troshock seizure test), sc Met (subcutaneous pentylenetetrazole seizure threshold test), and the rotorod test to evaluate neurotoxicity.

All tests were performed on male Carworth Farms No. 1 mice. All compounds were tested at three dosage levels (30, 100, and 300 mg/kg) at 30 min and 4 h after their intraperitoneal administration. Four animals are injected with each dose. Thirty minutes later, each animal is examined for toxicity in the rotorod test. Immediately thereafter, anticonvulsant activity is evaluated by subjecting one mouse to the MES test and another to the subcutaneous metrazole test. The same tests are repeated 4 h later on the two remaining mice. All compounds are solubilized in either 0.9% sodium chloride or 30% polyethylene glycol 400 and administered intraperitoneally in a volume of 0.01 ml/g. The ED_{50} and TD_{50} values and their confidence limits were determined by the method of Litchfield and Wilcoxin.[21] MES activity is defined as abolition of the hind limb tonic extensor component of the maximal electroshock seizure elicited in mice with a 60-Hz alternating current of 50 mA delivered for 0.2 s via corneal electrodes. sc Met activity is defined as failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s in duration).

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Alkaloids of *Vinca rosea* L. (*Catharanthus roseus* G. Don). 38. 4′-Dehydrated Derivatives[1]

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A series of 4′-dehydrated derivatives of various dimeric Vinca alkaloids has been synthesized to further define the structure–activity relationships of Vinca alkaloids with oncolytic potency. The concentrated sulfuric acid dehydration in most cases gave mixtures of the 3′,4′- and two isomeric 4′,20′-alkenes, which were isolated and characterized primarily by proton and ^{13}C NMR. Compounds tested for antitumor activity include the three dehydro isomers of 4-deacetylvinblastine, 4-deacetylvincristine, and 4-deacetylvinblastine-23-amide and some 4′-dehydrated derivatives epimeric at C-18′. Generally, the decrease in toxicity imparted by the new double bond was accompanied by a decrease in potency. An exception was 3′,4′-dehydro-4-deacetylvincristine, which showed a decrease in toxicity and increase in potency against at least one tumor in which vincristine itself has little effect.

Vinblastine (VLB, **1**)[2] and vincristine (VCR, **2**)[3] are dimeric indole alkaloids from *Catharanthus rosea* (*Vinca rosea*) used clinically in the treatment of various types of cancer. Despite their widely differing spectra of clinical activity and toxicities, VLB and VCR differ structurally only in the functional group on the dihydroindole nitrogen. Previous structure modification studies have shown that changes in the dihydroindole (vindoline) moiety have unpredictable results, causing either an increase or decrease in the oncolytic potency.[4,5] The effect of functional variation in the indole moiety (velbanamine) is evidenced in the activity of two other dimeric alkaloids isolated from *Catharanthus rosea*: leurosidine (**3**),[6] the 4′ epimer of VLB, and leurosine (**4**),[6] a 3′,4′-epoxide derivative. Although these compounds are somewhat lower in oncolytic potency as compared to VLB, their concomitant decrease in toxicity encouraged us to probe the effects of other functional changes in the velbanamine moiety. We have therefore prepared both the *exo*- and *endo*-4′-dehydro derivatives of several Vinca alkaloids. These serve to further define the structure–activity relationships at that position and provide intermediates to additional derivatives. Subsequent to the completion of our work, articles describing the synthesis of dehydrovinblastine have appeared[7,8] without reference to its biological activity. This paper reports the synthesis, isolation, and activities of a

Table I.[a] ^{1}H NMR Shifts Characteristic of Dehydro-4-deacetylvinblastine ($CDCl_3$, Me_4Si, δ = 0 ppm)

	Alkene	C-3′	C-20′	C-21′
9	Δ^3-DVLB	5.46		1.00 (t)
10	Δ^{4a}-DVLB		5.28	1.71 (d)
11	Δ^{4b}-DVLB		5.47	1.66 (d)

[a] Compounds are identified by structure number and also by trivial name where D = 4-deacetyl, DC = 18′-decarbomethoxy, Δ^3 = 3′,4′-dehydro, Δ^{4a} = 4′,20′-dehydro (e.g., 10), Δ^{4b} = 4′,20′-dehydro (e.g., 11), 18′-epi = 18′ epimer of that found in naturally occurring alkaloids.

Table II.[a] ^{1}H NMR Shifts Characteristic of C-18′ Stereochemistry ($CDCl_3$, Me_4Si, δ = 0 ppm)

		Compd	C-18′	C-22′	C-21
Natural	1	VLB		3.60	0.80
	15	DVLB		3.60	0.88
	9	Δ^3-DVLB		3.60	0.94
	10	Δ^{4a}-DVLB		3.60	0.91
	11	Δ^{4b}-DVLB		3.60	0.92
	16	Δ^3-VLB		3.55	0.81
	17	Δ^3-(DC)DVLB	5.5-6.0		0.81
	18	Δ^4-(DC)DVLB	5.6-6.0		0.85
	19	Vindoline			0.48
	20	D-vindoline			0.65
Epi	21	18′-Epi-Δ^3-DVLB[b]		3.75	0.73
	22	18′-Epi-Δ^3-VLB[b]		3.76	0.59
	23	18′-Epi-Δ^3-(DC)DVLB	4.57		0.63
	24	18′-Epi-Δ^4-(DC)DVLB	4.50		0.62

[a] See footnote a, Table I. [b] See ref 16.

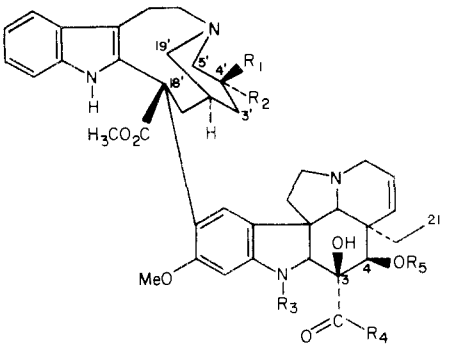
wide series of 4′-dehydro Vinca derivatives.

Chemistry. After extensive examination the process found to be most satisfactory for the conversion to olefins was a variation of that used by Büchi et al. in dehydrating 18′-decarbomethoxy-18′-cyano-3′,4′-dihydro-4′-hydroxycatharanthine (5).[9] The general reaction, accomplished by the addition of concentrated sulfuric acid to the dimeric alkaloid followed by careful neutralization, is sensitive to exacting conditions. Remarkably, this procedure is essentially without effect on the many functional groups of VLB, VCR, and other derivatives with the exception that deacylation occurs at the C-4 oxygen. This position may be reacetylated, however, using acetic anhydride as described by Hargrove.[4] These dehydro compounds are unstable, especially in solution, but increased stability is attained by conversion to the HCl or sulfate salts.

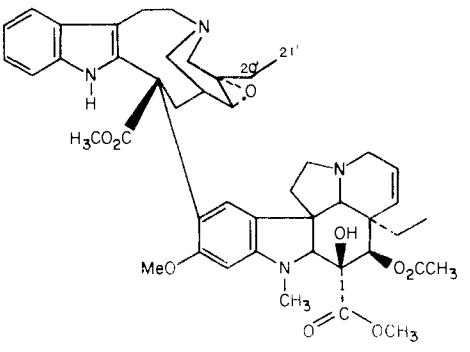
Dimeric alkaloids which have been dehydrated in this manner include VLB (1), VCR (2), leurosidine (3), 4-deacetylvinblastine-23-amide[5] (vindesine, VDS, 6), 18′-decarbomethoxyvinblastine (7), and 18′-epi-18′-decarbomethoxy-4-deacetylvinblastine (8). In most cases, three isomeric olefins, the 3′,4′-endo and the two 4′,20′-exo double bond derivatives (e.g., 9–11), are produced in an approximately 1:1:1 ratio. These isomers are readily identifiable by their ^{1}H NMR chemical shifts and coupling patterns of the vinylic C-3′ or C-20′ proton and the adjacent C-21′ methyl (see Table I).

Distinction between the cis- and trans-exo isomers becomes apparent from the shielding influence of the C-21′ methyl group on either C-20′ or C-3′ in the ^{13}C NMR spectrum. For example, the shielding effect of C-21′ causes the C-20′ to appear at δ 52.3 in the cis-exo isomer whereas the C-20′ appears at δ 60.5 in the trans-exo isomer. These assignments are based on correlation with the work of Dorman et al.[10]

In contrast to the formation of a mixture of three olefins in approximately equal amounts, 18′-decarbomethoxyvinblastine (7) and 18′-epi-18′-decarbomethoxy-4-deacetylvinblastine (8) are dehydrated to predominantly the endo double bond with only small amounts of exo compounds formed. This is analogous to the results of Büchi[9] and Kutney[11] who report a single analogous endo olefin in dehydrations of the monomers 5 and isovelbanamine (12).



	R_1	R_2	R_3	R_4	R_5
1	OH	Et	CH_3	OCH_3	Ac
2	OH	Et	CHO	OCH_3	Ac
3	Et	OH	CH_3	OCH_3	Ac
6	OH	Et	CH_3	NH_2	H
15	OH	Et	CH_3	OCH_3	H
28	OH	Et	CHO	OCH_3	H



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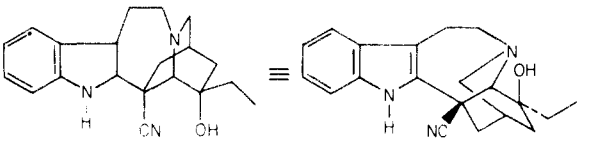

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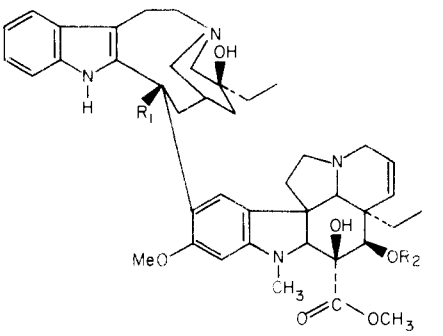
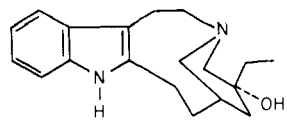


7, R_1 = β-H; R_2 = Ac
8, R_1 = α-H; R_2 = H
25, R_1 = β-H; R_2 = H

Previous papers concerned with the total synthesis of VLB have revealed coupling reactions of vindoline with derivatives of catharanthine (13) or cleavamine (14) which led to a 4′-dehydrovinblastine analogue.[7,8,12,13] In most cases, however, these syntheses result in stereochemistry at C-18′ inverted from that of VLB, whereas simple



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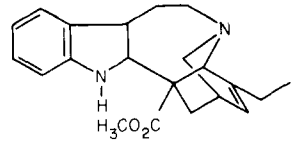

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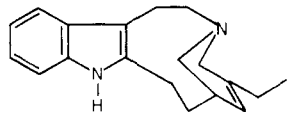

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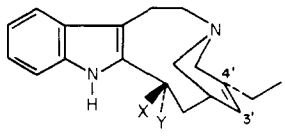
dehydration of the 4′-alcohol affords compounds which retain their original C-18′ stereochemistry. The proper identification of C-18′ stereochemistry is important in the evaluation of structure–activity data. Generally, the C-18′ stereochemistry like that found in nature is essential to retention of activity. C-18′ stereochemistry can be determined either by x-ray,[13] CD,[7,14] ^{13}C NMR,[15] or simple ^{1}H NMR spectra as indicated by this series of dehydro derivatives.

Table III.[a] Mitotic Accumulation of Chinese Hamster Ovary Cells[b]

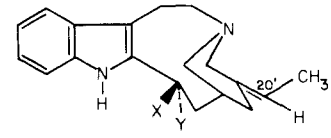
VLB Derivatives					
1 VLB	++	10^{-2}	26 Δ^{4a}-VLB	+	10^{1}
15 DVLB	++	10^{-3}	27 Δ^{4a}-3-Ac-VLB	++	10^{2}
25 (DC)-DVLB	–		17 Δ^{3}-18′-(DC)DVLB	+	10^{2}
9 Δ^{3}-DVLB	±	10^{-2}	18 Δ^{4}-18′-(DC)DVLB	±	10^{2}
10 Δ^{4a}-DVLB	±	10^{-2}	23 Δ^{3}-18′-Epi-18′-(DC)DVLB	–	
11 Δ^{4b}-DVLB	±	10^{-2}	24 Δ^{4}-18′-Epi-18′-(DC)DVLB	–	
VCR Derivatives			**VDS Derivatives**		
2 VCR	++	10^{-3}	6 VDS	++	10^{-3}
28 DVCR	±	10^{-3}	32 Δ^{3}-VDS	+	10^{-1}
29 Δ^{3}-DVCR	±	10^{-2}	33 Δ^{4a}-VDS	+	10^{-2}
30 Δ^{4a}-DVCR	+	10^{-1}	34 Δ^{4b}-VDS	+	10^{-3}
31 Δ^{4b}-DVCR	±	10^{-3} (++, 10^{-2})			

[a] See footnote *a*, Table I. [b] Minimal effective dose (μg/ml × 2) for accumulation in mitosis. Controls = 3.7% accumulation, – = 3-7%, ± = 7-10%, + = 10-15%, ++ = 15-40%, +++ = 40-50%.

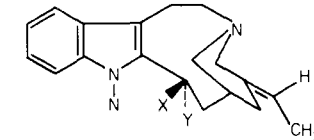
Dehydro Derivatives



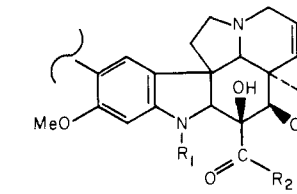
Δ^{3}	X	Y
9	CO_2CH_3	Z_1
16	CO_2CH_3	Z_2
17	H	Z_1
21	Z_1	CO_2CH_3
22	Z_2	CO_2CH_3
23	Z_1	H
29	CO_2CH_3	Z_3
32	CO_2CH_3	Z_4



Δ^{4a}	X	Y
10	CO_2CH_3	Z_1
18	H	Z_1[a]
24	Z_1	H[a]
26	CO_2CH_3	Z_2
27	CO_2CH_3	(3-$COCH_3$)-Z_2
30	CO_2CH_3	Z_3
33	CO_2CH_3	Z_4



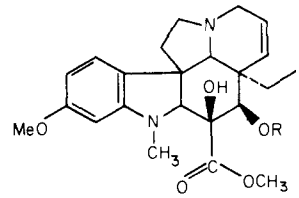
Δ^{4b}	X	Y
11	CO_2CH_3	Z_1
31	CO_2CH_3	Z_3
34	CO_2CH_3	Z_4



	R_1	R_2	R_3
Z_1	CH_3	OCH_3	H
Z_2	CH_3	OCH_3	$COCH_3$
Z_3	CHO	OCH_3	H
Z_4	CH_3	NH_2	H

[a] Stereochemistry at 20′ unknown.

The different configurations of the natural vs. unnatural C-18′ stereochemistry are reflected in distinctly different chemical shifts for the respective C-18′ substituent (carbomethoxy or hydrogen) and the vindoline C-21 methyl groups (Table II). This effect is explained by the use of models which show that the unnatural (epi) C-18′ stereochemistry prevents the folding of the velbanamine moiety over the vindoline portion as occurs in dimers of natural stereochemistry. Thus the C-21 methyl in dimers of unnatural C-18′ stereochemistry assumes the high-field shift characteristic of the unhindered C-21 methyl of the vindoline monomer. The effect of this folding in dimers



19, R = CO_2CH_3 (vindoline)
20, R = H

of natural stereochemistry on the C-18′ substituent is a downfield shift when the substituent is hydrogen and an upfield shift when it is carbomethoxy, relative to the unnatural C-18′ epimers (Table II).

Results

The Vinca alkaloids VLB and VCR are known to bind to tubulin, thus inhibiting mitosis and preventing cell division. Extensive structure–activity relationship studies with compounds 1–4, 6–8, and various other derivatives have suggested a positive correlation between the mitotic inhibition observed in Chinese hamster ovary (CHO) cells in vitro, with antitumor activity against P-1534(PJ) solid tumors in mice.[17] Therefore, mitotic arrest in cultured cells when dosed with the Vinca alkaloids may suggest potential oncolytic activity. The data from the dehydro derivatives in this assay (Table III) were used to select compounds for in vivo testing.

Assessment of in vivo antitumor activity was determined by measuring percentage inhibition of growth in solid tumors [Gardner lymphosarcoma, 755 adenocarcinoma, and the P-1534(J) leukemia] implanted subcutaneously as well as percentage prolongation of life in the B-16 melanoma following daily ip treatment with the drug. Results are given in Table IV for several dehydro derivatives against the Gardner lymphosarcoma. Of these, two dehydro Vinca dimers, **29** (Δ^{3}-DVCR) and **32** (Δ^{3}-VDS), which responded well in the GLS, were further evaluated in the other tumor systems.

Biological evaluation of this series of 15 4′-dehydrated Vinca derivatives has shown that a Δ^{3} or $\Delta^{4,20}$ double bond generally imparts lower toxicity and lower potency than

Table IV.[a] Percent Inhibition of Tumor Growth in the Gardner Lymphosarcoma in Vivo[b]

		Dose																	
	Compd	0.1	0.15	0.2	0.225	0.25	0.28	0.3	0.4	0.45	0.5	0.6	0.75	1.0	1.6	2.0	2.8	3.0	4.0	6.0
15	DVLB				28^{0}			84^{4}	91^{2}		100^{4}									
9	Δ^{3}-DVLB				0^{0}		12^{2}				15^{0}			9^{0}	26^{2}	22^{4}	Tx			
10	Δ^{4a}-DVLB				0^{0}					2^{0}					4^{2}		16^{0}	14^{0}	29^{0}	29^{0}
11	Δ^{4b}-DVLB				0^{0}		0^{0}								2^{0}		13^{0}		16^{4}	28^{0}
2	VCR	100^{0}	90^{0}	100^{2}		Tx		Tx												
29	Δ^{3}-DVCR					93^{2}		85^{2}	100^{4}	Tx										
30	Δ^{4a}-DVCR	2^{0}	8^{0}	11^{2}				19^{0}												
31	Δ^{4b}-DVCR	30^{0}	19^{0}	26^{2}				64^{2}												
6	VDS			100^{0}																
32	Δ^{3}-VDS								23^{2}		Tx	Tx	33^{1}	Tx						
33	Δ^{4a}-VDS								27^{4}	Tx	Tx	12^{2}								
34	Δ^{4b}-VDS								12^{2}	11^{0}	10^{0}	44^{4}								

[a] See footnote a, Table I. [b] Dose in mg/kg × 8-10 days; superscript indicates number of toxic deaths out of ten at conclusion of test at day 10, Tx = number of deaths ≥6; deaths in untreated controls were 3/35. These results are a combination of two tests run at various levels.

Table V.[a] Percent Inhibition of Tumor Growth in the P-1534(J) Leukemia in Vivo[b]

		Dose									
	Compd	0.2	0.25	0.3	0.35	0.4	0.6	0.8	1.0	1.25	1.5
29	Δ^{3}-DVCR	30^{1}	37^{2}	51^{0}		81^{3}					
32	Δ^{3}-VDS						7^{1}	13^{0}	19^{0}		
2	VCR	86^{5}	77^{2}								
1	VLB				54^{3}	76^{5}					
6	VDS	74^{0}	100^{2}	Tx							

[a] See footnote a, Table I. [b] Dose in mg/kg × 8-10 days; superscript indicates number of toxic deaths out of ten at conclusion of test at day 10, Tx = number of deaths ≥6; deaths in untreated controls were 4/30.

Table VI.[a] Percent Inhibition of Tumor Growth in the 755 Adenocarcinoma in Vivo[b]

		Dose									
	Compd	0.2	0.25	0.3	0.35	0.4	0.6	0.8	1.0	1.25	1.5
29	Δ^{3}-DVCR	0^{0}	14^{0}	28^{1}	17^{1}	18^{0}	Tx		Tx	Tx	
32	Δ^{3}-VDS						8^{0}	4^{3}	61^{5}		Tx
2	VCR	70^{0}	34^{0}								
1	VLB					37^{0}	31^{2}				
6	VDS	52^{0}	68^{2}	80^{3}							

[a] See footnote a, Table I. [b] Dose in mg/kg × 8-10 days; superscript indicates number of toxic deaths out of ten at conclusion of test at day 10, Tx = number of deaths ≥6; deaths in untreated controls were 6/60. These results are a combination of three tests run at various levels.

Table VII.[a] Percent Prolongation of Life in the B-16 Melanoma[b]

		Dose									
	Compd	0.2	0.25	0.3	0.35	0.4	0.6	0.8	1.0	1.25	1.5
29	Δ^{3}-DVCR	$^{0}79^{0}$	$^{1}116^{0}$	$^{0}133^{4}$		$^{1}112^{3}$					
32	Δ^{3}-VDS						$^{0}91^{0}$	$^{0}113^{1}$	$^{0}67^{2}$		
2	VCR	Tx	$^{1}49^{0}$								
1	VLB				$^{0}122^{3}$	$^{1}100^{1}$					
6	VDS	$^{2}132^{1}$	$^{3}66^{3}$	Tx							

[a] See footnote a, Table I. [b] Dose in mg/kg × 10 days; left superscript indicates number of toxic deaths; right superscript indicates number of survivors out of ten at day 60; Tx = number of survivors ≤4; controls life-span = 18.8 days.

found with the 4′-hydroxy analogues. In most cases, the decrease in potency makes these new derivatives less attractive as potential human antitumor drugs, in spite of the lower toxicity. However, one derivative, **29** (Δ^{3}-DVCR), appears to have a therapeutic effect paralleling that of VCR although requiring a two- to fourfold increase in dosage in the animal tumor systems examined. A significant improvement over that of VCR was seen in the response with Δ^{3}-DVCR in the B-16 melanoma, causing an increase in life-span and in the number of indefinite survivors which equaled or surpassed that seen with the two Vincas (VLB and VDS) most active in this system. Therefore, the combination of the 3′,4′ double bond and 4-deacetylation in VCR imparts both a decrease in toxicity and an increase in potency against at least one tumor in which VCR itself has little effect. Although the correlation of B-16 activity with clinical activity in solid tumors is reputed to be good, further comparison of **29** with other agents against other systems would be necessary before it could be recommended for clinical evaluation.

Experimental Section

^{1}H NMR 100-MHz spectra were recorded in $CDCl_3$ solution on a Varian Associates HA-100 instrument. Chemical shifts are recorded in parts per million (δ) relative to Me_4Si as internal standard. Mass spectra (MS) were obtained on a Varian MAT Model 731 double-focusing spectrometer.

Anhydro-4-deacetylvinblastine. The following procedure was used for all examples. Excess cold concentrated H_2SO_4 (6.34 g, 64.7 mmol, 50 equiv) was added to vinblastine sulfate (1.04 g, 1.3 mmol). Addition and subsequent stirring at room temperature

were carried out over a 30-min period, after which 70 ml of dry MeOH was added, followed by addition of solid Na_2CO_3 (20.36 g, 192.2 mmol). The mixture was stirred for 30 min. Saturated aqueous NaCl (250 ml) was added. This mixture was diluted to 500 ml and was stirred for 30 min. The suspension was then extracted four times with benzene. Removal of the solvent under vacuum left a mixture of three isomeric dehydro-4-deacetylvinblastine compounds. Separation by preparative TLC on silica gel with MeOH gave **9** (Δ^3-DVLB, 118.4 mg, 14% of theoretical yield), **10** (Δ^{4a}-DVLB, 101.3 mg, 12%), and **11** (Δ^{4b}-DVLB, 126.2 mg, 15%). Homogeneity of each isomer was shown by repeated TLC.[18]

9: NMR 0.94 and 0.99 (6 H, 2 t, C_{21}-H's and $C_{21'}$-H's), 3.75 (3 H, s, NCH_3), 3.60 (3 H, s, $C_{18'}$-CO_2CH_3), 3.81 (3 H, s, C_3-CO_2CH_3), 3.85 (3 H, s, CH_3OAr), 4.10 (1 H, m, C_4-H), 5.46 (1 H, m, $C_{3'}$-H), 5.80 (2 H, m, C_6-H, C_7-H), 6.11 (1 H, s, C_{17}-H), 6.61 (1 H, s, C_{14}-H), 7.15 and 7.5 (4 H, m, $C_{11'}$-$C_{14'}$-H's), 8.0 (1 H, br, NH), 9.4 (1 H, br, C_3-OH); MS 778, 764, (transmethylation peaks[19]) M^+ 750, 570, 427, 240, 188, 136, 135, 122, 121, 107, 106.

10: NMR 0.91 (3 H, t, C_{21}-H's), 1.72 (3 H, d, $C_{21'}$-H's), 2.75 (3 H, s, NCH_3), 3.60 (3 H, s, $C_{18'}$-CO_2CH_3), 3.79 (3 H, s, C_3-CO_2CH_3), 3.84 (3 H, s, CH_3OAr), 4.08 (1 H, m, C_4-H), 5.28 (1 H, m, $C_{20'}$-H), 5.80 (2 H, m, C_6-H, C_7-H), 6.08 (1 H, s, C_{17}-H), 6.55 (1 H, s, C_{14}-H), 7.14 and 7.48 (4 H, m, $C_{11'}$-$C_{14'}$-H's), 7.99 (1 H, br, NH), 9.4 (1 H, br, C_3-OH); MS 778, 764, (transmethylation peaks[19]) M^+ 750, 691, 633, 553, 525, 427, 337, 336, 240, 171, 167, 149, 136, 135, 122, 121, 107, 106.

11: NMR 0.92 (3 H, t, C_{21}-H's), 1.66 (3 H, d, $C_{21'}$-H's), 2.76 (3 H, s, NCH_3), 3.60 (3 H, s, $C_{18'}$-CO_2CH_3), 3.81 (3 H, s, C_3-CO_2CH_3), 3.85 (3 H, s, CH_3OAr), 4.09 (1 H, m, C_4-H), 5.47 (1 H, m, $C_{20'}$-H), 5.80 (2 H, m, C_6-H, C_7-H), 6.10 (1 H, s, C_{17}-H), 6.57 (1 H, s, C_{14}-H), 7.04 and 7.5 (4 H, m, $C_{11'}$-$C_{14'}$-H's), 7.96 (1 H, br, NH), 9.47 (1 H, br, C_3-OH); MS 778, 764, (transmethylation peaks[19]) M^+ 750, 691, 633, 620, 553, 525, 427, 337, 336, 240, 149, 136, 135, 122.

Biological Testing. Percent accumulation in mitosis for in vitro assays was determined after 6 h of incubation of CHO cells with a solution of the alkaloid. Dose ranging studies determined the minimum concentration of compound necessary to produce mitotic arrest greater than controls (Table III).

The Gardner lymphosarcoma (GLS) is a rapidly growing solid tumor which is sensitive to VCR, VDS, and 4-deacetylvinblastine. It is implanted by trocar using tumor fragments from a normal donor mouse (C_3H). Treatment is initiated 24 h after implantation and consists of ten daily consecutive doses. On the day following the final treatment, tumors in the treated and in the untreated control groups are measured by vernier caliper in two dimensions and are recorded along with animal weights. Percent inhibition (Table IV) is determined by T/C values of mean tumor diameter.

The P-1534(J) is a lymphocytic leukemia maintained and used for testing in a solid form by subcutaneous implantation in the DBA/2 mouse. This tumor was originally obtained from Jackson Memorial Laboratories, Bar Harbor, Me. Treatment and evaluation follow the same protocol as described for the GLS. This tumor responds well to VCR, VLB, and VDS.

The 755 tumor is an adenocarcinoma with a medium growth rate which responds to VCR and VLB. Implantation in the C-57BL/6 mouse, treatment, and evaluation are done as for the GLS.

The B-16 melanoma, obtained from Jackson Memorial Laboratories, is maintained in solid form in the C-57BL/6 mouse and tests are carried out in this strain or in the BDF_1. The tumor is palpable from 7 to 10 days and usually kills the host in 18–25 days after metastasis to the lungs, liver, and spleen. Tumors from donor mice are homogenized in the presence of Hanks balanced salt solution and the tumor brei is inoculated ip using 0.5 ml/mouse. Treatment is by ip route for ten daily doses. Evaluation is comprised of determining the day of death and comparing average prolongation of life-span of treated animals vs. average life-span of untreated controls [(T − C)/C × 100]. Indefinite survivors, animals alive at the end of the test (day 60 after inoculation), are listed separately. The B-16 responds well to VLB and VDS.

Evaluation of **29** (Δ^3-DVCR) and **32** (Δ^3-VDS) in the P-1534(J), 755, and B-16 systems is reported in Tables V–VII, respectively.

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