium hydroxide = 138 49 10 2 5) Fractions containing **3** were evaporated (290 mg, 76 3 %) and crystallized from acetone (210 mg, 43 7 %)

b) (Via Polonovski reaction) Catharanthine **1** hydrogensulfate (140 mg, 0 322 mmol) was dissolved in dichloromethane (2 5 $cm^3$) in the presence of diethylamine (0 05 $cm^3$) After 10 min stirring at room temperature, 3-chloro-peroxybenzoic acid (80 mg, 0 463 mmol) in 2-butanone and vindoline **2** base (150 mg, 0 328 mmol) in dichloromethane were added dropwise at -30°C The reaction mixture was cooled to -65°C and trifluoro-acetic anhydride (0 3 $cm^3$, 2 1 mmol) was added After stirring for 2 hours at -65°C the mixture was evaporated and the residue was dissolved in dichloromethane (5 $cm^3$) The solution was combined with a suspension of sodium borohydride (500 mg, 13 mmol) and ethanol (1 $cm^3$) The pH was adjusted with diethylamine (pH = 9-10) and the reaction mixture was washed with water (2 x 5 $cm^3$) The organic phase was dried over sodium sulfate and evaporated The residue (300 mg) was purified in the same way as above After crystallizing from acetone **3** was obtained (100 mg, 0 124 mmol) in 38 5 % yield

IR (KBr) 1740 $cm^{-1}$ (C=O), 2250-2240 $cm^{-1}$ ($BH_3$)
MS FAB m/e 807 ($M^+$), 793, 733, 717, 541, 351

**Stability investigation on AVLB 4 and its borane complex 3**

Both compounds were stored at room temperature in closed phials for a week The samples were examined every 24 hours 1 0 Mg of both **4** and **3** was dissolved in 0 5 $cm^3$ dichloromethane and small amounts (1, 5 and 10 ul) were investigated by TLC After 24 hours the base **4** had already partially decomposed, and leurosine appeared in ca 30 % After 48 hours the white crystals of the base **4** had changed into yellow-brown and the amount of leurosine had increased to 50 % The AVLB borane complex **3** remained unchanged during the whole week

Spectroscopy

NMR spectra of the borane complex **3** were recorded on a Bruker AM-400 spectrometer operating at 400, 100 and 128 MHz for $^1H$, $^{13}C$ and $^{11}B$ nuclei, respectively, with internal deuterium lock at ambient temperature (25°C) in $CDCl_3$, and at 40°C in $C_6D_6$ NMR measurements on the AVLB base were carried out on a Varian VXR-300 instrument (300 MHz for $^1H$ and 75 MHz for $^{13}C$) at 28°C in $CDCl_3$ Chemical shifts are given relative to $\delta$TMS=0 00 ppm The COSY (COSY-90, magnitude mode), HETCOR and NOE experiments were recorded by using the standard spectrometer software packages The HETCOR experiments were run with $^1H$ decoupling in the $F_1$ dimension [17] NOEs were measured in non-degassed samples, typically 2 5 s pre-irradiation times were used (NOE intensities did not appear to vary significantly with longer saturation periods, in accordance with the short cross-relaxation times associated with a molecule of this size) FIDs were exponentially multiplied prior to Fourier transformation (LB = 1 Hz) Subsaturating irradiation power levels were varied [53-62L (Bruker), and DLP=25-30 (Varian)] according to target-signal widths to achieve good selectivity For the same reason, irradiation of the $BH_3$ signal was carried out with simultaneous $^{11}B$ decoupling

**Acknowledgements**

We are grateful to J Tamás for mass spectral measurements and to S Holly for infra red investigations

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