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STRUCTURAL STUDIES OF VINBLASTINE ALKALOIDS BY EXCITON COUPLED CIRCULAR DICHROISM

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IN HONOUR OF PROFESSOR ANTONIO G. GONZALEZ

Key Word Index—*Catharanthus roseus*; Apocynaceae; circular dichroism; exciton coupling; absolute configuration; vinblastine alkaloids.

Abstract—SCF-CI-dipole velocity MO calculations have shown that the bisignate circular dichroic curves of vinblastine/vincristine alkaloids at *ca* 210 and 220–230 nm are due to exciton coupling between the indoline and indole moieties. Furthermore, a combination of X-ray crystal structure data with MM2 local energy minimization provides a convenient means for estimation of the preferred solution conformation.

INTRODUCTION

Initial reports of hypoglycaemic activity from a tea derived from the pantropical plant *Catharanthus roseus* (L.) G. Don prompted, in depth, phytochemical screening by two independent groups: Svoboda and Johnson at the Lilly Research Laboratories, and Noble and co-workers at the University of Toronto. During the course of this screening. Over 75 alkaloids were isolated, some of which demonstrated diuretic, hypoglycaemic and antiviral activity. However, bone marrow depression (leukopaenia) was observed as well as significant activity against murine leukaemia [1]. The active principles, vinblastine and vincristine were independently reported in 1958 by Svoboda and Noble and determined to possess a unique bisindole alkaloid structure [2–5]. Because of the significant clinical antitumour activity against Hodgkin's and non-Hodgkins's lymphomas, acute lymphoblastic leukaemia, breast carcinoma Wilms' tumour, Ewing's sarcoma, neuroblastoma, heptoblastoma and small cell lung cancer, vinblastine and vincristine have achieved a prominent role in modern cancer chemotherapy.

While numerous biochemical effects have been observed for the vinca alkaloids, the primary mode of action consists of an interaction with α- and β-tubulin heterodimeric subunits of microtubules, which could be considered one of the most strategic subcellular targets for chemotherapeutic action [6]. The high affinity binding site of vinblastine/vincristine on the subunits tubulin is a discrete one, and is different than that of taxol, colchicine and podophyllotoxin [7]. This binding prevents the normal process of polymerization of the subunits into microtubules and the resulting self-association process leads to tubulin–vinblastine aggregates. There has also been a secondary low-affinity binding site found on the walls of microtubules, which is responsible for the separation of protofilaments and the formation of protofilaments coiled into spirals. These effects produce mitotic arrest, which ultimately results in cell death, either as a direct consequence or due to other effects in the cell cycle (cytotoxic effects have been observed in the G1 and S phases of non-proliferating cells).

During the course of development of these alkaloids as chemotherapeutic agents, the unique observation was made that there is an exquisite sensitivity [8,9] in the structure–activity relationships concerning the stereochemistry at C-16′, C-14′ and C-20′. In vinblastine and vincristine, the configuration at the C-16′ stereogenic centre is *S* while at C-14′ it is *R* and at C-20′ is *S*. The inversion of C-16′ configuration from *S* to *R* results in a complete loss of activity as does C-14′ conversion from *R* to *S* [10]. The position at C-20′ has also demonstrated to be especially critical concerning the interaction with tubulin [9]. Another observation concerning the unique configurational–conformational relationships of these alkaloids reported that vinblastine epimers at $C_{20'}$ that lack antimicrotubule activity did not exhibit mitotic arrest, but modulated intrinsic and acquired drug resistance [11].

Based on these observations, a detailed study of the stereochemical aspects of vinblastine was initiated using circular dichroic (CD) spectroscopy in conjunction with molecular modelling.

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RESULTS AND DISCUSSION

The absolute stereochemistry of vincristine (**2**) and its close analogue, vinblastine (**1**), was determined by X-ray analysis 30 years ago by Monchief and Lipscomb [12]. Numerous new analogues have been described since then, assigned either to the natural or unnatural vinblastine series with respect to the crucial C-16′ centre. However, an unambiguous stereochemical determination of the stereogenic centres of these binary indole–indoline alkaloids still remains a difficult task which depends heavily on enantioselective and diastereoselective synthetic considerations.

Kutney *et al.* first showed that CD spectroscopy could be applied successfully to determine the configurations at C-16′ for the structural assignments of vinblastine alkaloids [13]. It was also shown that the C-16′ stereochemistry could be deduced from ^{13}C NMR chemical shifts [14]. In the case of CD spectroscopy, when the Cotton effects (CEs) at *ca* 210 and 220–230 nm are negative and positive, respectively, the configuration at this centre is *S*, the physiologically active form, and vice versa. We have interpreted this empirical, but useful, trend concerning the C-16′ configuration on a non-empirical theoretical basis, the results of which are presented in the following. Further results and discussions stemming from the UV and CD data for a variety of vinblastine analogues with different configurations at C-16′, C-14′ and C-20′ [15, 16, Dong, J. *et al.*, unpublished data] will be published separately [Dong, J. *et al.*, unpublished data].

The UV spectrum of **1** is characterized by an intense 214 nm band (ε 46 200) with a shoulder at 228 nm (Fig. 1); as assigned earlier [13], they arise from ^1B_a transitions of the indoline (217 nm) and indole (225 nm) chromophores, respectively. Similarly, the 260 nm band (ε 15 000) is due to the ^1L_a transition of the indoline moiety, while the overlapping bands at *ca* 293 nm (ε 10 500) are from ^1L_a and ^1L_b transition of the indole chromophore and the ^1L_b transition of the indoline chromophore. It can be seen that the two ^1B_a transition bands give rise to two intense CD signals in **1**, i.e. a negative CE at 214 nm and a positive CE at 228 nm. Thus, the two intense absorption bands below 230 nm of the indoline and indole chromophores, as suggested earlier [13], are most likely coupled to yield characteristic split CD curves, the signs of which can be correlated with the absolute stereochemistry. As shown in Fig. 2, an unnatural analogue **3** with the *R* configuration at C-16′ exhibits a CD with CEs of opposite signs at these two wavelengths. However, the correlation between the signs of CEs and mutual orientation of respective electric transition dipole moments remained unclear. The theoretical basis of this effect has been clarified and is described below.

In exciton coupled circular dichroism (ECCD) [17–21] the interaction between chirally disposed strong electric transition dipoles leads to 'split' Cotton effects, the signs of which are directly related to the chiral twist between the corresponding chromophores. The π-electron SCF-CI-dipole velocity MO method, the details of which were introduced and developed by Harada and co-workers

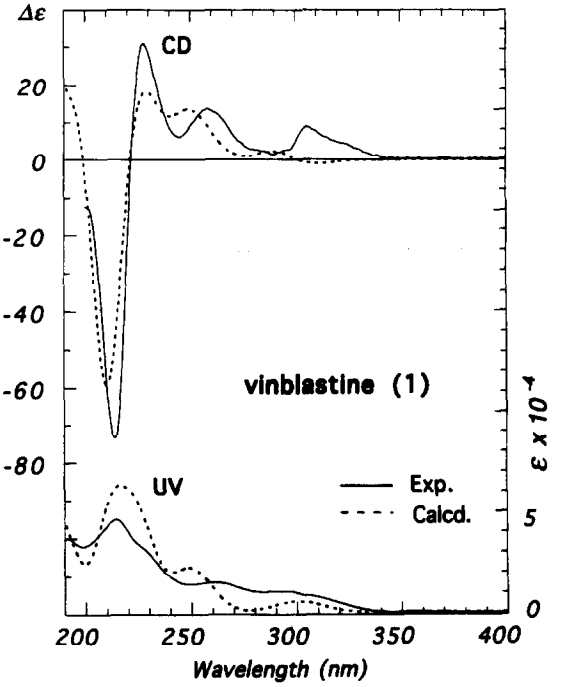


Fig. 1. Solid line; experimental CD and UV spectra of vinblastine free base (**1**) in acetonitrile; dashed line: calculated UV and CD spectra of minimum energy conformation **A** of **1**.

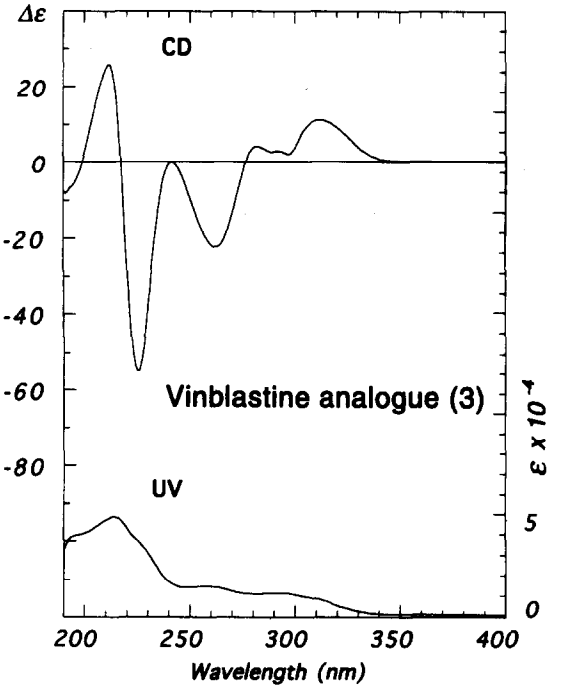
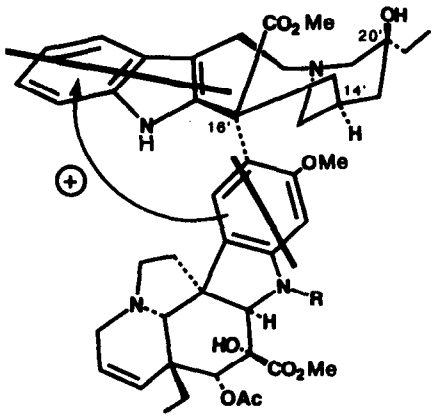


Fig. 2. Experimental CD and UV spectra of unnatural analogue free base **3** in acetonitrile.

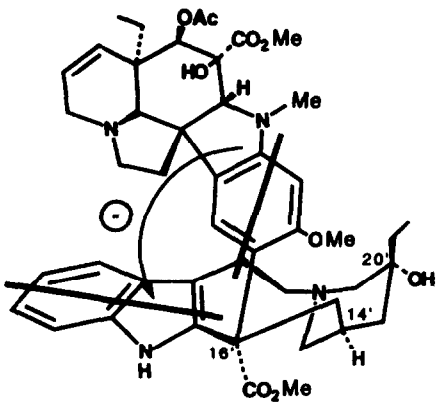
[20, 22] has become an important tool for assigning the absolute configuration of a variety of twisted and conjugated π-electron systems based on the calculated CD curves. Since these calculations in general were



R

Vinblastine (1) Me

Vincristine (2) CHO



Unnatural analogue (3)

performed on rigid or on estimated most stable conformations, we established the vinblastine solution conformation prior to CD calculations.

In 1966 the X-ray analysis of vincristine methiodide dihydrate, $(C_{47}H_{59}O_{10}N_4^+)I^-.2H_2O$ [12] revealed the solid state conformations of both **2** and **1**. However, since to the best of our knowledge, no rigorous solution conformational studies on **1** had been carried out, we used the X-ray data as the input for MacroModel local conformational search [23]. All atoms in vincristine methiodide dihydrate unrelated to **1** were excluded prior to performing local energy minimization in chloroform solution with MM2 force field [24]. MM2 calculations indicated that the vinblastine free base conformation **A** has a minimum energy of 502 kJ mol^{-1}, which is almost six times lower than that of the input conformation (2759 kJ mol^{-1}), although the final structure of **A** (Fig. 3) is seemingly close to the starting geometry. It follows that the molecular framework of the vinblastine type of bisindole alkaloids is relatively rigid.

Using the atomic coordinates obtained by MM2 calculations, we applied the π-electron SCF-CI-DV MO method to calculate the UV and CD spectra of the vinblastine **A** conformation (Fig. 1, dashed line). We assumed that the π-electron systems of the indole and indoline chromophores make the most significant contributions to the electric transition dipole moments, giving rise to the exciton coupled CEs. The nitrogen atoms in the indole and indoline chromophores were treated as singly and doubly charged species, respectively. The parameters for the calculation were adjusted to fit the UV spectra of the two separate moieties of **1**, namely, vindoline and cleavamine. The dipole strengths and orientation of the electric transition moments were obtained from the UV calculation of the two separated chromophores; the projection angle between two ^1B_a transitions was estimated to be +85.7°.

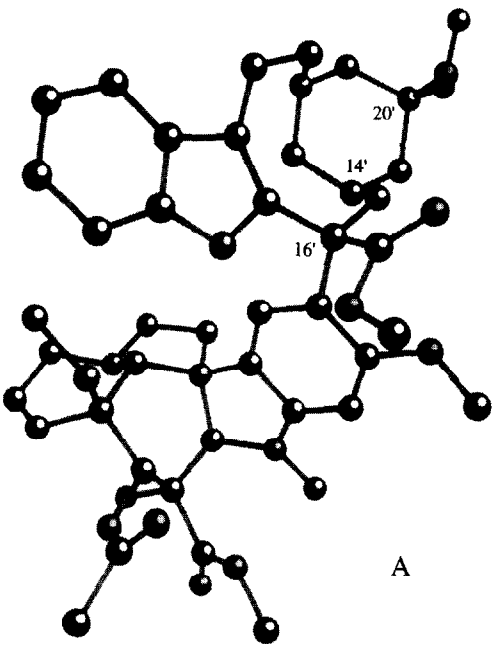


Fig. 3. Stereoscopic view of vinblastine conformation **A** calculated by MacroModel V4.5.

As shown in Fig. 1, the theoretical CD calculations yielded three principal CEs: positive bands at 250 nm ($\Delta\varepsilon$ + 13.4), an intense, positively split exciton couplet with a first CE at 228 nm ($\Delta\varepsilon$ + 18.5) due to the indole moiety, and a second strong negative CE at 211 nm ($\Delta\varepsilon$ − 60) arising from the indoline moiety. Although CEs around 230–210 nm derived from theoretical calculations are somewhat weaker than the experimental CEs, the calculated curves are in good agreement with the observed CD data, including the sign, position and shape of the corresponding CEs. The calculated CD of analogue

3 (not shown), also closely simulating the experimental curve, will be described in a full paper.

The above calculations correlate the UV and CD spectra of vinblastine free base (1) with its C-16′ absolute configuration, or more precisely, with the chiral sense of twist between the indole/indoline chromophores in its lower energy conformation. They not only establish that the observed intense split CD bands at *ca* 230–210 nm are indeed due to exciton coupling between indole and indoline chromophores, but also show that the X-ray crystal structure combined with MM2 local energy minimization provides a convenient means for estimation of the preferred conformation in solution.

EXPERIMENTAL

Vinblastine free base was obtained from an aqueous solution of vinblastine sulphate after extraction with HPLC grade CH_2Cl_2 and 10% $NH_3.H_2O$. After being dried over $MgSO_4$, the CH_2Cl_2 extract was evapd *in vacuo*. Vinblastine free base was kept under $-70°$.

UV/VIS and CD spectra were recorded as MeCN (spectrophotometric grade) solns on a Perkin-Elmer Lambda 4B UV/VIS spectrophotometer and JASCO J-720 spectropolarimeter, respectively. In all cases, the estimation of soln concn was based on an exact sample's weight. Molecular modelling calculations were performed with MacroModel V4.5 on a Silicon Graphics 3D workstation in the Chemistry Department of Columbia University. The π-electron SCF-CI-DV- MO numerical calculations were carried out on an IBM PC 486/DX2 computer.

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