Structure-Activity Relationships of Dimeric *Catharanthus* Alkaloids. 1. Deacetylvinblastine Amide (Vindesine) Sulfate[1,2]

Charles J. Barnett, George J. Cullinan, Koert Gerzon,* Richard C. Hoying, William E. Jones, William M. Newlon, Gerald A. Poore, Robert L. Robison, Martin J. Sweeney, Glen C. Todd,

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206

Richard W. Dyke, and Robert L. Nelson

The Lilly Laboratories for Clinical Research, Wishard Memorial Hospital, Indianapolis, Indiana 46206.
Received May 17, 1977

Exploration of the effects of "minor" structural differences on the antitumor activity and toxicity of dimeric *Catharanthus* alkaloids resulted in the preparation of deacetylvinblastine amide (vindesine, VDS) from either vinblastine (VLB) or deacetylvinblastine. Adequate amounts of vindesine for biological testing were prepared by preferential hydrazinolysis of the C_{23}-ester in the vindoline moiety of VLB, followed by hydrogenolysis of the resulting deacetylvinblastine hydrazide. Vindesine in its activity spectrum against rodent tumor systems resembles vincristine (VCR) rather than its parent VLB, while its neurotoxic potential appears to be less than that of VCR. The experimental models developed to estimate this potential include in vitro measurements of axoplasmic transport effects in the cat sciatic nerve and the estimation of neuromuscular disturbances in chickens and monkeys by vindesine in comparison with VCR. A radioimmunoassay for VLB, VCR, and VDS, developed by means of deacetylvinblastine acid azide, has been used to study the pharmacokinetics of vindesine in man. The clinical investigation of vindesine is in progress. Deacetylvinblastine, in contrast to earlier reports, showed activity against several murine tumor systems.

The molecular structures of the dimeric *Catharanthus* alkaloids vinblastine (VLB, **1**) and vincristine (VCR, **2**) differ only in the nature of the substituent, a methyl or a formyl group, respectively, on the vindoline N_a atom[3] (see Figure 1). Despite this "minor" difference between these two anticancer agents, they differ significantly in their clinical usefulness and clinical toxicity.[4] To illustrate these differences, a principal use of VCR—usually in combination with other agents[5]—is in the treatment of acute lymphocytic leukemia in children, while the chief use of VLB, singly or in combination, is in the treatment of Hodgkin's disease. Bone marrow toxicity is a dose-limiting factor in clinical therapy with VLB whereas VCR in children and in adults frequently produces neuropathy which necessitates discontinuation of therapy.[6]

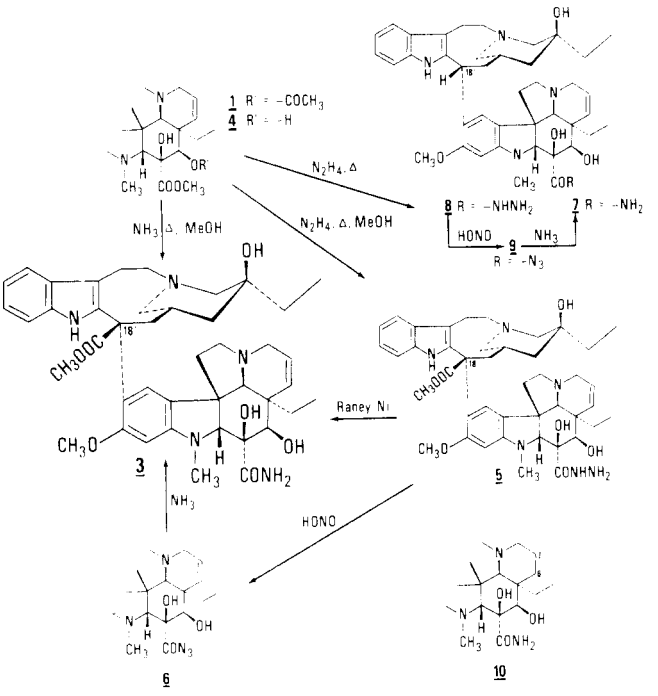
In order to explore the effect of other "minor" structural changes on experimental activity and toxicity, a systematic modification of accessible functions in the VLB molecule was initiated. The tumor inhibitory qualities of the analogues prepared were assessed primarily against the Ridgeway osteogenic sarcoma (ROS)[7] and the Gardner lymphosarcoma (GLS)[8] systems in the mouse. Selected agents were further evaluated in other murine tumors, such as the B16 melanoma,[9,10] leukemias (P388,[9] L1210,[9] and P1534[4]), and ascites.

It was hoped that the resulting information would furnish an understanding of structure–activity relationships (SAR) among the members of this class of oncolytic alkaloids and possibly lead to novel agents with an expanded activity spectrum and/or reduced toxicity. As neurotoxic symptoms encountered in clinical therapy with VCR have a dose-limiting effect, the search for experimental models which would permit an assessment of the neurotoxic potential of selected new agents is an essential element of the present investigation.

In this paper we present the chemistry leading to the synthesis of deacetylvinblastine amide (vindesine, VDS, **3**) and some of its biological activities. Preliminary observations on the clinical evaluation of VDS are noted. The experimental antitumor activities of a number of N-substituted deacetylvinblastine amides[1b] and a survey of SAR among this group of VDS congeners will be described in a subsequent paper.[11]

Chemistry. Vindesine (Figure 2) is prepared (see Scheme I) from VLB (**1**), vinblastine sulfate, or deacetylvinblastine (**4**)[12] (a) by preferential ammonolysis of the C_{23}-carbomethoxy function coupled with loss of the acetyl group in the vindoline moiety, (b) by preferential hydrazinolysis of the C_{23}-ester and deacetylation, followed by hydrogenolysis of the resulting deacetylvinblastine monohydrazide (**5**), and (c) by reaction of deacetylvinblastine acid azide (**6**), obtained from the hydrazide **5** by nitrosation, with ammonia in chloroform solution.

Scheme I



(a) Reaction of vinblastine sulfate, free base (**1**), or **4** at elevated temperatures in anhydrous methanol with excess ammonia produces vindesine (**3**) and small amounts of deacetyl-C_{18}-decarbomethoxyvinblastine amide (**7**). Column chromatography followed by crystallization from ethanol affords the amide **3** in 30–40% yield and 10–15% of the by-product **7**.

The singlet at δ 3.58, representing the C_{18}-$COOCH_3$ group in the spectra of VDS (Figure 2), **1**, and catharanthine,[4b] is absent in the proton NMR spectrum of **7**. This evidence, together with the IR spectrum (amide C=O only) and the mass spectral data (M^+ 695, M^+ 753 for **3**),

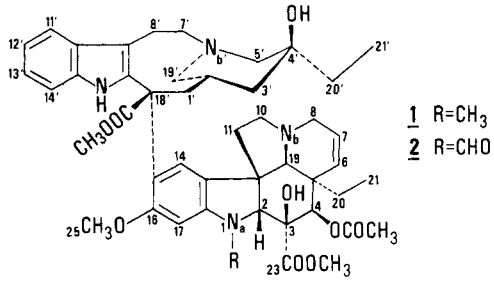


Figure 1. Molecular structures of vinblastine (VLB, 1) and vincristine (VCR, 2).

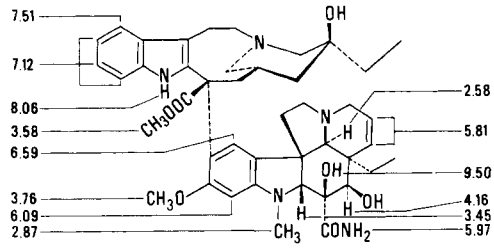


Figure 2. Values (ppm) of assigned signals in the NMR spectrum of vindesine in $CDCl_3$ solution using Me_4Si as internal standard.

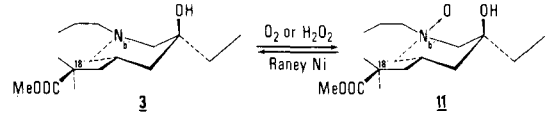
supports the decarbomethoxy structure **7**. The physical–chemical data (e.g., shoulder at 224 nm in the UV spectrum) of this amide **7** were the same as those of authentic amide **7** prepared from the known deacetyldecarbomethoxyvinblastine hydrazide (**8**)[13] via the corresponding azide **9**.

The structures of **3**, the decarbomethoxy species **7** and **8**, and by-products are supported by physical–chemical data (see Experimental Section) including circular dichroism data relevant to the stereochemical configuration at the $C_{18'}$ center.[14] ^{13}C NMR spectra of VDS (**3**) and of VLB (**1**) and its derivatives have been reported.[15]

(b) Hydrazinolysis of vinblastine sulfate, **1**, or **4** at moderate temperatures in methanol solution proceeds preferentially to yield—without the need for chromatography—the crystalline monohydrazide **5** containing only traces of the decarbomethoxy hydrazide **8**, the latter being the exclusive product formed in neat hydrazine under reflux conditions.[13] The monohydrazide **5**, a versatile intermediate, upon hydrogenolysis with Raney nickel catalyst[16] in methanol solution provides vindesine (**3**) and small amounts of the dihydro analogue **10**. High-pressure liquid chromatography and subsequent crystallization from an ethanol–methanol mixture provide purified vindesine free base in about 20% overall yield based on vinblastine sulfate (30% based on **5**) and a 2–3% yield of the dihydroamide **10**.

The signal of the vinyl protons seen at δ 5.81 in the NMR spectrum of **3** is absent in the spectrum of **10**, indicating the $C_6{=}C_7$ bond in the vindoline moiety to be the site of hydrogen uptake. Susceptibility to reduction of this double bond in vindoline and VLB has been observed.[17] Also, a comparison of the mass spectrum of the dihydroamide **10** with that of vindesine (and other alkaloids[18]) reveals an increase of two mass units in the parent ion (M^+ 755) as well as in the transmethylated[18b] indolenine moiety (m/e 414) and fragments derived therefrom (m/e 227, 124), whereas the indole (carbomethoxyvelbanamine) moiety and its major fragment (m/e 355 and 154, respectively) are common to both spectra. The increase of the 124/122 intensity ratio[18b] in the mass spectrum of **10** (see Experimental Section) further supports the 6,7-dihydrovindesine structure **10** shown.

(c) In the third preparation vindesine is produced in the reaction of deacetylvinblastine acid azide (**6**), prepared from the hydrazide **5** and nitrous acid, with excess ammonia in chloroform solution. In paper 2[11] of this series the preparation of N-substituted VDS congeners from the azide **6** and substituted amines will be described. A radioimmunoassay sensitive to VLB, VCR, and VDS has been developed[19] which involves as antigen the reaction product[11] of the azide **6** and bovine serum albumen.



3 **11**

Figure 3. Interconversion of vindesine and vindesine $N_{b'}$-oxide (**11**).

Of the three methods, the hydrazinolysis–hydrogenolysis procedure (b) is preferred for the larger scale preparation of vindesine because of its efficiency, simplicity, and relative ease of purification of the product.

Upon prolonged storage of vindesine free base, a new, more polar product is formed. After isolation this is characterized as vindesine $N_{b'}$-oxide (**11**) having physical properties, e.g., $pK_a' = 6.6$ compared to 7.4 in **3**, identical with those of this oxide **11** synthesized independently from VDS and hydrogen peroxide. Vindesine is regenerated by treatment of **11** with Raney nickel under the conditions of its original preparation from **5** (Figure 3).

The water-soluble monosulfate salt of vindesine, used in initial animal antitumor assays, was formed by titration of **3** in ethanolic solution with 1% ethanolic sulfuric acid to pH 4.2[20] and precipitation of the salt with 2-propanol. Alternatively, an aqueous solution of pH 4.2 containing the monosulfate (obtained by adding to a suspension of **3** the stoichiometric amount of dilute sulfuric acid) is filtered to remove traces of undissolved material and then lyophilized in portions. Vindesine sulfate may be stored at refrigerator temperatures for prolonged periods of time without discernible loss of potency.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Microelemental analyses were done on samples heated briefly (block-dried) at 120 °C.

UV spectra were obtained in methanol solution using a Cary 15 spectrophotometer. Circular dichroism spectra in MeOH solution were read on a Cary 60 recording spectropolarimeter with 6002 CD accessory. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. IR spectra were recorded in $CHCl_3$ solution for the free base alkaloids and as a Nujol mull for the sulfate salts on a Perkin-Elmer 457A spectrophotometer.

^1H NMR 100- and 220-MHz spectra were recorded in $CDCl_3$ solution on Varian Associates HA-100 and H-220 instruments, respectively. The presence of exchangeable protons, e.g., in $-CONH_2$, $-CONHNH_2$, and $-OH$ functions, was confirmed by D_2O shake. Chemical shifts are recorded in parts per million (δ) relative to tetramethylsilane [$(CH_3)_4Si$] as internal standard.

Mass spectra (MS) including high-resolution spectra (HRMS) were obtained on a Varian MAT Model 731 double-focusing spectrometer. Samples were inserted into the ion source by direct probe. The Varian MAT 731 transmitter using a field desorption ion source with tungsten emitters[21] served to obtain mass spectral analyses.

TLC was performed on EM-reagents precoated silica gel F-254 plates (5 × 10, 5 × 20, and 20 × 20 cm). The presence of alkaloid materials was detected by fluorescence in shortwave (254 nm) UV light and by spraying with ceric ammonium sulfate reagent.[22] Solvent systems used in TLC work include the following: (A) EtOAc–EtOH (3:1); (B) EtOAc–EtOH (1:1); (C) $CHCl_3$–Et_2NH (20:1); (D) Et_2O–MeOH–$MeNH_2$ (20:4:1); (E) acetone–MeOH (1:1). Solvent systems used in two-dimensional TLC are listed in the order of usage.

EM-precoated TLC plates of 2-mm thickness of silica gel G F-254 were used for preparative chromatography. For column

chromatography the absorbents used were Woelm silica gel (activity IV) and Woelm neutral alumina (activity I).

In high-pressure liquid chromatography (HPLC) on a preparative scale, we made use of Woelm silica 02747 containing 20% surface water and columns of 1 in. × 24 ft dimension under 100–300 psi of pressure.

Deacetylvinblastine Amide (Vindesine) (3). Method A. To a stirred solution of vinblastine sulfate (10 g, 11 mmol) in 100 mL of water, an excess of 6 N NH_4OH was added slowly ro raise the pH to 10–11. The resulting suspension of vinblastine free base 1 was extracted with three 150-mL portions of CH_2Cl_2. The combined extracts were washed with three 50-mL portions of water, dried three times (Na_2SO_4), and taken to dryness under reduced pressure. The residue of amorphous VLB (8.71 g, 97.7%) was dissolved without further purification in 200 mL of anhydrous methanol. Next, anhydrous liquid ammonia (200 mL) was added quickly, and the reaction mixture was maintained in a sealed vessel at about 100 °C for 60 h.

The reaction vessel was cooled, the contents were removed, and the excess ammonia and solvent were evaporated under reduced pressure. TLC (system A) revealed that the residue, weighing 6.4 g, contained several components: a major product **3** (R_f 0.2), which was slightly more polar than the (relay) deacetylvinblastine[12] (**4**) (R_f 0.24); a fast-moving by-product **7** (R_f 0.41) which gave a blue coloration with ceric ammonium sulfate spray; and some polar products (R_f 0.0–0.1). The amorphous residue was dissolved in 10 mL of ethanol, applied to a 2.5 × 12 in. silica gel column, and eluted with EtOAc–EtOH (1:1).

Early fractions contained the decarbomethoxy product **7**. Subsequent fractions contained both **7** and **3** (2.59 g) and later fractions only **3** (1.59 g). Alkaloidal material retained on the column was eluted with MeOH; the residue (0.8 g, R_f 0.0), presumably containing the ammonium salt of deacetylvinblastine acid,[12] was discarded. Rechromatography of the mixture of **3** and **7** and combination of the respective products yielded a total of 3.5 g (42%) of vindesine and 1.1 g (15%) of the decarbomethoxyamide **7**.

Physical–chemical data characterizing vindesine are described under method B. Data for **7** are reported in the section on its preparation from **8**.

Method B. Deacetylvinblastine Monohydrazide (5). To a mixture of 120 mL of anhydrous hydrazine and 120 mL of anhydrous methanol was added 44 g (53 mmol) of VLB (free base, **1**) prepared from 50 g of vinblastine sulfate. The mixture was stirred under a nitrogen atmosphere at 60 ± 2 °C for 24 h. Progress of the hydrazinolysis was monitored by TLC (systems A and C) by extracting 0.5-mL aliquots diluted with 1 mL of water with CH_2Cl_2. When conversion to the monohydrazide **5** was complete, only trace amounts (estimated 2–4%) of the decarbomethoxyhydrazide[13] **8** (R_f 0.25, system A) were noted.

The cooled reaction mixture was poured into 1200 mL of water and the resulting suspension extracted with 400 mL of CH_2Cl_2. The aqueous phase was extracted three times with 200-mL portions of CH_2Cl_2, and the combined extracts were washed first with 200 mL of water and then with 200 mL of saturated NaCl solution. The solution was dried (Na_2SO_4) for 1 h with occasional swirling. Evaporation of the CH_2Cl_2 afforded 35.2 g (83% yield based on vinblastine sulfate) of crude deacetylvinblastine monohydrazide (**5**) as an amorphous powder which was suitably pure for hydrogenolysis. A sample of **5** crystallized from CH_2Cl_2 on standing for 18 h at 4 °C: mp 210–220 °C dec; $[\alpha]^{25}_D$ +37.1° (*c* 0.12, MeOH); TLC R_f 0.34 (C); IR ν_{max} 3685, 3600, and 3440 (OH and hydrazide NH), 1735 ($COOCH_3$), 1672 cm^{-1} (hydrazide C=O); ^1H NMR 3.58 (3 H, s, $C_{18'}$-$COOCH_3$), 4.15 [1 H, s, $-C_4H(OH)$], 8.04 (1 H, s, indole NH), 8.26 (1 H, s, $-CONHNH_2$), 9.52 (1 H, br s, C_3-OH); MS M^+ 768; electrometric titration (66% DMF) pK_a' 7.50, 5.55, and <4.0. Anal. ($C_{43}H_{56}N_6O_7$) C, H, N. Mol wt: found (HRMS), 768.4207; calcd, 768.4210.

Hydrogenolysis of 5. To a freshly prepared slurry of 150 g of Raney nickel in 850 mL of methanol was added a solution of 33.15 g (43 mmol) of the monohydrazide **5** in about 400 mL of methanol. Additional methanol was added to a volume of 1500 mL. The mixture was stirred mechanically and heated under reflux for 7 h and allowed to cool to room temperature, and the nickel was removed by filtration with the aid of talc. To prevent self-ignition of the pyrophoric catalyst, a layer of solvent was maintained above the filter at all times. The clarified solution was concentrated under reduced pressure and the residual crude product, about 28 g, was allowed to crystallize from methanol (120 mL), yielding 18.7 g (57% based on **5**) of vindesine. TLC (system B) revealed vindesine (R_f 0.28) as the major component and a slightly faster minor component (R_f 0.33) recognized as the 6,7-dihydro analogue **10** (see below).

HPLC (silica gel, adjusted to activity V) of a sample (8 g) of this product with 11 L of an Et_2O–Et_2NH–H_2O (75:25:0.75) mixture as the eluting solvent, while monitoring (500 mL) fractions of the effluent by TLC (using the same solvent mixture), gave a large forerun (3930 mL), fractions 1–2 containing **3** and **10** (1.15 g), fractions 3–10 containing only **3** (6.3 g), and fractions 11–16 containing vindesine and slower moving impurities (R_f 0.1–0.2).

The amorphous product (6.3 g) crystallized from EtOH–MeOH yielding 4.2 g of purified vindesine (30% based on **5**, 20% overall based on vinblastine sulfate), mp 230–232 °C dec. The product compares favorably in TLC (R_f 0.34, D), in two-dimensional TLC (MeOH, D), and in physical properties with **3** prepared by method A: CD curve, nm ($\Delta\epsilon$), min 213 (−86), max 225, 260, and 305 (+44, +22, and +9); $[\alpha]^{25}_D$ +39.4° (*c* 1.0, MeOH); UV λ_{max} 214, 266, 288, and 296 nm (ϵ 53 400, 17 450, 13 950, and 12 500); IR ν_{max} 3655 and 3560 (OH), 3495 (indole NH), 3455 and 3380 (amide NH), 1715 ($COOCH_3$), 1680 cm^{-1} ($CONH_2$); ^1H NMR, cf. Figure 2; electrometric titration (66% DMF) pK_a' 5.39 and 7.36, (H_2O) 6.04 and 7.67; MS M^+ 753, *m/e* 767 and 781 (transmethylation peaks[18b]), 722, 709, 695, 694, 651, 571, 412, 355, 225, 154, 124, 122; intensity ratio 124/122 = 1.5. ^{13}C NMR has been reported elsewhere.[15] Anal. Calcd for $C_{43}H_{55}N_5O_7$: C, 68.50; H, 7.35; N, 9.29; O, 14.86. Found: C, 68.73; H, 7.42; N, 9.16; O, 14.92. Mol wt: found (HRMS), 753.4086; calcd, 753.4101. Mol wt: *m/e* 124 peak found (HRMS), 124.1126; calcd for $C_8H_{14}N$,[18b] 124.1126.

Rechromatography of the mixture of **3** and **10** (fractions 1–2) gave 155 mg of amorphous 6,7-dihydrovindesine (**10**): UV λ_{max} 214, 266, and 288 (sh), 296 nm (ϵ 46 420, 13 000, 12 000, and 11 360); IR ν_{max} 1681 cm^{-1} ($-CONH_2$); ^1H NMR 2.30 (1 H, s, C_{19}-H), 2.83 (3 H, s, N_a-CH_3), 3.45 (1 H, s, C_2-H), 3.58 (3 H, s, $C_{18'}$-$COOCH_3$), 3.76 (3 H, s, C_{16}-OCH_3), 4.40 [1 H, s, $C_4H(OH)$], 5.80 (2 H, m, $-C_{23}ONH_2$) (5.81 signal for C_6-H and C_7-H in **3** is absent in **10**), 6.09 (1 H, s, C_{17}-H), 6.55 (1 H, s, C_{14}-H), 7.13 (3 H, m, $C_{11',12',13'}$-H), 7.51 (1 H, m, C_{14}-H), 7.98 (1 H, s, indole NH), and 9.98 (1 H, br s, C_3-OH); MS M^+ 755, *m/e* 769 (transmethylation peak[18b]), 724, 711, 697, 696, 653, 414, 355, 227, 154, 124 (s), 122 (w), intensity ratio 124/122 = 20.0; electrometric titration (66% DMF) pK_a' 5.48 and 7.20. Anal. Calcd for $C_{43}H_{57}N_5O_7$: C, 68.32; H, 7.60; N, 9.26. Found: C, 69.19; H, 7.84; N, 8.77. Mol. wt: found (HRMS), 755.4248; calcd, 755.4258.

Vindesine Sulfate. Initial Procedure.[20] A solution of vindesine (**3**, 600 mg) in ethanol (10 mL) was titrated with 1% ethanolic sulfuric acid until an aliquot diluted with a fivefold portion of water had a pH of 4.2. Addition of an equal volume of *i*-PrOH gave a precipitate of vindesine sulfate. Twice the salt was dissolved in ethanol and precipitated with *i*-PrOH to give 150 mg of amorphous vindesine sulfate incorporating an undetermined amount of solvent: mp >250 °C; IR ν_{max} 1675 cm^{-1} (amide C=O). Anal. Calcd for $C_{43}H_{55}N_5O_7 \cdot H_2SO_4$: C, 60.62; H, 6.74; N, 8.22; S, 3.68. Found: C, 60.92; H, 6.86; N, 8.45; S, 3.60.

Recovery of **3** from the sulfate gave vindesine (R_f 0.20, A) with IR, UV, and NMR spectra indistinguishable from those of authentic **3**.

Alternate Procedure. To a solution of mannitol (310 g) in water (5 L), vindesine (55.4 g) was added with mechanical stirring. To this suspension 10% (w/v) H_2SO_4 (66 mL) was added dropwise; the resulting solution (pH 4.2) was diluted to 7.2 L and filtered. Portions containing 10 mg of the sulfate were lyophilized and stored at 2–8 °C in a closed vessel.

Method C. Deacetylvinblastine Acid Azide (6). To a cooled solution (4 °C) of deacetylvinblastine hydrazide (**5**, 678 mg, 0.9 mmol) in 15 mL of MeOH and 50 mL of 1 N HCl, 140 mg (2 mmol) of $NaNO_2$ was added at once, and the reaction mixture was stirred for 10 min at 4 °C. The red-brown solution was adjusted to pH 8.0 with cold 5% $NaHCO_3$ solution (10 mL) and the acid azide **6** was extracted rapidly with three successive portions of 100 mL of CH_2Cl_2. The dried solution (Na_2SO_4) was reduced to a volume of 20 mL, 2 mL of which was used for characterization of the azide. The intensity of the $-CON_3$ band

at 2135 cm^{-1} in the IR spectrum of this solution remained unchanged for several days at 0 °C.

Excess anhydrous ammonia gas was bubbled into the solution of the acid azide and the mixture was allowed to stand at 0 °C for 18 h. The presence of deacetylvinblastine amide was detected by TLC (R_f 0.20, A). The product (120 mg), purified as described for method A, possessed the physical characteristics (e.g., two-dimensional TLC, MeOH–system D) of vindesine prepared by methods A or B.

Deacetyldecarbomethoxyvinblastine Hydrazide (8). Compound 8 was prepared according to Neuss et al.[3,13] A sample crystallized from MeOH: mp 220–225 °C (lit.[3] 210–214 °C); $[\alpha]^{25}_D$ +31.25 (*c* 1.0, MeOH); TLC R_f 0.63 (B), 0.90 (E); UV λ_{max} 212.5, sh 224, 258, 287, and 294 nm (ϵ 53 390, 37 500, 13 500, 11 100, and 11 200); CD curve, nm ($\Delta\epsilon$), min <200 and 256 (–59 and –4.5), max 227.5, 277.5, and 306 (+44.5, +10.0, and +9.0); IR ν_{max} 3690, 3570, and 3440 (OH, amide NH), 3480 (indole NH), 1650 cm^{-1} (hydrazide C=O); ^1H NMR, see ref 3 and 13; electrometric titration (66% DMF) pK_a' 5.20 and 6.53; MS M^+ 710, *m/e* 695, 650, 593, 569, 427, 154, 124, 122; intensity ratio 124/122 = 0.9.

Deacetyldecarbomethoxyvinblastine Amide (7) from 8. Deacetyldecarbomethoxyvinblastine hydrazide (8) (5.0 g, 7.0 mmol) was dissolved in 60 mL of MeOH and 200 mL of 1 N HCl. $NaNO_2$ (500 mg, 7.0 mmol) was added to the cooled solution (4 °C) which was stirred for 5 min, made basic (pH 8) with 5% $NaHCO_3$, and extracted three times with 150-mL portions of CH_2Cl_2. The dried solution (Na_2SO_4) was concentrated to 5–10 mL under reduced pressure, and the concentrate was dissolved in 100 mL of methanol and 25 mL of anhydrous ammonia. After 18 h at 4 °C the solvents and excess ammonia were removed and the residue was dissolved in 100 mL of CH_2Cl_2. The solution was washed twice with 10 mL of 5% $NaHCO_3$, dried (Na_2SO_4), and taken to dryness. The residue, dissolved in 5 mL of EtOH, was applied to a silica gel column and eluted with an EtOAc–EtOH (3:1) solvent mixture. Fractions containing the desired amide 7 (R_f 0.39, A) were combined and yielded 970 mg of amorphous product possessing physical characteristics (R_f, UV, IR, ^1H NMR, MS) indistinguishable from those of 7 obtained in the ammonolysis of 1 (see method A). A sample was recrystallized from CH_2Cl_2 and afforded white, electrostatic crystals: mp 210–213 °C; UV λ_{max} 213, sh 225, 260, 287, and 295 nm (ϵ 49 500, 37 000, 14 000, 11 500, and 11 800); CD curve, nm ($\Delta\epsilon$), min <200 and 257 (–60 and –6.0), max 228, 278, and 306 (+46.2, +9.5, and +9.0); $[\alpha]^{25}_D$ +33.2°, $[\alpha]^{25}_{365}$ +50.2° (*c* 0.5, MeOH); IR ν_{max} 3675 (OH), 3520 (indole NH), 3470 and 3400 (OH, amide NH), 1690 (amide C=O), and 1565 cm^{-1} ($CONH_2$ deformation); ^1H NMR 2.78 (3 H, s, NCH_3), 3.40 (1 H, s, C_2-H), 3.80 (3 H, s, $-OCH_3$), 4.10 (1 H, m, C_4-H), 5.56 (2 H, m, $-CONH_2$), 5.81 (2 H, m, C_6=C_7 vinyl protons), 5.91 (1 H, d, $C_{18'}$-H, *J* = 12 Hz), 6.02 (1 H, s, C_{17}-H), 7.00 (4 H, m, $C_{14,11',12',13'}$-H), 7.44 (1 H, m, $C_{14'}$-H), 8.14 (1 H, br s, indole NH), 9.40 (1 H, br s, C_4-OH); MS M^+ 695, *m/e* 593, 554, 412, 294–297, 154, 124, 122; intensity ratio 124/122 = 0.9; electrometric titration (66% DMF) pK_a' 5.17 and 6.55. Anal. ($C_{41}H_{53}N_5O_5$) N; C: calcd, 70.76; found, 69.87. H: calcd, 7.68; found, 8.11. Mol wt: found (HRMS), 695.4029; calcd, 695.4047.

For bioassay, the sulfate salt of 7 was prepared in ethanolic 1% sulfuric acid in the usual manner:[20] mp >250 °C; IR ν_{max} 1670 cm^{-1} (amide C=O). Treatment of the sulfate (cf. method A) with 6 N NH_4OH reconstituted the free base with properties (IR; UV; R_f 0.40, *a*) of the original amide 7.

Vindesine N_b-Oxide (11). A 250-mg sample of vindesine was stored in a closed container at 4 °C for approximately 2 months. Estimation by TLC (system E) revealed the formation of approximately 15% of the N_b-oxide (R_f 0.25; R_f for 3, 0.55). Preparative TLC (system E) and extraction of the slow zone with methanol gave 11 and absorbant material. The solids were suspended in $CHCl_3$, the mixture was filtered, and the filtrate was concentrated under reduced pressure. The amorphous product (20 mg) possessed the physical properties (IR, TLC systems A and E) of vindesine N_b-oxide prepared from 3 and H_2O_2 (below).

To a mechanically stirred suspension of vindesine (10 g, 13.3 mmol) in 500 mL of water at room temperature was added 300 mL of 3% aqueous H_2O_2 over 15 min. The mixture was stirred for 6 h at which time most of the vindesine had dissolved. The product was extracted with CH_2Cl_2 (3 × 200 mL); the combined extracts were washed first with water and then saturated NaCl and dried (Na_2SO_4). Evaporation of solvent afforded 8.34 g (83%) crude N_b-oxide. Crystallization from MeOH gave 3.0 g of 11, mp 227–232 °C dec, and additional crops (2.8 g): IR ν_{max} 3500, 3460, and 3390 (amide NH, OH), 1715 ($COOCH_3$), and 1680 cm^{-1} ($-CONH_2$); ^1H, s, C_{14}-H), other signals resemble those for 3, Figure 2; MS (electron impact) M^+ 753 (calcd for $C_{43}H_{55}N_5O_8$, 769); MS (field desorption,[21] 10-μ emitter with 23 mA) M^+ 769; electrometric titration (66% DMF) pK_a' 5.4 and 6.6.

Reduction of N_b-Oxide 11 to 3. To a suspension of 2.5 g of Raney nickel (W-2) in 30 mL of MeOH was added a solution of vindesine N_b-oxide (0.5 g) in 5 mL of MeOH. The mixture was stirred and heated under reflux for 5 h. The flask was cooled and the nickel removed by filtration with talc. The filtrate was evaporated to dryness, affording vindesine base (0.44 g), identified by TLC (R_f 0.55, E) and IR comparison with an authentic sample.

Table I. Activity of Vindesine in Comparison with VCR, Deacetylvinblastine, and VLB against the Ridgeway Osteogenic Sarcoma and the Gardner Lymphosarcoma in Mice

Alkaloid (sulfate salts)	Dose range,[a] mg/kg/day	Antitumor effect[b] ROS	Antitumor effect[b] GLS	Acute LD_{50} (iv), mice, mg/kg
Vincristine	0.1–0.2	+++	+++[c]	2.1 ± 0.14
Vindesine	0.2–0.3	+++	+++	6.3 ± 0.6
Deacetylvinblastine	0.3–0.4	++	++	5.8 ± 0.5
Vinblastine	0.1–0.35	±	±	10.0 ± 0.8

[a] Treatment ip on days 1–9. [b] +++ = 75–100% inhibition of tumor growth compared to that of controls; ++ = 50–75% inhibition; ± = less than 25% inhibition. The methods for these and other tumor assays have been previously described.[30] [c] See ref 28.

Biological Observations

Antitumor Activities. The discovery in 1958 of vinblastine (1) as an antiproliferative factor in leaf extracts of the periwinkle plant (*Catharanthus roseus* G. Don, *Vinca rosea* Linn.) at the University of Western Ontario[23] and, independently, at the Lilly Research Laboratories[24] and the further discovery in these extracts of vincristine[25] (leurocristine), of leurosidine,[25] and of leurosine[26] were made possible largely through the use of the P1534 murine leukemia. This lymphocytic leukemia, which had earlier served to detect and predict clinical activity of neoplastic agents,[27] was found to be exquisitively sensitive, especially to the action of vinblastine and vincristine.[4b,26] However, only a few years later the response of this leukemia had begun to decline,[4b] especially in the case of VLB, and by the time of the present work (ca. 1972) the P1534 leukemia was essentially insensitive to *Catharanthus* agents. For our VLB modification studies, aimed at emulating the superior antitumor effects—but not the neurotoxicity—of VCR, the Ridgeway osteogenic sarcoma (ROS),[7] and the Gardner lymphosarcoma (GLS),[8] solid murine tumors sensitive to VCR but not to VLB[28] served as primary assay systems.

The activities of vindesine against the ROS, GLS, and other murine tumor systems, described also elsewhere,[1,29] are summarized below.

The sensitive response of the ROS and the GLS murine tumors to VCR and to VDS, the lesser response to deacetylvinblastine, and the lack of response to the parent alkaloid VLB itself are shown in Table I. That the growth in these two experimental systems is effectively inhibited by VDS at dose levels approaching those of VCR represents a *qualitative* difference in antitumor activity of VDS relative to that of VLB.

Our demonstration of the activity of VDS, administered ip, against the Ridgeway osteogenic sarcoma was confirmed

Table II. Activity of Vindesine in Comparison with VLB, VCR, and Deacetylvinblastine against P1534(J) Leukemia[a] in Mice

Alkaloid agent (sulfate salts)	Dose,[b] mg/kg/day	Antitumor effect[c]
Vindesine	0.25[d]	100
	0.2	74
Vinblastine	0.35[d]	54
Vincristine	0.25[d]	77
Deacetyl-vinblastine	0.2[d]	96
	0.1	41

[a] Tumor implanted subcutaneously. The method used in this assay has been described recently.[30] [b] Treatment ip on days 1-9. [c] Inhibition (percent) of tumor growth compared to untreated controls. [d] Highest nontoxic dose.

at the National Cancer Institute, Laboratory of Experimental Chemotherapy, through the courtesy of Drs. Abraham Goldin and Randall K. Johnson.[31] Orally administered VDS was found to be ineffective against the ROS-GLS systems.

Against the P1534(J) leukemia strain,[30] implanted subcutaneously, VDS shows activity of the same degree as that of VCR, VLB, and deacetylvinblastine (Table II). These four agents—according to test results provided by the southern Research Institute (courtesy of Messrs. Montgomery and Laster)[32]—have also similar potency in prolonging the life span of mice bearing the P388 leukemia[9] over that of untreated controls (Table III). The activity of VDS against the P388 leukemia was confirmed at the Lilly Research Laboratories, at the National Cancer Institute,[31] and also at the National Service Center, Drug Development Branch (NCI), through the courtesy of Dr. Harry Wood.[33]

Against the murine B16 (ip) melanoma VDS, according to test results kindly provided by Dr. Randall K. Johnson, National Cancer Institute,[31] appears to be superior to VCR in increasing survival time (Table IV, experiment I). This observation was confirmed at the Lilly Laboratories and at the Drug Development Branch, National Cancer Institute.[33] The activity of deacetylvinblastine is comparable to that of VDS.[34]

VDS also inhibits growth of the adenocarcinoma 755 and prolongs survival time of mice bearing the Walker, Ehrlich, and S180 ascites.[29] On the other hand, VDS—like VCR and VLB—showed only marginal or no activity against the L1210, C1498, and L5178Y leukemias, the Lewis lung carcinoma, and the X5563 plasma cell myeloma. VDS does not increase survival time[31] of mice bearing the P388/VCR strain of leukemia, a strain which was shown to be resistant to maytansine[35] as well as to VCR.

Additional in vivo and in vitro biological assay systems which have been used for the comparison of VDS with VLB, VCR, and deacetylvinblastine include growth inhibition of the Friend leukemia virus in mice,[36] mitotic arrest of Chinese hamster ovary cells in tissue culture,[37] and binding affinity to pig brain tubulin.[38] SAR among the alkaloids in these systems[11] parallel those seen in the antitumor assays described above.

Finally, activities against the ROS and GLS of intermediates **4** and **5** and by-products **7**, **8**, **10**, and **11** of the vindesine preparation are compared with that of VDS (see Table V). The reduced potency of dihydrovindesine (**10**), the marginal activity and much decreased acute toxicity of vindesine N_b-oxide (**11**), and virtual lack of activity of the C_{18}-decarbomethoxy species **7** and **8**, compounds which retain the "natural" stereochemistry[14] at $C_{18'}$, all *reexemplify* the high degree of chemical and stereochemical specificity required for optimum antitumor effect.

Table III. Activity of Vindesine in Comparison with Vinblastine, Vincristine, and Deacetylvinblastine against the P388 Leukemia[a,b] in Mice

Alkaloid agent (sulfate salts)	Dose, mg/kg/day, schedule (ip)		40th-Day survivors/ total	Median[c]	
	Days 1-9	Day 1 only		Life span, days	Increase of life span, %
Controls, 10^6 cells			0/20	11.0	
Vindesine	0.9[d]		4/10	30.5	177
	0.6		4/10	34.5	213
	0.4		3/10	30.0	172
	0.27		4/10	30.0	172
	0.10		0/10	23.0	109
		13.5[d]	4/10	36.5	231
		9.0	3/10	32.0	190
		6.0	1/10	23.0	109
		4.0	0/10	19.5	77
		2.7	0/10	21.5	95
		1.8	0/10	17.0	54
Vinblastine	0.9[d]		3/10	23.0	109
	0.6		5/10	>40	>263
	0.4		0/10	19	72
	0.27		1/10	25.5	131
		9.0[d]	1/09	25.0	127
		6.0	2/10	23.0	109
		4.0	0/10	21.5	95
		2.7	0/10	20.0	81
Vincristine	0.33[d]		3/10	31.0	181
	0.22		0/10	25.5	131
		4.0[d]	1/10	24.0	118
		2.7	2/10	27.0	100
		1.8	0/10	20.0	81
		1.2	0/10	18.5	68
Deacetyl-vinblastine	0.6[d]		7/10	>40.0	>263
	0.4		7/10	>40.0	>263
	0.27		4/10	31.0	181
	0.18		2/10	26.0	136
		9.0[d]	5/10	>40.0	>263
		6.0	2/10	33.0	200
		4.0	4/10	35.0	218
		1.7	1/10	28.0	154
		1.8	1/10	28.5	159

[a] Data kindly provided by Messrs. Laster and Montgomery, Kettering Meyer Laboratories, Southern Research Institute, Birmingham, Ala. [b] P388 ascites implanted ip. A detailed description of the methods used is given in ref 9. [c] Median life span calculated by using all deaths and survivors. [d] Highest nontoxic dose.

Animal Toxicology. Toxicological studies, including acute and 3-month toxicity estimations in mice, rats, and dogs, have been reported previously.[39] Signs of acute toxicity observed with VDS in mice and rats resemble those seen with VLB and VCR. The LD_{50} values in these species for VDS of 6.3 and 2.0 mg/kg, respectively, are between those for VLB (10.0 and 2.9 mg/kg) and VCR (2.1 and 1.0 mg/kg); in mice the LD_{50} of deacetylvinblastine is 5.8 mg/kg.

The primary toxicity of vindesine in animals was observed in the rapidly proliferating cells that produce the intestinal mucosa, blood cells, sperm, etc. A dose of vindesine administered once a week was tolerated better than the same dose divided and given more frequently. Functional or structural changes in nerve tissue could not be demonstrated in these studies.[39]

Special attention was given to the experimental assessment of the neurotoxic potential of vindesine. We are greatly indebted to Drs. Sidney Ochs and Robert Worth, Indiana University Medical School, for measuring the effects of vindesine in comparison to those of VLB and VCR on axoplasmic transport in the dorsal root ganglion (L7) of the cat sciatic nerve.[40] In this in vitro system

Table IV. Activity of Vindesine in Comparison with Vincristine, Vinblastine, and Deacetylvinblastine against the B16 Melanoma[a] in Mice

Alkaloid agent (sulfate salts)	Dose, ip, mg/kg/day	Treatment: Days 1, 5, 9 MST (range), days	Days 1, 5, 9 ILS (surv), %	Days 5, 9, 13 MST (range), days	Days 5, 9, 13 ILS (surv), %
		Experiment I			
Untreated controls		20.0 (16-38)	(0/30)	20.0 (16-38)	(0/30)
Vindesine	3.00	47.5 (10-54)	138 (1/10)	29.5 (16-37)	48
	1.80	48.5 (16-52)	142 (2/10)	39.0 (16-50)	90
	1.08	42.0 (21-52)	110 (1/10)	31.5 (25-41)	58
	0.648	35.0 (31-51)	75	27.0 (24-37)	35
	0.389	37.0 (21-38)	85	25.0 (15-32)	25
Vincristine	1.67	35.0 (26-41)	75	26.0 (16-33)	30
	1.0	32.5 (21-36)	62	29.5 (16-36)	48
	0.60	30.5 (22-32)	52	24.5 (16-39)	22
	0.36	26.0 (19-31)	30	24.0 (20-32)	20
	0.216	24.0 (20-27)	20	24.5 (21-54)	22 (1/10)
Vinblastine	3.0	40.5 (12-51)	102 (1/10)	32.5 (15-36)	62
	1.80	38.5 (28-46)	92	25.0 (20-31)	25 (1/10)
	1.08	35.5 (29-44)	78 (1/10)	27.5 (22-36)	39
	0.648	33.5 (23-41)	68	28.0 (22-36)	40
	0.389	32.0 (20-40)	60	22.5 (15-27)	12
		Experiment II			
Vindesine	0.9	39.3 (11-56)	60 (5/10)	38.3 (26-47)	78
Deacetylvinblastine	1.2	41.5 (13-56)	69 (3/10)	45.2 (11-51)	110 (1/10)
	0.9	45.3 (9-56)	84 (6/10)	48.7 (12-61)	126
	0.6	51.5 (15-56)	109 (7/10)	41.1 (14-54)	91 (1/10)
	0.3	49.3 (34-60)	100	41.1 (31-53)	91
	0.15	42.1 (13-51)	71	33.7 (26-48)	57 (1/10)
Control		24.6 (9-50)	(0/30)	21.5 (17-31)	(0/30)

[a] Experiment I done at the National Cancer Institute (Dr. R. K. Johnson):[31] 0.25 mL of a 20% (w/v) brei of B16 melanoma[9] implanted ip on day 0 into male B6D2F$_1$ mice. MST (range) = medium survival time in days (range of individual animals deaths); ILS (surv) = percent increase in life span of survivors/total on day 56. Experiment II was done at the Lilly Laboratories: 0.5 mL of tumor brei implanted ip in C57BL/6 mice. Experiment terminated at 61 days.

Table V. Relative Activities against the ROS and GLS in Mice of Intermediate and By-Products in the Vindesine Preparation

No.	Alkaloid agent (sulfate salts)	Inhibn of ROS and GLS (dose range)[a]
3	Vindesine	+++ (0.2-0.3)
5	Deacetylvinblastine hydrazide[b]	+++ (0.2-0.3)
10	Dihydrovindesine	++ (0.5-0.7)
11	Vindesine $N_{b'}$-oxide[b,c]	+ (0.5-0.8)
7	$C_{18'}$-Decarbomethoxy-vindesine	± (1.0-2.0)
8	$C_{18'}$-Decarbomethoxy-deacetylvinblastine hydrazide[b]	± (1.0-2.0)

[a] +++ = 75-100% inhibition of tumor growth; ++ = 50-75% inhibition; + = 25-50% inhibition; ± = less than 25% inhibition. Dose range = mg/kg/day for 9 days, ip route. [b] Free base. [c] $LD_0 > 10.0$.

permitting study of fast transport of synthesized labeled protein, VCR appears to inhibit this transport more effectively than VLB. VDS resembles VLB rather than VCR as an inhibitor of fast axoplasmic transport.[41,42]

In the search for a suitable animal model for neurotoxic estimation, it was observed that, in the chicken, VCR given iv elicits neuromuscular disturbance, e.g., loss of balance, while VDS does not cause such disturbance at the same or higher doses given for a longer time.[43] Similarly, when chronic doses of these agents were administered iv to monkeys in amounts causing an equal degree of leukopenia, symptoms of peripheral neuropathy, resembling those seen in patients receiving VCR, were observed in the group of animals receiving VCR but not in those receiving VDS.[43]

Neither the in vitro nerve-ganglion system nor the chicken and monkey assay is necessarily predictive of clinical effects of VDS or other alkaloid agents. Nevertheless, that VDS in these animal experiments was seen to cause less neurotoxicity than VCR raises the hope that VDS may also prove to be less neurotoxic in man.

Pharmacology. The effects of vindesine administered iv on blood pressure, respiration, and heart rate have been examined in the cat.[44] The tissue distribution of [^{3}H]-VDS, prepared by exchange tritiation,[45] has been studied in the rat.[46] Blood and tissue levels of [^{3}H]-VDS following oral administration were shown to be very low, about 1–3% of corresponding levels after iv administration.

As part of a phase I clinical evaluation (see below), the pharmacokinetics of VDS in humans, as determined by radioimmunoassay,[19] have been reported from the Lilly Laboratory for Clinical Research[47] and the Johns Hopkins Oncology Center.[48] The comparative pharmacokinetics of VDS, VLB, and VCR in man have been determined recently.[49]

Discussion

In the present investigation of dimeric *Catharanthus* alkaloids, the first objective, namely, exploring the effects of "minor" structural differences on antitumor activity and toxicity, has been attained in the preparation of VDS. This VLB modification product resembles VCR rather than VLB in its experimental antitumor spectrum, while its neurotoxicity appears potentially to be less than that of VCR, as estimated by comparative response in the chicken and monkey.

The clinical implications of these experimental findings can be ascertained only by a judicious evaluation of VDS in man, an investigation now in progress at the Lilly Laboratories for Clinical Research (Indianapolis), at the

Memorial Sloan-Kettering Center (New York City),[50] at the University of Texas M. D. Anderson Hospital (Houston),[51] and elsewhere.[52]

The decision to evaluate VDS in selected cancer patients was based on the following (four) preclinical observations.

(1) Though not included among the tumor systems (P388, L1210, B16, and LL) of high predictivity used for screening of new agents by the NCI, Drug Development Program,[53] the ROS has been described as a solid tumor system with a satisfactory degree of predictivity.[7] Therefore, the effective growth inhibition of the ROS by VDS—but not VLB—served to select VDS for clinical evaluation.

(2) Though less active than VCR against the ROS tumor, VDS has similar activity as VCR against the P1534(J) (sc) leukemia[30] (Table II) and the P388 leukemia (Table III). VDS is more effective than VCR in extending the life span of mice bearing the B16 melanoma (Table IV).

Among the group of N-substituted VDS congeners[1b] to be reported in detail,[11] some (e.g., *N*-β-hydroxyethylvindestine, compound VII in ref 1b) approach or surpass VDS in activity against a single tumor system (ROS), but none appear superior to VDS in terms of *collective* activity.

Deacetylvinblastine hydrazide (**5**), tested as the free base against the ROS (Table V), compares favorably with VDS but is not attractive as a candidate for further evaluation since it lacks adequate chemical stability. Hydrolysis of the hydrazide group may release small amounts of hydrazine,[54] a chemical reported to possess carcinogenic potential.[55]

Deacetylvinblastine (**4**) is slightly less active than VDS against the ROS–GLS tumors (Table I). Activity of deacetylvinblastine, especially against the P1534(J) (sc) leukemia, is in contrast with the lack of activity against the original P1534 (ip) leukemia reported for this agent (**4**) from these laboratories in 1963–1964.[7,12] Since the unavailability of the original, VLB- and VCR-sensitive P1534 precludes verification of the earlier report,[12] a dilemma remains. Deacetylvinblastine (**4**) and the quality of its biological activity are of considerable importance in view of its reported occurrence as a metabolite of VLB in the dog[56] and in man.[57,58]

The reduced activity against the ROS of 6,7-dihydrovindesine (**10**) relative to that of VDS (Table V) is reminiscent of a similar relationship recorded[59] for dihydrovinblastine[60] and VLB. The much reduced acute toxicity of vindesine N_b-oxide (**11**) is considered as noteworthy.

The lack of demonstrable activity observed for $C_{18'}$-decarbomethoxyvindesine (**7**) indicates that the $C_{18'}$-ester group in VDS (and other dimers) is necessary for biological activity.

In terms of *collective* (experimental antitumor) activity, VDS, a representative member of the group of deacetylvinblastine amides, appears to have optimum qualities.

(3) The reduced neurotoxic potential of VDS relative to that of VCR, suggested by the in vitro and in vivo findings mentioned above, reinforces its candidacy for clinical evaluation.

Binding of VCR to nerve tubulin has been suggested[61] as a mode through which neurotoxicity may be expressed but the molecular basis for these events remains to be formulated. Lipophilicity is reported to enhance transport of uncharged molecules across cell membranes of the peripheral nerve[62] and the blood–brain barrier.[63] Although no direct relationship between the lipophilicity[64] and the degree of neurotoxicity of the small number of dimeric alkaloid agents now in use is evident at this time, the regional increase of polarity associated with the conversion of VLB to VDS appears as a desirable feature (Figure 4).

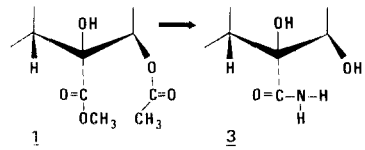


Figure 4. Regional increase of polarity in conversion of vinblastine to vindesine.

Studies now in progress with [^{3}H]-VDS and [^{3}H]-VLB, and planned with [^{3}H]-VCR, comparing uptake of these alkaloids by cat sciatic nerve in vitro[65] as well as studies of the disposition and tissue levels of [^{3}H]-VDS in rats[46] hopefully may lead to some understanding of the complex interplay of factors affecting neurotoxicity.

(4) Finally, aspects of chemical stability, feasibility of preparation, and pharmaceutical acceptability may be decisive in favoring one clinical candidate over another equally active one. Vindesine sulfate, prepared as described in the Experimental Section, adequately fulfills requirements in these areas.

Thus, because of the favorable nature of the above four observations, the clinical evaluation of VDS was undertaken.

Preliminary Clinical Observations. Phase I–II clinical trials with vindesine were carried out in the Lilly Laboratories for Clinical Research. Following dose-ranging studies, it was found that 3–4 mg/m^2 once weekly, given intravenously by the "push" technique, provides an acceptable balance between efficacy and toxicity. Complete remissions were obtained in adult nonlymphocytic leukemia and acute lymphocytic leukemia of childhood. Greater than partial remissions have occurred in single instances of malignant melanoma (patient on treatment since July 1976 with continuing regression of metastases), acute myelomonocytic leukemia, IV-B Hodgkin's disease, male breast cancer, and non-Hodgkin's lymphoma.

Vindesine at the dose mentioned above has produced mild neutropenia with nadir and recovery time almost identical with Velban. The platelet-sparing effect seen with Oncovin has been observed also with vindesine and a few cases of elevation of platelet count in patients not initially thrombocytopenic were noted. Alopecia likewise has occurred in several patients; its exact incidence has not been determined.

Clinical neurotoxicity quantitatively different from that seen with Oncovin has occurred. Numbness of the fingertips has appeared in some patients. However, this has not progressed, as would be the case with Oncovin, even with continued weekly doses of vindesine. In our experience to date, neurotoxicity has not required that therapy be discontinued.[66]

Acknowledgment. We wish to thank Mr. John L. Occolowitz and Mr. James L. Gilliam for the mass spectral data; Dr. Douglas E. Dorman, Mr. Thomas K. Elzey, and Mr. Jonathan W. Paschal for NMR studies; Mr. George M. Maciak and Mrs. Mary Ann Bogan for microelemental analyses, titrations, and partition coefficient determinations;[64] Mr. Paul D. Vernon for the circular dichroism determinations; Mr. Raymond L. Hussey and Dr. David L. Sondack for material and product quality assay work; and Messrs. L. Kent Hoffman, Richard A. Bimm, and James E. Hines for the large-scale preparation and HPLC purification of VDS. For constant, dedicated attention to tumor assay work our appreciation is extended to Mrs.

Virginia Bothwell and to Messrs. Edward F. Priller, John L. Richardson, and Donald E. Dudley. Special thanks are due to Drs. Gordon H. Svoboda, Norbert Neuss, and Marvin Gorman for consent and advice. Support and guidance in biological matters given by Dr. Irving S. Johnson is gratefully acknowledged. The contribution of Drs. Sidney Ochs and Robert Worth, School of Medicine, Indiana University, in providing critical information on axoplasmic transport is acknowledged with gratitude. We are indebted to Dr. John A. Montgomery and Mr. W. Russell Laster, Jr., Southern Research Institute, for the P388 data; to Drs. Abraham Goldin and Randall K. Johnson, National Cancer Institute, for B16 melanoma data; and to Dr. Harry B. Wood, Jr., NCI, Drug Development Program, for complementary testing data. We also gladly acknowledge the help of Mrs. Wilma Bauer and Mr. Robert A. Conrad in the preparation of the manuscript.

References and Notes

(1) (a) Presented in part at the XIth International Cancer Congress, Florence, Italy, Oct 23, 1974, panel 14, p 137; (b) 9th International Congress of Chemotherapy, London, July 13–18, 1975, abstract SC-19; and (c) the 172nd National Meeting of the American Chemical Society, San Francisco, Calif., Aug 29–Sept 3, 1976, Abstracts, MEDI 81.
(2) Vindesine is the USAN council approved name for deacetylvinblastine amide. Other approved nonproprietary names cited are vinblastine and vincristine, supplied as Velban (vinblastine sulfate, Lilly) and Oncovin (vincristine sulfate, Lilly), respectively. In the biological or clinical use of these agents, the names or abbreviations denote the sulfate salts.
(3) N. Neuss, M. Gorman, W. Hargrove, N. J. Cone, K. Biemann, G. Büchi, and R. E. Manning, *J. Am. Chem. Soc.*, **86**, 1440 (1964).
(4) (a) R. C. DeConti and W. A. Creasey, "The Catharanthus Alkaloids", W. I. Taylor and N. R. Farnsworth, Ed., Marcel Dekker, New York, N.Y., 1975, pp 237–278; (b) I. S. Johnson, J. G. Armstrong, M. Gorman, and J. P. Burnett, Jr., *Cancer Res.*, **23** (8), 1390 (1963).
(5) V. T. DeVita, Jr., A. A. Serpick, and P. P. Carbone, *Ann. Intern. Med.*, **73**, 881 (1970); M. A. Goldsmith and S. K. Carter, *Cancer (Philadelphia)*, **33**, 1 (1974).
(6) (a) H. D. Weiss, M. D. Walker, and P. H. Wiernik, *N. Engl. J. Med.*, **291** (3), 127 (1974); (b) S. Rosenthal and S. Kaufman, *Ann. Intern. Med.*, **80**, 733 (1974); (c) H. Gröbe and D. Palm, *Monatsschr. Kinderheilkd.*, **120** (1), 23 (1972).
(7) (a) F. M. Schabel, W. R. Laster, and J. G. Mayo, *Prog. Chemother. (Antibacterial, Antiviral, Antineoplast.), Proc. Int. Congr. Chemother., 8th, 1973*, 3 (1974); (b) K. Sugiura and C. C. Stock, *Cancer*, **5**, 382 (1952).
(8) W. U. Gardner, T. S. Dougherty, and W. L. Williams, *Cancer Res.*, **4**, 73 (1944).
(9) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3** (2), 9 (1972).
(10) M. M. Lippman, W. R. Laster, B. J. Abbott, J. Venditti, and M. Baratta, *Cancer Res.*, **35**, 939 (1975).
(11) C. J. Barnett, W. W. Bromer, G. J. Cullinan, K. Gerzon, G. A. Poore, and M. J. Sweeney, unpublished results.
(12) W. W. Hargrove, *Lloydia*, **27** (4), 340 (1964).
(13) N. Neuss, M. Gorman, N. J. Cone, and L. L. Huckstep, *Tetrahedron Lett.*, 783 (1968).
(14) J. P. Kutney, D. E. Gregonis, R. Imhof, I. Itoh, E. Jahngen, A. I. Scott, and W. K. Chan, *J. Am. Chem. Soc.*, **97**, 5013 (1975).
(15) D. E. Dorman and J. W. Paschal, *Org. Magn. Reson.*, **8**, 413 (1976).
(16) C. Ainsworth, U.S. Patent 2 756 235 (July 24, 1956).
(17) (a) N. Neuss and M. Gorman, U.S. Patent 3 352 868 (Nov 14, 1967); (b) R. E. McMahon, *Experientia*, **19**, 434 (1963).
(18) (a) K. Biemann, *Lloydia*, **27**, 397 (1964); (b) P. Bommer, W. McMurray, and K. Biemann, *J. Am. Chem. Soc.*, **86**, 1439 (1964).
(19) Presented by M. A. Root, K. Gerzon, and R. W. Dyke at the Federation of Analytical Chemistry and Spectroscopy Societies Meeting, Indianapolis, Ind., Oct 6–10, 1975, Abstract 183.
(20) G. H. Svoboda, U.S. Patent 3 225 030 (Dec 21, 1965).
(21) M. D. Migahed and H. D. Beckey, *Int. J. Mass. Spectrom. Ion Phys.*, **7**, 1 (1971).
(22) (a) I. M. Jakovljevic, L. D. Seay, and R. W. Shaffer, *J. Pharm. Sci.*, **53**, 553 (1964); (b) N. J. Cone, R. Miller, and N. Neuss, *ibid.*, **52**, 688 (1963).
(23) R. L. Noble, C. T. Beer, and J. H. Cutts, *Biochem. Pharmacol.*, **1**, 347 (1958); *Ann. N.Y. Acad. Sci.*, **76**, 882 (1958).
(24) (a) I. S. Johnson, H. F. Wright, and G. H. Svoboda, *J. Lab. Clin. Med.*, **54**, 830 (1959); (b) I. S. Johnson, H. F. Wright, G. H. Svoboda, and J. Vlantis, *Cancer Res.*, **20**, 1016 (1960).
(25) G. H. Svoboda, *Lloydia*, **24**, 173 (1961).
(26) G. H. Svoboda, I. S. Johnson, M. Gorman, and N. Neuss, *J. Pharm. Sci.*, **51**, 707 (1962).
(27) K. Gerzon, J. E. Cochran, Jr., L. A. White, R. Monahan, E. V. Krumkalns, R. E. Scroggs, and J. Mills, *J. Med. Chem.*, **1**, 223 (1959).
(28) According to ref 26 above, Table VII, VCR is not active against the GLS carried in C_3H mice but recent experiments show this tumor to be inhibited quite effectively by this agent (Table I).
(29) M. J. Sweeney, G. A. Poore, G. J. Cullinan, and K. Gerzon, *Cancer Res.*, submitted for publication.
(30) J. C. Miller, G. E. Gutowski, G. A. Poore, and G. B. Boder, *J. Med. Chem.*, **20**, 409 (1977).
(31) Dr. Randall K. Johnson, personal communication to K.G. (June 24, 1975, and Dec 15, 1976).
(32) Dr. John A. Montgomery, personal communication to K.G. (March 21, 1974).
(33) Dr. Harry B. Wood, Jr., and Dr. Florence White, personal communication to K.G. (Sept 22, 1975).
(34) The comparison of deacetylvinblastine and VDS was done in a separate test (see experiment II, Table IV).
(35) M. K. Wolpert-Defilippes, R. H. Adamson, R. L. Cysyk, and D. G. Johns, *Biochem. Pharmacol.*, **24**, 751 (1975).
(36) Vindesine at 0.2 and at 0.25 mg/kg/day for 10 days iv inhibited spleen enlargement of mice (DBA/2) bearing the Friend leukemia virus to the extent of 80 and 90%, respectively, relative to that of untreated controls: Dr. D. C. DeLong, personal communication to K.G. (May 5, 1974). Methods used have been described in D. C. DeLong, L. A. Baker, N. R. Easton, and R. D. Dillard, *Proc. Soc. Exp. Biol. Med.*, **127**, 845 (1968).
(37) Techniques have been described: (a) I. S. Johnson, W. W. Hargrove, P. N. Harris, H. F. Wright, and G. B. Boder, *Cancer Res.*, **26**, 2431 (1966); (b) G. H. Svoboda, G. A. Poore, P. J. Simpson, and G. B. Boder, *J. Pharm. Sci.*, **55**, 758 (1966).
(38) (a) W. W. Bromer and J. W. Kirk, unpublished experiments; (b) R. J. Owellen, C. A. Hartke, R. M. Dickerson, and F. O. Hains, *Cancer Res.*, **36**, 1499 (1976).
(39) G. C. Todd, W. R. Gibson, and D. M. Morton, *J. Toxicol. Environ. Health*, 1, 843 (1976).
(40) S. Ochs, *Ann. N.Y. Acad. Sci.*, **228**, 202 (1974).
(41) S. Ochs and R. Worth, *Proc. Am. Assoc. Cancer Res.*, **16** (Abstract 278), 70 (1974).
(42) (a) S. Ochs, Abstract, 6th International Symposium for Pharmacology, Helsinki, Finland, July 20–25, 1975; (b) Proceedings of the 6th International Congress of Pharmacology, Vol. 2 (Neurotransmission), Finnish Pharmacology Society, Helsinki, 1975, pp 161–174.
(43) Glen C. Todd, unpublished observations.
(44) R. Nickander, unpublished observations.
(45) [^{3}H]Vindesine was provided by the Amersham/Searle Corp.
(46) H. W. Culp, W. D. Daniels, and R. E. McMahon, *Cancer Res.*, **37**, 3053 (1977).
(47) R. L. Nelson, M. A. Root, R. W. Dyke, and S. Ahmadzai, *Proc. Am. Assoc. Cancer Res.*, **17** (Abstract 118), 30 (1976).
(48) (a) R. J. Owellen and F. O. Hains, *Proc. Am. Assoc. Cancer Res.*, **17** (Abstract 406), 102 (1976); (b) R. J. Owellen, M. A. Root, and F. O. Hains, *Cancer Res.*, **37**, 2603 (1977).

(49) R. L. Nelson, R. W. Dyke, and M. A. Root, *Clin. Pharmacol. Ther.*, 21, 112 (1977).
(50) V. Currie, P. Wong, R. Tan, C. Tan, and I. Krakoff, *Proc. Am. Assoc. Cancer Res.*, 17 (Abstract 694), 174 (1976).
(51) G. P. Bodey and E. J. Freireich, *Proc. Am. Assoc. Cancer Res.*, 17 (Abstract 510), 128 (1976).
(52) R. H. Blum and D. M. Dawson, *Proc. Am. Assoc. Cancer Res.*, 17 (Abstract 429), 108 (1976).
(53) J. M. Venditti, "Pharmacological Basis of Cancer Chemotherapy", Williams and Wilkins, Baltimore, Md., 1975, p 245.
(54) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan, and U. C. Quarck, *J. Am. Chem. Soc.*, 78, 6396 (1956).
(55) L. Severi and C. Biancifiori, *J. Natl. Cancer Inst.*, 41, 331 (1968); B. Toth, *ibid.*, 42, 469 (1969).
(56) W. A. Creasey, A. I. Scott, C. C. Wei, J. Kutcher, A. Schwartz, and J. C. Marsh, *Cancer Res.*, 35, 1116 (1975); W. A. Creasey, personal communication to K.G. (Feb 7, 1977).
(57) R. J. Owellen, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 34, 808 (1975).
(58) R. J. Owellen, D. W. Donigian, C. A. Hartke, and F. O. Hains, *Biochem. Pharmacol.*, 26, 1213 (1977).
(59) R. L. Noble, C. T. Beer, and R. W. McIntyre, *Cancer*, 20, 885 (1967).
(60) N. Neuss and M. Gorman, U.S. Patent 3 352 868 (Nov 14, 1967).
(61) M. L. Shelanski and H. Wisniewski, *Arch. Neurol.*, 20, 199 (1969); R. J. Owellen, D. W. Donigian, C. A. Hartke, R. M. Dickerson, and M. J. Kuhar, *Cancer Res.*, 34, 3180 (1974); L. S. Green, J. A. Donoso, I. E. Heller-Bettinger, and F. E. Sampson, *Ann. Neurol.*, 1, 255 (1977).
(62) J. M. Ritchie and P. Greengard, *Annu. Rev. Pharmacol.*, 6, 405 (1966).
(63) (a) D. P. Rall and C. G. Zubrod, *Annu. Rev. Pharmacol.*, 2, 109 (1962); (b) S. I. Rapoport, "Blood–Brain Barrier in Physiology and Medicine", Raven Press, New York, N.Y., 1976, Chapter VI.
(64) A. Leo, C. Hansch, and D. Elkins [*Chem. Rev.*, 71, 613 (1971)] reported values of log P of 3.72 and 2.82 for VLB and VCR, respectively, where P is the partition coefficient between octanol and water. Experiments in our laboratories conducted at pH 7.4 have determined log P^* (apparent partition coefficient) values of 2.87, 2.15, and 2.62 for VLB, VCR, and VDS, respectively. Owellen recently reported[58] values for VLB (3.65), VCR (2.57), VDS (1.27), and deacetylvinblastine (2.76); log P 1.27 for VDS is a calculated value.
(65) Z. Iqbal and S. Ochs, unpublished experiments.
(66) R. W. Dyke and R. L. Nelson, *Cancer Treatment Rev.*, 4 (2), 135 (1977).

Nucleosides. 107. Synthesis of 5-(β-D-Arabinofuranosyl)isocytosine and Related *C*-Nucleosides[1]

C. K. Chu, U. Reichman, K. A. Watanabe,* and J. J. Fox

Laboratory of Organic Chemistry, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Scienes, Cornell University, New York, New York 10021. *Received July 6, 1977*

The synthesis of 5-(β-D-arabinofuranosyl)isocytosine (ψ-*ara*-isoC) (**7**), an isostere of the antileukemic agent, *ara*-C, was achieved. 5-(β-D-Ribofuranosyl)isocytosine (**4**, ψ-isocytidine, also an antileukemic agent) was converted into 4,2′-anhydro-5-(β-D-arabinofuranosyl)isocytosine (anhydro-ψ-*ara*-isoC) (**14**) by treatment with α-acetoxyisobutyryl chloride or *o*-acetoxybenzoyl chloride, followed by removal of the protecting groups. The anhydro nucleoside was hydrolyzed with 10% NaOH to give ψ-*ara*-isoC (**7**). Treatment of anhydro-ψ-*ara*-isoC with NH_3MeOH gave both α and β isomers of 2,4-diaminopyrimidine *C*-nucleosides (**18a,b**). A total synthesis of ψ-*ara*-isoC from 2,3,5-tri-*O*-benzyl-D-arabinofuranose (**8**) was attempted. The benzyl sugar **8** was converted by Wittig reaction with (ethoxycarbonylmethylene)triphenylphosphorane to ethyl 2-(tri-*O*-benzyl-D-arabinofuranosyl)acetate (**9**) which was formylated and then methylated to give 3-methoxy-2-arabinosylacrylate **10b**. Cyclization of the latter with guanidine followed by debenzylation with BCl_3–CH_2Cl_2 gave, however, the α isomer of ψ-*ara*-isoC as the sole isolable product. Treatment of ψ-uridine (**18**) with α-acetoxyisobutyryl chloride gave 4,2′-anhydro-5-(β-D-arabinofuranosyl)uracil (**19**, anhydro-ψ-*ara*-U) or 2′-chloro-2′-deoxy-ψ-uridine (**20**) depending upon the reaction conditions. 5-(β-D-Arabinofuranosyl)uracil (ψ-*ara*-U, **23**) and 5-(β-D-arabinofuranosyl)cytosine (ψ-*ara*-C, **24**) were also prepared from anhydro-ψ-*ara*-U (**22**). All the new *C*-nucleosides showed no significant inhibitory activity against leukemic cells in culture even though they are closely related structurally to the antileukemic agents, *ara*-C and ψ-isocytidine.

1-(β-D-Arabinofuranosyl)cytosine (**1**, *ara*-C) is a potent drug against acute myeloblastic leukemia.[2] This drug is converted in vivo into the 5′-triphosphate (*ara*-CTP) which is a strong inhibitor of mammalian DNA polymerase.[3] However, *ara*-C undergoes rapid deamination in vivo by cytidine deaminase to give an inactive metabolite, *ara*-U[4] (**2**). Leukemic cells develop resistance to *ara*-C by decreasing the activities of kinases[5] (which catalyze the phosphorylation of *ara*-C) or by increasing the deaminase activity.[6] Recently, it was found that 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine (**3a**, AAC)[7] or its 5-fluoro analogue (**3b**, AAFC)[8] are not substrates of deaminase but are slowly hydrolyzed under the physiological conditions giving rise to their respective arabino nucleosides (see Chart I).

5-(β-D-Ribofuranosyl)isocytosine (**4**, ψ-isocytidine),[9,10] an isostere of both cytidine (**5**) and 5-azacytidine (**6**), is active in vitro and in vivo against various *ara*-C resistant lines of mouse leukemia[11] and is currently undergoing phase I clinical trials. ψ-Isocytidine is not deaminated by cytidine deaminase from mouse kidney.[12] This report deals with the synthesis of *C*-nucleoside analogues and/or isosteres of *ara*-C and of ψ-isocytidine. A preliminary report of a portion of this work has appeared.[13]

Our first approach to the synthesis of 5-(β-D-arabinofuranosyl)isocytosine (**7**, ψ-*ara*-isoC) utilized 2,3,5-tri-*O*-benzyl-D-arabinose (**8**) which, on treatment with (ethoxycarbonylmethylene)triphenylphosphorane in acetonitrile, gave ethyl 2-(2,3,5-tri-*O*-benzyl-D-arabinofuranosyl)acetate (**9**) in good yield as a mixture of glycosyl isomers. The major isomer was isolated as an analytically pure liquid after chromatography on a silica gel column. The purified isomer **9** was formylated with ethyl formate in the presence of sodium hydride to afford the crude

0022-2623/78/1821-0096$01.00/0