1160 cm^{-1}; MS, *m/e* 461. Anal. ($C_{21}H_{30}N_6O_4S$) C, H, N, S.

Pharmacology. Receptor Binding. N^6-[^{3}H]Cyclohexyladenosine binding[5b] in rat brain was performed with use of triplicate incubations for 60 min at 25 °C in 2 mL of 50 mM Tris-HCl buffer (pH 7.7) with 20 mg wet weight of rat brain membranes (whole brain minus brainstem and cerebellum), 1 nM N^6-[^{3}H]cyclohexyladenosine (30 Ci/mmol), and 0.1 unit/mL of adenosine deaminase.

In Vitro Adenosine Antagonism. Two rate hearts were isolated and perfused in parallel by the Langendorff method[10] at physiological temperature, pH, and pressure. After 30 min, (*R*)-PIA (1.5×10^{-8} M) was introduced into the oxygenating reservoir, decreasing heart rate and increasing coronary flow (40–50% each). After stabilization, the drug was administered at increasing log doses, and the effect on heart rate and coronary flow was measured. Reversal of (*R*)-PIA-induced heart rate and coronary flow effects are interpreted as receptor antagonism. For each drug, the experiment was run in triplicate, thus giving a total of six observations at each dose. The results are shown in Figure 2.

Data Processing. Correlations, regressions, and factor analyses were run on an IBM 3081 machine using the SAS program package.[26] In eq 1–5, the figures in parentheses are the standard errors of the regression coefficinets. For a given equation, *n* is the number of compounds, *r* is the correlation coefficient, *F* is a significance test, and *s* is the standard error of the estimate.

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Registry No. 1, 75922-48-4; 2, 2850-37-5; 3, 961-45-5; 4, 93214-97-2; 5, 85872-53-3; 6, 85884-03-3; 7, 78164-01-9; 8, 85872-56-6; 9, 18830-58-5; 10, 93215-02-2; 11, 93215-01-1; 12, 96445-28-2; 13, 85872-60-2; 14, 93214-98-3; 15, 93215-00-0; 16, 85872-51-1; 17, 85884-04-4; 18, 78146-60-8; 19, 85872-55-5; 20, 85872-57-7; 21, 973-69-3; 22, 85872-59-9; 23, 85872-63-5; 24, 93214-92-7; 25, 78146-61-9; 26, 85872-54-4; 27, 85872-61-3; 28, 85872-52-2; 29, 85872-67-9; 30, 93214-82-5; 31, 85872-65-7; 32, 78146-59-5; 33, 85872-64-6; 34, 85872-68-0; 35, 93214-90-5; 36, 93214-85-8; 37, 54013-58-0; 38, 93214-89-2; 39, 93214-91-6; 40, 63325-99-5; 41, 93214-86-9; 42, 57196-70-0; 43, 29064-02-6; 44, 85872-58-8; 45, 93214-87-0; 46, 93214-88-1; 47, 57281-09-1; 48, 93214-84-7; 49, 54013-59-1; 50, 85872-66-8; 51, 1094-63-9; 52, 93214-99-4; 53, 967-42-0; 54, 93214-83-6; 55, 85872-69-1; 56, 93215-04-4; 57, 80206-91-3; 58, 96445-29-3; 59, 89073-61-0; 60, 89073-47-2; 61, 89073-54-1; 62, 96445-30-6; 63, 96445-31-7; 64, 89073-49-4; 65, 96445-38-4; 65·HCl, 89073-51-8; 66, 89073-53-0; 67, 89073-55-2; 68, 89073-52-9; 69, 96445-32-8; 70, 96445-39-5; 70·HCl, 96445-33-9; 71, 96445-34-0; 72, 89073-57-4; 73, 89073-58-5; 74, 96445-35-1; 75, 96445-40-8; 75·HCl, 96445-36-2; 76, 96445-37-3; IV ($R_1 = R_3 = (CH_2)_2CH_3$), 81250-34-2; IX ($R_1 = R_3 = (CH_2)_2CH_3$, 4-SO_3H), 89073-57-4; 4-$HO_2CC_6H_4SO_3H$·K, 5399-63-3; $H_2N(CH_2)_2N(CH_3)_2$, 108-00-9.

Supplementary Material Available: Values for the 28 parameters used as input to the factor analysis (5 pages). Ordering information is given on any current masthead page.

Vinblastin-23-oyl Amino Acid Derivatives: Chemistry, Physicochemical Data, Toxicity, and Antitumor Activities against P388 and L1210 Leukemias

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The dimeric alkaloids vinblastine (VLB) and vincristine (VCR) differ structurally only in the functional group on the dihydroindole nitrogen. The semisynthetic derivative vindesine (VDS) differs slightly from VLB by having an amide group instead of an ester group. However, these minor distinctions are responsible for profound differences in the oncolytic spectrum, potency, and toxicity of these compounds. Vinblastin-23-oyl amino acid derivatives were synthesized by linking amino acid carboxylic esters to the vinblastin-23-oyl moiety through an amide linkage. Studies were extended to explore the influence of the nature of the amino acid, the ester alkyl chain lengths, the stereoisomerism of the amino acid, or the reacetylation of the hydroxyl group (position O-4) of the vindoline moiety. The present study deals with the synthesis of 21 vinblastin-23-oyl amino acid derivatives, some of their physicochemical data, the acute toxicity in mice, and therapeutic activities of these derivatives against the P388 and L1210 leukemias in comparison with VDS, VBL, and VCR.

The antitumor alkaloids vinblastine (VLB) and vincristine (VCR)[1] are extracted from the periwinkle plant *Catharanthus roseus* G. Don.[2] They possess a basic structure comprising an indole and a dihydroindole nucleus linked together.

Mitotic arrest and cytotoxicity are the principal biological actions cited.[3] Furthermore, their antitumor activities and toxic side effects are clearly related to certain structural features of the alkaloids. VLB differs in molecular structure from VCR in that it contains a methyl group instead of a formyl group and this minor structural difference leads to a different antitumor spectrum, potency, and toxicity.[4] Deacetylvinblastine amide (VDS)[4] is a semisynthetic derivative of VLB and it differs slightly from VLB by having an amide group in place of the ester

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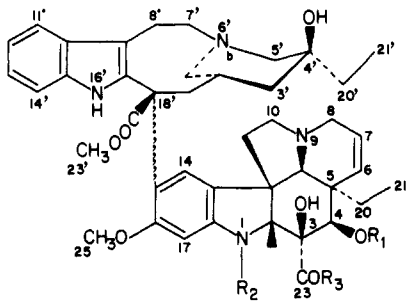
Chart I



	R_1	R_2	R_3
VBL	$COCH_3$	CH_3	OCH_3
VCR	$COCH_3$	CHO	OCH_3
VDS	H	CH_3	NH_2
Va.a.E	H	CH_3	$NHCHR^5(COOR^4)$

group at position C-23 (Chart I).

VLB is useful in the treatment of various malignancies, such as Hodgkin's disease, and VCR has shown effectiveness in acute lymphoblastic leukemia.[4] Use of the former is limited by leucopenia and the latter by neurotoxicity.[4]

Oncolytic activity of these dimeric alkaloids has been related to the mitotic spindle poisoning resulting in mitotic arrest.[5] These alkaloids also change the rate of axoplasmic transport[6] by producing an alteration in neurotubules.[7,8] Cytotoxicity and peripheral neuropathy may be the result of the primary mode of action of these alkaloids by binding to tubuline.[9]

Vinblastin-23-oyl amino acid derivatives were synthesized by linking amino acid carboxylic esters to the vinblastin-23-oyl moiety through an amide linkage (Chart I) (V.a.a.E.). A few representative amino acids were chosen from the four main classes on the basis of the polarity of their side chains. Further, the influence of structural alterations such as (1) the presence of a carrier amino acid at the C-23-oyl moiety, (2) the nature of the amino acid carboxylic ester, (3) the alkyl chain length of the amino acid, (4) the stereoisomerism of the amino acid, and (5) the reacetylation of the hydroxyl group (position O-4) of the vindoline moiety on the therapeutic activity are explored and discussed in comparison with VDS, VLB, and VCR.

Deacetylvinblastine acid azide (**5**) was readily prepared[4] from VLB by hydrazinolysis of the ester group of the dihydroindole moiety and deacetylation followed by nitrosation of the resulting deacetylvinblastine monohydrazide (**4**).

Deacetylvinblastine azide (**5**) reacts with amino esters in dichloromethane at room temperature to give good yields of compounds of general structure **6** (Scheme I).

Several amino acid derivatives (Chart II) were obtained in good yields and readily purified by column chromatography. The free bases were characterized.

To explore the relation existing between chemotherapeutic activity and the amino acid derivatives linked to vinblastine, the following modified amino acid carboxylic

Scheme I

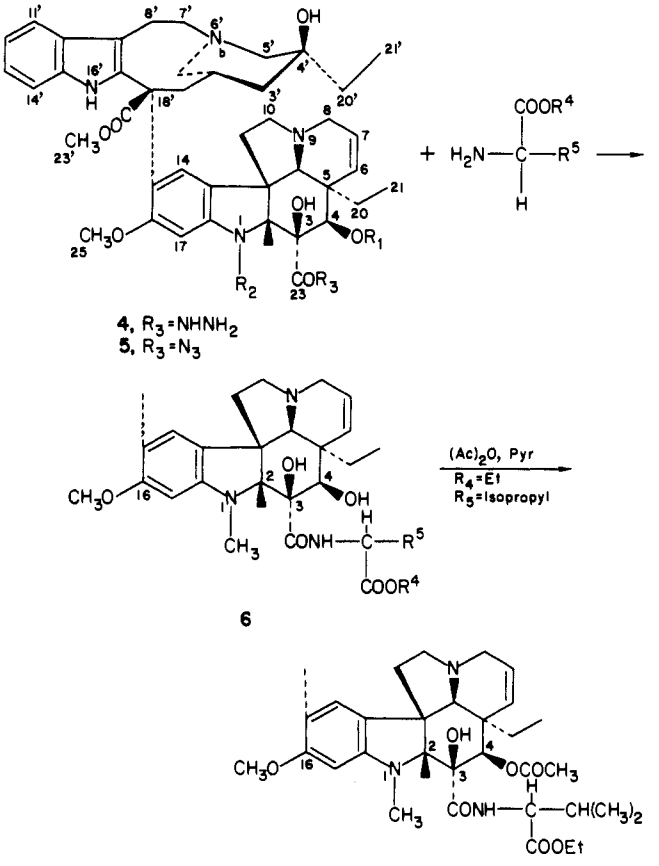
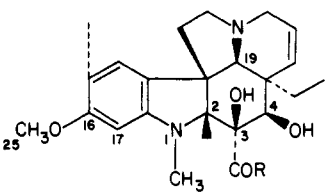


Chart II



no.	COR	no.	COR
8	CO-L-Leu-OMe	18	CO-L-Trp-O-*n*-butyl
9	CO-L-Leu-OEt	19	CO-D-Trp-OEt
10	CO-D-Leu-OEt	20	CO-L-Ala-OEt
11	CO-L-Leu-OBu	21	CO-L-Val-OEt
12	CO-L-Leu-O-octyl	22	CO-L-Phe-OEt
13	CO-L-Leu-amide	23	CO-L-Ser-OEt
14	CO-L-Ile-OMe	24	CO-L-Tyr-OEt
15	CO-L-Ile-OEt	25	CO-εTFA-Lys-OEt
16	CO-L-Trp-OMe	26	CO-Glu-OEt
17	CO-L-Trp-OEt	27	CO-Val-Trp-OEt

derivatives were compared to their respective methyl esters: L-leucine ethyl ester (**9**), L-isoleucine ethyl ester (**15**), and L-tryptophan ethyl ester (**17**). L-Leucine *n*-butyl ester (**11**), L-leucine octyl ester (**12**), and L-Trp *n*-butyl ester (**18**) were synthesized for their long alkyl chains. L-Leucine amide (**13**) was coupled to test the role of amide groups. Also, D-leucine ethyl ester (**10**) and D-tryptophan ethyl ester (**18**) were chosen to study the influence of stereoisomerism of the amino acid.

Experimental Section

Vinblastine sulfate was obtained from OMNICHEM, Louvain-la-Neuve, Belgium.

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Optical rotations were obtained (g/100 mL, $CHCl_3$ solution) on a LTD polarimeter, infrared spectra were recorded (ν cm^{-1}, $CHCl_3$ or KBr) on a Perkin-Elmer 197 instru-

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ment, and ultraviolet spectra were obtained (CH_3OH, max, nm, log ϵ) on a Perkin-Elmer, Coleman 1240 instrument.

^1H NMR spectra were recorded ($CDCl_3$, Me_4Si, $\delta = 0$ ppm) on Varian T 60 and Brucker WH 360 spectrometers (s, d, t, dd, m, ind indicate singlet, doublet, triplet, doublet of doublets, multiplet, and indole, respectively). Mass spectra were obtained by desorption chemical ionization on a Varian Mat-411 instrument.

TLC was performed on Merck precoated silica gel F-254 plates (5 × 20, 20 × 20 cm). TLC solvent systems used were the following: (A) ethyl acetate–ethanol (3:1), (B) ethyl acetate–ethanol (1:1), (C) chloroform–diethylamine (20:1), (D) 2-propanol–ethyl acetate–cyclohexane (40:20:40), (E) ether–methanol saturated with ammonia gas (96:4), (F) ether–methanol saturated with ammonia gas (86:14). The presence of alkaloid materials was detected by fluorescence under shortwave UV light and by spraying with ceric ammonium sulfate reagent. Analytical grade amino acids and their respective esters were purchased from Serva Fine Biochemicals, Heidelberg.

Reagents and solvents were recrystallized or distilled prior to use.

The chemotherapeutic activities were evaluated on the experimental L1210 and P388 leukemias. Murine L1210 leukemic cells were given by Drs. C. Gosse and J. Morizet at Villejuif (France). The leukemic cells are maintained in our laboratory by intraperitoneal (ip) inoculation into DBA_2 mice (Charles River, St. Aubin-lez-Elbeuf, France). For the chemotherapeutic trials, the leukemic cells were inoculated (iv or ip) into DBA_2 (10^4 or 10^5 iv) or BDF_1 (10^6 ip) female mice on day 0. The drugs, as sulfate salts, were dissolved in physiological saline solutions and injected intravenously (iv) or intraperitoneally following different schedules. The results are expressed in terms of (a) increase in lifespan (ILS) relative to that of animals receiving no therapy and (b) survivors up to 60 days.

Acute intravenous toxicity studies of the derivatives **15** and **16** were carried out on female mice (average weight 20–24 g) of the strains NMRI (Iffa, Credo, St. Germain-sur-Arbresle, France) and Swiss and CD_1 (Charles River, France). The doses were logarithmically spaced. Following careful observation for 14 days, the LD_{50} was calculated by use of a linear regression analysis.

Deacetylvinblastine Monohydrazide[4] (4). Vinblastine (0.876 g, 1.08 mmol) was added to a mixture of 5 mL of anhydrous hydrazine and 5 mL of anhydrous ethanol. The solution was stirred under an argon atmosphere at 60 °C for 24 h.

The cooled reaction mixture was poured into 50 mL of deionized water and extracted with 5 × 50 mL of CH_2Cl_2. The extracts were washed successively with 50 mL of water and 100 mL of saturated NaCl solution and then dried over Na_2SO_4. After evaporation of the solvent, compound **4** was isolated (0.793 g, 94%): $[\alpha]_D$ +37° (c 0.1, MeOH); NMR δ 8.04 (1 H, s, NH ind), 8.25 (1 H, s, $CONHNH_2$), 9.5 (1 H, br s, C^3-OH).

Deacetylvinblastine Acid Azide[4] (5). Deacetylvinblastine monohydrazide (**4**; 1.0 g, 1.3 mmol) was dissolved in a mixture of 23 mL of methanol and 74 mL of 1 N HCl. The solution was cooled to −10 °C and then $NaNO_2$ (207 mg, 3 mmol) was added at once with stirring. After 10 min the pH of the brownish-red solution was adjusted to 8.8 with cold, saturated $NaHCO_3$ solution. Deacetylvinblastine acid azide (**5**) was extracted rapidly with 4 × 50 mL of CH_2Cl_2 and washed with a saturated NaCl solution. The extracts were dried over Na_2SO_4 concentrated to a volume of 40 mL and characterized by IR spectroscopy (2135 cm^{-1}, CON_3).

General Reaction of Deacetylvinblastine Acid Azide (5) and Amino Esters. A solution of equimolar quantities of deacetylvinblastine acid azide (**5**; ca. 2 mmol) and the amino ester (base) in 40 mL of CH_2Cl_2 was stirred at 4 °C for 60 h. The solvent was evaporated. The residue was chromatographed on a silica column eluting with the following solvent system: Et_2O–MeOH saturated with NH_3 (from 96:4 to 85:15). The appropriate fractions are combined to yield the coupled derivative.

Compound 8 from 5 and L-Leu-OMe: yield 68%; mp 172 °C; $[\alpha]_D$ +67° (c 0.27, $CHCl_3$); IR 1740 (ester), 1680 (amide) cm^{-1}; MS, 910 (25, $M^+ + 28$), 896 (78, $M^+ + 14$), 883 (26, $M^+ + 1$), 882 (36, M^+); NMR δ 3.83 (3 H, s, OCH_3), 3.80 (3 H, s, $COOCH_3$), 3.63 (3 H, s, OCH_3), 2.8 (3 H, s, NCH_3), 0.96 (12 H, m, $C^{21'}$-H_3 + isopropyl).

Compound 9 from 5 and L-Leu-OEt: yield 49%; mp 169 °C; $[\alpha]_D$ +60° (c 0.35, $CHCl_3$); IR 1735 (ester), 1665 (amide) cm^{-1}; MS, 924 (6, $M^+ + 28$), 910 (56, $M^+ + 14$), 897 (62, $M^+ + 1$), 896 (100, M^+); NMR (360 MHz) δ 4.2 (2 H, q, CH_2 ester), 3.77 (3 H, s, $COOCH_3$), 3.66 (3 H, s, OCH_3), 2.77 (3 H, s, NCH_3), 0.92 (12 H, m).

Compound 10 from 5 and D-Leu-OEt: yield 74%; mp 181 °C; $[\alpha]_D$ +70° (c 0.28, $CHCl_3$); IR 1735 (ester), 1665 (amide) cm^{-1}; MS, 924 (14, $M^+ + 28$), 910 (32, $M^+ + 14$), 897 (38, $M^+ + 1$), 896 (66, M^+); NMR δ 4.26 (2 H, q, $COOCH_2$), 3.83 (3 H, s, OCH_3), 3.66 (3 H, s, OCH_3), 2.90 (3 H, s, N^1-CH_3), 1.3 (3 H, t, $COOCH_2CH_3$), 0.96 (12 H, m, $C^{21'}$-H_3 + C^{21}-H_3 + isopropyl).

Compound 11 from 5 and L-Leu-OBu: yield 58%; mp 158 °C; $[\alpha]_D$ +74° (c 0.26, $CHCl_3$); IR 1740 (ester), 1670 (amide); MS, 925 (5, $M^+ + 28$), 938 (31, $M^+ + 14$), 925 (5, $M^+ + 1$); NMR δ 4.2 (2 H, t, $COOCH_2$), 3.83 (3 H, s, OCH_3), 3.63 (3 H, s, OCH_3), 2.8 (3 H, s, NCH_3), 1 (15 H, m).

Compound 12 from 5 and L-Leu-O-*n*-octyl: yield 37%; mp 145 °C; $[\alpha]_D$ +54° (c 0.33, $CHCl_3$); IR 1735 (ester), 1665 (amide) cm^{-1}; MS, 981 ($M^+ + 1$), 980 (M^+); NMR δ 4.12 (2 H, t, $COOCH_2$-octyl), 3.95 (1 H, t, C^4-H), 3.75 (3 H, s, $COOCH_3$), 3.66 (3 H, s, OCH_3), 2.77 (3 H, s, NCH_3), 1.33 (10 H, m), 0.88 (15 H, m).

Compound 13 from 5 and L-Leu-amide: yield 42%; IR 1720 (ester), 1670 (amide) cm^{-1}; MS, 867 (M^+); NMR (360 MHz) δ 8.04 (1 H, s, NH), 4.5 (1 H, m, C*-H), 4.12 (1 H, d, C^4-H), 3.78 (3 H, s, OCH_3 ester), 3.6 (3 H, s, OCH_3), 2.75 (3 H, s, NCH_3).

Compound 14 from 5 and L-Ile-OMe: yield 52%; $[\alpha]_D$ +66° (c 0.13, $CHCl_3$); IR 1730 (ester), 1665 (amide) cm^{-1}; MS, 896 ($M^+ + 14$), 882 (M^+); NMR (360 MHz) δ 3.77 (3 H, s, $COOCH_3$), 3.75 (3 H, s, $COOCH_3$), 3.6 (3 H, s, OCH_3), 2.73 (3 H, s, NCH_3), 2.58 (1 H, s, C^{19}-H), 0.92 (12 H, m).

Compound 15 from 5 and L-Ile-OEt: yield 58%; $[\alpha]_D$ +32° (c 0.44, EtOH); UV 214 (4.42), 268 (3.82), 282 (3.71), 295 (3.64) nm; IR 3465, 3040, 2970, 2940, 2880, 1736 (ester), 1670 (amide), 1612, 1500, 1460, 1225 (ester); MS, 924 (7), 910 (11, $M^+ + 14$), 897 (23, $M^+ + 1$), 896 (44, M^+), 867 (11.3), 837 (18.7), 836 (26.6), 822 (4), 709 (10), 650 (21), 571 (13), 570 (34.7), 366 (21.7), 154 (100), 126 (11); NMR (360 MHz) δ 9.46 (1 H, br s, C^3-OH), 8.03 (1 H, br s, NH), 7.55 (1 H, d, $J = 7$, 2 Hz), 7.51 (1 H, d), 7.23–7.06 (3 H, m), 6.58 (1 H, s, C^{14}-H), 6.06 (1 H, s, C^{17}-H), 5.85 (1 H, dd, C^7-H), 5.78 (1 H, d, C^6-H), 4.61 (1 H, m), 4.21 (2 H, q, $COOCH_2$), 4.15 (1 H, d, C^4-H), 3.96 (1 H, t), 3.76 (3 H, s, OCH_3 ester), 3.6 (3 H, s, OCH_3), 3.46 (1 H, s, C^{10}-H), 2.73 (3 H, s, NCH_3), 1.25 (3 H, t, $COOCH_2CH_3$), 0.91 (12 H, m).

Compound 16 from 5 and L-Trp-OMe: yield 41%; $[\alpha]_D$ +94° (c 1.82, $CHCl_3$); IR 1730 (ester), 1660 (amide) cm^{-1}; MS, 970 ($M^+ + 15$), 956 ($M^+ + 1$), 955 (M^+); NMR (360 MHz) δ 9.5 (1 H, br s, C^3-OH), 8.06 (1 H, s, NH ind), 8.03 (1 H, s, NH ind), 7.58 (3 H, m), 7.38–7.06 (7 H, m), 6.41 (1 H, s, C^{14}-H), 3.75 (3 H, s), 3.61 (3 H, s), 3.58 (3 H, s, $COOCH_3$), 2.6 (3 H, s, NCH_3).

Compound 17 from 5 and L-Trp-OEt: yield 60%; $[\alpha]_D$ +80° (c 0.51, $CHCl_3$); UV 225 (5.15), 267 (4.67), 290 (4.55) nm; IR 3460, 3400, 3040, 2960, 2940, 2880, 1730 (ester), 1665 (amide), 1610, 1500, 1460, 1225, 740 cm^{-1}; MS, 998 ($M^+ + 29$), 984 ($M^+ + 15$), 970 ($M^+ + 1$), 926, 391, 279, 165, 108, 35; NMR (360 MHz) δ 9.35 (1 H, s, C^3-OH), 8.23 (1 H, s, NH ind), 8.03 (1 H, s, NH ind), 7.7 (1 H, d), 7.66 (1 H, d), 7.58 (1 H, d), 7.51 (1 H, d), 7.3–7.04 (5 H, m), 6.58 (1 H, s, C^{14}-H), 6.06 (1 H, s, C^{17}-H), 5.83 (1 H, dd, C^7-H), 5.78 (1 H, d, C^6-H), 4.93 (1 H, s), 4.18 (1 H, d, C^{14}-H), 4 (2 H, m, CH_2 ester), 3.76 (3 H, s, $COOCH_3$), 3.60 (3 H, s, OCH_3), 3.45 (1 H, s), 2.77 (3 H, s, NCH_3), 1.15 (3 H, t, CH_3 ester), 0.85 (7 H, m). Anal. Calcd for $C_{56}H_{68}N_6O_9$: C, 68.76; H, 7.11; N, 8.59; O, 15.5. Found: C, 69.39; H, 7.07; N, 8.67; O, 14.85.

Compound 18 from 5 and L-Trp-O-*n*-butyl ester: yield 65%; IR 1730 (ester), 1665 (amide); MS, 1012 ($M^+ + 15$), 998 ($M^+ + 1$), 997 (M^+); NMR (360 MHz) δ 8.28 (1 H, s, NH ind), 8.05 (1 H, s, NH ind), 7.68 (1 H, d), 7.58 (1 H, d), 7.5 (1 H, d), 7.34 (1 H, d), 7.2–7.04 (6 H, m), 4 (3 H, m, $COOCH_2$ + $H^{1'}$-A), 3.75 (3 H, s, OCH_3 ester), 3.6 (3 H, s, OCH_3), 2.78 (3 H, s, NCH_3), 1.5 (4 H, m), 1.24 (4 H, m), 0.9 (10 H, m).

Compound 19 from 5 and D-Trp-OEt: yield 59%; $[\alpha]_D$ +70° (c 0.33, $CHCl_3$); MS, 984 ($M^+ + 15$), 970 ($M^+ + 1$), 969 (M^+); IR 1725 (ester), 1665 (amide); NMR δ 8.1 (1 H, s), 8.02 (1 H, s), 7.76 (1 H, d), 7.65 (1 H, d), 7.51 (1 H, d), 7.35 (1 H, d), 7.2–7.05 (6 H, m), 6.53 (1 H, s, C^{14}-H), 4.1 (3 H, m, C^4-H + $COOCH_2$), 3.75 (3 H, s, $COOCH_3$), 3.6 (3 H, s, OCH_3), 2.43 (3 H, s, NCH_3), 1.16 (3 H, t, $COOCH_2CH_3$), 0.93 (8 H, m).

Table I. Activity of Vinblastine, Vindesine, and Amino Acid Derivatives against the P388 Leukemia[a] Implanted Intravenously in DBA_2 Mice

no.	amino acid deriv[b]	dose, mg/kg per day	no. of mice	ILS,[c] %	30th day survivors/total	60th day survivors/total
	vinblastine	3	10	30.8	0/10	0/10
		4	10[d]	42.3	0/10	0/10
		5	10	47.1	0/10	0/10
		6	10	48	0/10	0/10
		7	9	59.3	1/9	0/9
		8	9	72.9	0/9	0/9
		9	10	49.5	1/10	0/10
		10	10	81	4/10	0/10
		11	10	−40	0/10	0/10
	vindesine	2	10	27.1	0/10	0/10
		2.5	10[g]	19	0/10	0/10
		3	10[d]	23	0/10	0/10
		4	10	25.5	0/10	0/10
		5	10	30.2	0/10	0/10
		6	10	32	0/10	0/10
		7	10	24	0/10	0/10
		8	10	−46	0/10	0/10
	vincristine	0.5	10	4	0/10	0/10
		1	10	16	0/10	0/10
		1.5	10	20.8	0/10	0/10
		2	10	−46	0/10	0/10
8	V-L-Leu-OMe	10	10	41	1/10	0/10
		10.5	10	53.3	0/10	0/10
		11	10	41	0/10	0/10
		11.5	10	57	0/10	0/10
		12	10	53.8	3/10	2/10
		12.5	10	59	1/10	0/10
		13	10	74.3	3/10	3/10
		15	10	55.2	4/10	1/10
9	V-L-Leu-OEt	20	10	52.4	2/10	1/10
		22	10	90.5	4/10	1/10
		24	10	75.4	1/10	0/10
		26	10	53.5	1/10	0/10
		28	10	71.9	1/10	1/10
		30	10	84.2	2/10	1/10
		34	10	−48	2/10	1/10
10	V-D-Leu-OEt	6	10	26.7	0/10	0/10
		8	10	46.5	2/10	1/10
		10	10	>146	7/10	2/10
		11	10	155	3/10	0/10
		12.5	10	>146	6/10	1/10
		14	10	−30	2/10	0/10
11	V-L-Leu-O-*n*-Bu	55	10	23	0/10	0/10
		60	10	26	0/10	0/10
		65	10	13.8	0/10	0/10
		70	10	26.7	0/10	0/10
		80	10	27.6	0/10	0/10
12	V-L-Leu-O-*n*-octyl	40	10	−0.9	0/10	0/10
		60	10	7.8	0/10	0/10
		80	8	9.5	0/8	0/8
13	V-L-Leu-amide	5	10	1.7	0/10	0/10
		15	10	8.7	0/10	0/10
		20	10	1.7	0/10	0/10
		30	10	1.7	0/10	0/10
		40	10	19	0/10	0/10
		50	10	15.5	0/10	0/10
14	V-L-Ile-OMe	3	10	41.3	0/10	0/10
		5	10	66.5	3/10	1/10
		6	10[d]	117.6	3/10	0/10
		7	10	164	5/10	2/10
		8	10	149	3/10	3/10
		9	10	−48.3	4/10	4/10
15	V-L-Ile-OEt	4	10	60	0/10	0/10
		5	9	60.4	1/9	0/9
		6	10	73.5	1/10	0/10
		7	10	56	2/10	0/10
		8	10[d]	>183	8/10	0/10
		9	10	−34	3/10	0/10
16	V-L-Trp-OMe	30	10	33.9	0/10	0/10
		40	10	42.8	0/10	0/10
		50	10	109.8	1/10	1/10
		55	10	85.8	2/10	1/10
		60	10	132	3/10	2/10
		70	10	145.3	5/10	3/10

Table I (Continued)

no.	amino acid deriv[b]	dose, mg/kg per day	no. of mice	ILS,[c] %	30th day survivors/total	60th day survivors/total
17	V-L-Trp-OEt	20	10	43.9	0/10	0/10
		40	10	58.8	5/10	4/10
		50	10	>161	7/10	5/10
		55	10[f]	>183	7/10	1/10
		56	10	113	4/10	2/10
		58	10	122	2/10	0/10
		60	10[f]	>178	8/10	3/10
		65	9[e]	>173	7/9	7/9
		70	11	160	5/11	0/11
18	V-L-Trp-O-*n*-Bu	40	10	63	1/10	1/10
		50	10	31.6	2/10	2/10
		55	10	>163	7/10	5/10
		60	10	140	5/10	4/10
		70	10	−52	5/10	5/10
		80	10	>216	7/10	7/10
19	V-D-Trp-OEt	20	10	18.7	0/10	0/10
		30	10	41.4	1/10	0/10
		40	10	53.8	0/10	0/10
		45	10	85	2/10	0/10
		50	9	−59	0/9	0/9
20	V-L-Ala-OEt	5	10	27.6	0/10	0/10
		10	10	36.8	2/10	2/10
		12.5	10	46	1/10	1/10
		15	10	39	0/10	0/10
		25	10	63.8	2/10	1/10
21	V-L-Val-OEt	4	10	13.5	1/10	1/10
		5	10	17.2	0/10	0/10
		7	10	40	0/10	0/10
		10	10	53.4	0/10	0/10
		12.5	10	82.6	2/10	0/10
		14	10	108	2/10	0/10
		15	10	76	3/10	2/10
		17.5	9	−22.2	0/9	0/9
22	V-L-Phe-OEt	50	10	21.5	1/10	1/10
		55	10	36.4	1/10	0/10
		60	10[d]	13.3	0/10	0/10
		75	10[d]	26.6	0/10	0/10
		80	10	41.7	0/10	0/10
23	V-L-Ser-OEt	5	10	16	0/10	0/10
		7.5	10	26	0/10	0/10
		10	10	32	0/10	0/10
24	V-L-Tyr-OEt	20	10	29.9	0/10	0/10
		40	10	53.3	1/10	1/10
		50	10	49.5	1/10	0/10
		60	10	49.5	3/10	2/10
		70	10	−49.5	0/10	0/10
25	V-L-ε-trifluoroacetyl-Lys-OEt	20	10	19.6	0/10	0/10
		30	10	16.8	0/10	0/10
		40	10	19	0/10	0/10
		50	10	27.6	0/10	0/10
		60	10	31	0/10	0/10
26	V-L-Glu-OEt	10	10	−0.9	0/10	0/10
		20	10	10	0/10	0/10
		40	10	34.5	0/10	0/10
		50	10	−2.8	0/10	0/10

[a] Female DBA_2 mice were inoculated iv on day 0 with 10^4 P388 cells. Treatment is given iv on day 1. [b] Compounds were tested as their sulfate salts. [c] % ILS: percent increase in lifespan by using all deaths and survivors following the equation [(T/C × 100) − 100]. Maximal median survival time (MST) = 30 days. Controls average day of death 10.7 days. [d-g] The majority of experiments have been confirmed three times. Some have been confirmed more than five times (*d*), 10 times (*e*), 20 times (*f*), and 30 times (*g*).

Compound 20 from 5 and L-Ala-OEt: yield 50%; IR 1740 (ester), 1670 (amide); MS, 854 ($M^+ + 1$); NMR (360 MHz) δ 4.2 (2 H, q, $COOCH_2$), 3.96 (1 H, t), 3.76 (3 H, s, OCH_3 ester), 3.6 (3 H, s, OCH_3), 3.48 (1 H, s), 2.8 (3 H, s, NCH_3), 1.46 (3 H, d, CH_3), 1.3 (3 H, t, $COOCH_2CH_3$).

Compound 21 from 5 and L-Val-OEt: yield 63%; IR 1730-(ester), 1720 (ester), 1670 (amide); MS, 897 ($M^+ + 15$), 882 (M^+); NMR (360 MHz) δ 4.21 (2 H, q, $COOCH_2$), 4.15 (1 H, d, C^4-H), 3.76 (3 H, s), 3.6 (3 H, s, OCH_3), 2.73 (3 H, s, NCH_3), 1.31 (9 H, m), 0.96 (14 H, m).

Compound 22 from 5 and L-Phe-OEt: yield 66%; mp 154 °C; $[\alpha]_D$ +75° (c 1.2, $CHCl_3$); IR 1735 (ester), 1660 (amide) cm^{-1}; MS, 958 (17), 944 (41), 930 (35) M^+; NMR δ 8.1 (1 H, s, $N^{16'}$-H), 7.6 (1 H, d), 7.5 (1 H, d), 7.3–7.02 (7 H, m), 4.16 (2 H, q, $COOCH_2$), 3.8 (3 H, s, OCH_3), 3.56 (3 H, s, OCH_3), 2.74 (3 H, s, NCH_3), 1.20 (3 H, t, CH_3 ester).

Compound 23 from 5 and L-Ser-OEt: $[\alpha]_D$ +65° (c 0.7, $CHCl_3$); IR 1730 (ester), 1665 (amide) cm^{-1}; MS, 885 ($M^+ + 15$), 870 (M^+); NMR δ 7.9 (1 H, d, OH), 7.52 (2 H, m), 7.20 (3 H, m), 4.3 (2 H, q, $COOCH_2$), 3.83 (3 H, s, OCH_3), 3.66 (3 H, s, OCH_3), 2.67 (3 H, s, NCH_3), 1.34 (3 H, t, $COOCH_2CH_3$).

Compound 24 from 5 and L-Tyr-OEt: yield 48%; $[\alpha]_D$ +64° (c 0.108, $CHCl_3$); IR 1715 (ester), 1660 (amide) cm^{-1}; MS, 961 ($M^+ + 15$), 947 ($M^+ + 1$), 946 (M^+); NMR (360 MHz) δ 7.55 (2 H, m), 7.21–7.06 (3 H, m), 7.03 (2 H, d, arom Tyr, J = 7.5 Hz), 6.7 (2 H, d, arom Tyr, J = 7.5 Hz), 4.13 (3 H, $COOCH_2$, C^4-H), 3.76 (3 H, s, $COOCH_3$), 3.6 (3 H, s, OCH_3), 2.71 (3 H, s, NH_3), 1.21 (3 H, t, $COOCH_2CH_3$).

Compound 25 from 5 and L-ε-trifluoroacetyl-Lys-OEt: yield 32%; IR 1740 (ester), 1720 (ester), 1670 (amide) cm^{-1}; MS, 1022

Table II. Influence of the Schedule on the Activity of VLB, VDS, and V-L-Trp-OEt against the P388 Leukemia[a] (10^4 Cells)

no.	drug[c]	dose, mg/kg per day	schedule day	no. of mice	ILS,[b] %	30th day survivors/total	60th day survivors/total
	VLB	4	1	10[d]	42.3	0/10	0/10
		4	1, 5, 9	10	112	5/10	2/10
	VDS	3	1	10[d]	23	0/10	0/10
		3	1, 5, 9	9	49.2	0/9	0/9
17	V-L-Trp-OEt	60	1	10[f]	>224	8/10	3/10
		60	1, 5, 9	9	>426	9/9	9/9
	VLB	3	1	10	30.8	0/10	0/10
		3	1, 8, 15	12	108	0/12	0/12
	VDS	2.5	1	10[e]	19	0/10	0/10
		2.5	1, 8, 15	12	43.2	0/12	0/12
17	V-L-Trp-OEt	55	1	10[f]	>261	7/10	1/10
		55	1, 8, 15	10	>426	8/10	8/10

[a] DBA_2 mice were inoculated iv with 10^4 leukemic cells. [b] % ILS: percent increase in lifespan by using all deaths and survivors following the equation [(T/C × 100) − 100]. Maximal median survival time (MST) = 60 days. [c] Compounds were tested as their sulfate salts. [d-f] The majority of experiments have been confirmed three times. Some have been confirmed more than five times (*d*), 10 times (*e*), and 20 times (*f*).

($M^+ + 14$), 1008 (M^+); NMR (360 MHz) δ 4.22 (3 H, m), 3.76 (3 H, s, $COOCH_3$), 3.58 (3 H, s, OCH_3), 2.73 (3 H, s, NCH_3), 2.60 (1 H, s).

Compound 26 from 5 and L-Glu-OEt: yield 55%; mp 149 °C; $[\alpha]_D$ +59° (c 2, $CHCl_3$); IR 1730 (ester), 1665 (amide) cm^{-1}; MS, 967 (28, $M^+ + 28$), 953 (62, $M^+ + 14$), 939 (38, M^+); NMR δ 4.23 (2 H, q, $COOCH_2$), 4.16 (2 H, q, $COOCH_2$), 3.8 (3 H, s, OCH_3), 3.64 (3 H, s, OCH_3), 2.8 (3 H, s, NCH_3), 1.33 (3 H, t, CH_3), 1.26 (3 H, t, CH_3).

Acetylation of Compound 21. Compound **21** (100 mg, 0.11 mmol) was dissolved in 2.5 mL of pyridine and 2.5 mL of acetic anhydride. The solution was stirred at room temperature for 48 h. CH_3OH was then added, and the solvents were evaporated. This procedure was repeated four times. The residue was dissolved in CH_2Cl_2 and washed three times with water and once with saturated NaCl. The CH_2Cl_2 solution was dried with Na_2SO_4 and evaporated. The residue was chromatographed on silica gel and eluted with the solvent system Et_2O–CH_3OH saturated with NH_3 (96:4). The desired fractions were combined and evaporated, giving 26 mg of compound **7** (Scheme I) (24% yield): MS, 953 ($M^+ + 29$), 939 ($M^+ + 15$), 925 ($M^+ + 1$), 924 (M^+); IR 1740 (ester), 1690 (amide) cm^{-1}; NMR (360 MHz) δ 4.57 (1 H, dd, C*a.a.), 4.20 (2 H, q, $COOCH_2$), 3.77 (3 H, s, $COOCH_3$), 3.60 (3 H, s, OCH_3), 2.68 (3 H, s, NCH_3), 2.01 (3 H, s, $OCOCH_3$).

Results

Chemistry. The structure of the synthesized vinca derivatives was confirmed by different analytical procedures.

The presence of the peptidic bond was evident from the carbonyl absorption in the infrared spectrum. The carbonyl frequency of the peptidic bond appeared at 1660–1680 cm^{-1}.

In the NMR spectrum (360 or 60 MHz), all the principal signals are very similar to those found in the individual NMR spectra of deacetyl-VLB and amino esters.

The signal for the asymmetric carbon of the amino ester is detected between 4.55 and 4.95 ppm depending on the nature of the amino ester. The signal for the acyl ester of the amino acid is detected at 4.2 ppm ($CO\mathit{OCH_2}CH_3$) and between 1.15 and 1.3 ppm ($COOCH_2\mathit{CH_3}$). The signal for the methyl ester group is detected at ca. 3.6 ppm. The alkyl lateral chains CH_2 and CH_3 of the amino acids were detected between 0.92 and 1.3 ppm. The tryptophan derivative presents a signal at 8.2 ppm due to the indolic NH and the aromatic hydrogen signals between 7.66 and 7.31 ppm. The phenylalanine and tyrosine derivatives also show their aromatic hydrogen signals between 7.6 and 6.7 ppm. Finally the glutamic derivative shows signals between 4.23 and 4.16 ppm ($COO\mathit{CH_2}CH_3$) and 1.33 and 1.26 ($COOCH_2\mathit{CH_3}$), which are characteristic to the side-chain esters and the amino acid carboxylic group.

Acetylation of the compound **21** with acetic anhydride and pyridine yielded the corresponding *O*-acetyl compound **7** (Scheme I). It should be noted that under these conditions, the two tertiary hydroxy groups of **2** are not acetylated.

In the NMR spectrum of **7** the acetyl group is detected at 2.01 ppm. The signal of the vinyl proton C^6-H seen at 5.78 in the starting material appears now at 5.28 ppm. The peak of the proton of the carbon-4 bearing the acetoxyl moiety shifts from 4.15 to 5.53 in **7**. The same difference was observed between the spectra of vindoline and deacetylvindoline[10] or between vinblastine and deacetylvinblastine.[11]

The mass spectra exhibit the molecular ions and also the presence of ions at $M^+ + 14$ or $M^+ + 28$. These ions are formed through intra- and intermolecular transmethylation processes that are well-known to occur during mass spectroscopy of compounds related to vinblastine.[12]

Reaction of the azide **5** with the dipeptide Val-Trp-OEt provides compound **27**.

The yields of these derivatives varied from 32% to 74%, most of them being close to 60%.

Antitumor Activities. The therapeutic activities of all these vinblastin-23-oyl derivatives were assayed on murine tumor systems P388 and L1210 leukemias.

To explore the effect of structural differences on antitumor efficiency, murine leukemia P388 (Tables I–III) served as the primary assay system. When 10^4 P388 leukemic cells are injected iv into DBA_2 female mice (Table I, single-injection schedule), VDS and VCR are inactive while VLB induces a maximal increase in lifespan of 81% and 40% survivors on day 30. There are no survivors at 60 days.

The nonpolar compounds **10** and **14–18** (Table I) appear to be superior to VLB in prolonging the lifespan of mice bearing the P388 leukemia over that of untreated controls. The activity of these compounds is further confirmed by the presence of long-term survivors. The derivatives **8**, **9**, **19**, **21**, and **24** (Table I) are as active as VLB. Replacement of the ester group with an amide group (**13**, Table I) leads to an inactive compound. Gradual loss of activity accompanies the introduction of longer alkyl ester chains in the leucyl derivatives **11** and **12** (Table I). Congeners **20**, **22**, **23**, **25**, and **26** (Table I) are poorly active or inactive.

Table II points out the influence of the administration schedule. Inoculated mice were treated with VLB, VDS,

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(11) De Bruyn, A.; De Taeye, L.; Anteunis, M. J. O. *Bull. Soc. Chim. Belg.* **1980**, *89*, 8.
(12) Biemann, K. *Lloydia* **1964**, *27*, 397.

Table III. Activity of VLB, VDS, V-L-Ile-OEt, and V-L-Trp-OEt against the P388 Leukemia[a] Implanted Intravenously in DBA_2 Mice (10^5 Cells)

no.	drug[c]	dose, mg/kg per day	schedule day	no. of mice	ILS,[b] %	60th day survivors/total
	VLB	4	1	10	52	0/10
	VDS	2	1	10	28	0/10
		2.5	1	10	32	0/10
		4	1	10	43	0/10
15	V-L-Ile-OEt	6	1	10	74	0/10
		7	1	9	-37	0/9
17	V-L-Trp-OEt	65	1	10	82	0/10
	VLB	4	1, 8, 15	7	58	0/7
		4	1, 8, 15	7	35	1/7
	VDS	2	1, 8, 15	7	31	0/7
		2.5	1, 8, 15	7	31	1/7
		2.5	1, 8, 15	7	13	0/7
15	V-L-Ile-OEt	6	1, 8, 15	7	150	0/7
		6	1, 8, 15	7	200	3/7
		8	1, 8, 15	7	119	0/7
		8	1, 8, 15	7	>482	4/7
		10	1, 8, 15	7	-41	0/7
17	V-L-Trp-OEt	55	1, 8, 15	7	53	0/7
		65	1, 8, 15	7	88	0/7
		65	1, 8, 15	7	40	2/7
		75	1, 8, 15	7	108	2/7
		75	1, 8, 15	7	109	0/7

[a] Female DBA_2 mice were inoculated iv on day 0 with 10^5 P388 cells. [b] Compounds were tested as their sulfate salts. [c] % ILS: percent increase in lifespan by using all deaths and survivors following the equation [(T/C × 100) - 100]. Maximal median survival time (MST) = 60 days. Average day of death for controls 10.3 days.

or derivative 17 either in a one-injection schedule or following two different three-injection schedules. In every case, the three-injection schedule gives higher increases in lifespan. However, the schedule markedly influences the activity of compound 17: treatment with this derivative following those three-injection schedules results in an increase in lifespan of 426% with 100% and 80% long-term survivors respectively for the doses of 60 and 55 mg/kg.

The activities of VLB, VDS, and derivatives 15 and 17 on mice inoculated with 10^5 P388 leukemic cells are summarized in Table III. The three-injection schedule leads to better results; however, in this scheme, VDS is inactive and VLB induces a maximal increase in lifespan of 58% and few long-term survivors. Compound 15 gives the best ILS, i.e., 482% with a dose of 8 mg/kg per day, producing 57% of survivors on day 60. At optimal treatment with compound 17, a 108% increase in lifespan was noted with 30% long-term survivors.

Table IV shows the response of the parent alkaloids to the L1210 leukemia inoculated intravenously. Among the derivatives tested, 14–17 and 21 bring about an increase in lifespan of 60%, with long-term survivors for compound 17. Moreover, this derivative, injected according to a three-injection schedule, induces an increase in lifespan of 92%. Hence, this derivative 17 was selected for further examination of its activity against P388 leukemia inoculated ip, in comparison with VLB, VCR, and VDS.

In a set of experiments (Table V), increasing doses of VLB, VDS, VCR, and compound 17 were given on day 1. The maximal increase in. lifespan obtained were 63%, 150%, and 64% for VLB, VDS, and VCR, respectively; compound 17 gives better results as the maximal increase in lifespan afforded by this derivative amounts to 216%. At nontoxic doses, long-term survivors are only recorded with this latest derivative.

In another experiment, the optimal dose has been injected in nine daily doses; in this case, VCR yields an increase in lifespan of 146% but no long-term survivors were observed. With compound 17, we notice an increase in lifespan of 144% and 40% survivors on day 30.

Animal Toxicology. The comparative LD_{50} values for VLB, VDS, VCR, and compound 17 in mice are presented in Table VI. The acute signs of toxicity were similar for all agents: frequent signs of toxicity were locomotor inactivity, loss of weight, and diarrhea. Death usually occurred 4–7 days after dosing. The condition of surviving animals improved slowly during observation days 7–14. Comparatively, the LD_{50} value for derivative 17 is 4, 10, and 30 times higher than LD_{50} values of VLB, VDS, and VCR, respectively. The LD_{50} value for compound 15, of 17.7 mg/kg, is between those of VLB (27.4 mg/kg) and VDS (13.8 mg/kg). The LD_{50} values for VLB, VDS, and compound 17 on NMRI mice are respectively 18.6, 10.7, and 82 mg/kg.

Discussion

The present paper deals with synthesis and evaluation of experimental chemotherapeutic activities of 23-oyl amino acids derivatives in comparison with those of the parent alkaloids VLB, VDS, and VCR.

Although derivatives of vinca were previously screened ip or sc against P388 leukemia, we selected first of all the experimental models of P388 and L1210 leukemias implanted intravenously as an attempt to simulate human leukemia.

Out of the congeners examined, derivatives 10 and 14–18 are more effective in extending the lifespan of iv inoculated mice with P388 leukemia (Tables I–IV) when compared to the parent alkaloids.

The results indicate that the activity of the derivatives depends on the nature of the amino acid linked to the vinblastin-23-oyl moiety.

On the basis of the polarity of the side chains of the four main classes of amino acids, we can conclude that the activity is linked to the amino acids representing the nonpolar or hydrophobic amino acids (Leu, Ile, Val, Trp), with the exception of phenylalanine.

Apparently the presence of an amino acid at the C-23-oyl moiety increases the therapeutic potency if there is a carboxyl ester present. Indeed, introducing an amide group (13) in place of the ester group causes loss of the activity.

Amino acid ethyl or methyl esters (8, 9; 14, 15; 16, 17) have similar potency in prolonging the lifespan of mice bearing the P388 leukemia. When the alkyl chain length increases (11, 12), the activity diminishes in the leucyl

Table IV. Comparative Activity of Vinblastine, Vindesine, Vincristine, and Amino Acid Derivatives against the L1210 Leukemia[a]

no.	drug	dose, iv, mg/kg per day	schedule day	no. of mice	ILS, %	30th day survivors/total	60th day survivors/total
	vinblastine	3	1	10	30.3	0/10	0/10
		4	1	10	31.9	0/10	0/10
		5	1	10	37	0/10	0/10
		6	1	10	24.7	0/10	0/10
		7	1	10	35	0/10	0/10
		8	1	10	57.6	0/10	0/10
		9	1	10	-21.6	0/10	0/10
	vindesine	2.5	1	10[e]	13.3	0/10	0/10
		3	1	10	11.5	0/10	0/10
		5	1	10	25.7	0/10	0/10
		7	1	10	-9.1	0/10	0/10
	vincristine	0.5	1	10	1	0/10	0/10
		1	1	10	10.7	0/10	0/10
		1.5	1	10	12	0/10	0/10
		2	1	10	16	0/10	0/10
		2.5	1	10	30	0/10	0/10
		3	1	10	28.8	0/10	0/10
		3.5	1	10	14.7	0/10	0/10
		4	1	10	24	0/10	0/10
		4.5	1	10	20	0/10	0/10
		5	1	10	36	0/10	0/10
		5.5	1	10	26.6	0/10	0/10
		6.5	1	10	-37	0/10	0/10
8	V-L-Leu-OMe	10.5	1	10	24.7	0/10	0/10
		11.5	1	10	27	0/10	0/10
		12.5	1	10	17.6	0/10	0/10
9	V-L-Leu-OEt	20	1	10	26.2	0/10	0/10
		22	1	10	30.9	0/10	0/10
		24	1	10	38.1	0/10	0/10
		30	1	10	-26	0/10	0/10
10	V-D-Leu-OEt	9	1	10	27	0/10	0/10
		10	1	10	29.4	0/10	0/10
14	V-L-Ile-OMe	6	1	10	65.7	0/10	0/10
		7	1	10	64	0/10	0/10
15	V-L-Ile-OEt	4	1	9	20	0/9	0/9
		5	1	10	36	0/10	0/10
		6	1	10	41.3	0/10	0/10
		7	1	9	-27	0/9	0/9
		8	1	10	49	0/10	0/10
		9	1	10	64	0/10	0/10
		10	1	9	-27	0/9	0/9
16	V-L-Trp-OMe	50	1	10	47.4	0/10	0/10
		55	1	10	60.2	0/10	0/10
17	V-L-Trp-OEt	55	1	10	55.3	0/10	0/10
		58	1	10	48.7	0/10	0/10
		60	1	10	58	0/10	0/10
		65	1	10	71	0/10	0/10
		70	1	10	61.8	1/10	1/10
		45	1, 5, 9	10	92	0/10	0/10
18	V-L-Trp-O-*n*-Bu	50	1	10	44	0/10	0/10
19	V-D-Trp-OEt	40	1	10	21.8	0/10	0/10
		45	1	10	34.6	0/10	0/10
20	V-L-Ala-OEt	12.5	1	10	50	0/10	0/10
21	V-L-Val-OEt	15	1	10	67	0/10	0/10
22	V-L-Phe-OEt	55	1	10	0	0/10	0/10
		60	1	10	13.9	0/10	0/10
		65	1	10	3.5	0/10	0/10
		70	1	10	13	0/10	0/10
		75	1	10	25	0/10	0/10

[a] 10^4 L1210 cells were inoculated iv on day 0 into DBA_2 female mice. [b] Compounds tested as their sulfate salts. [c] % ILS: percent increase in lifespan by using all deaths and survivors following the equation [(T/C × 100) - 100]. Maximal median survival time (MST)= 30 days. Controls average day of death 7.5 days. [e] The majority of experiments have been confirmed three times. Some have been confirmed more than 10 times (*e*).

derivatives. In contrast, the *n*-butyl tryptophanyl derivative (18) is a highly effective agent in the P388 system.

Of the D isomers prepared, the D-Leu derivative (**10**) is more active than the L-Leu derivative **9**, while the biological activity of D-Trp (derivative **19**) is less than that of L-Trp (compound **17**).

As far as stereoisomerism is concerned, we notice that the presence of the R group "iso" induces activity at lower doses (**8** and **9** compared to **14** and **15**).

Tables II and III attest the superior activity of compound **17** in the P388 model.

Though parent alkaloids have weak activity against the L1210 leukemia, some amino acid derivatives have shown, for the first time, activity against this system (Table V).

All the natural vinca alkaloids[13,14] as well as the semi-

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Table V. Activity against the P388 Leukemia[a] Implanted Intraperitoneally in BDF_1 Mice

no.	drug	dose, ip, mg/kg per day	schedule day	no. of mice	ILS,[b] %	30th day survivors/total	60th day survivors/total
	VLB	2	1	10	54	0/10	0/10
		3	1	10	63	0/10	0/10
		4	1	10	0	0/10	0/10
		6	1	10	-61	0/10	0/10
	VCR	1	1	10	47	0/10	0/10
		2	1	10	53	0/10	0/10
		2.7	1	11	64	0/11	0/11
	VDS	2	1	10	89.5	0/10	0/10
		2.5	1	11	150	0/11	0/11
		3	1	10	89	0/10	0/10
		6	1	10	-47	2/10	0/10
17	V-L-Trp-OEt	50	1	10	132	1/10	1/10
		55	1	10	130	2/10	1/10
		60	1	10	>216	6/10	1/10
		65	1	10	110	1/10	1/10
		70	1	10	149	0/10	0/10
		75	1	10	138	1/10	0/10
		80	1	10	126	0/10	0/10
		90	1	10	132	4/10	1/10
		100	1	10	-47	1/10	1/10
	VLB	0.33	1-9	10	74	1/10	0/10
	VCR	0.22	1-9	10	146	0/10	0/10
	VDS	0.30	1-9	10	95	1/10	0/10
17	V-L-Trp-OEt	7.77	1-9	10	144	4/10	0/10

[a] 10^6 cell inoculum implanted ip on day 0 into BDF_1 female mice. Drugs are given ip following the indicated schedule. [b] % ILS: percent increase in lifespan by using all deaths and survivors following the equation [(T/C × 100) - 100]. Maximal median survival time (MST) = 30 days.

Table VI. Comparative Acute LD_{50} Values of Intravenously Administered Drugs

strain of mice	no.	drug	no. of doses	no. of mice	LD_{50} (day 14), mg/kg
Swiss		VLB	4	40	26
	17	V-L-Trp-OEt	4	40	94
CD_1		VLB	4	40	28
	17	V-L-Trp-OEt	5	50	110
NMRI		VLB	7	100	27.4
		VDS	8	100	13.8
	17	V-L-Trp-OEt	22	269	100.8

synthetic derivatives vinglycinate,[15,16] VDS,[4,17] and N-substituted deacetylvinblastine amide sulfate[18] have been screened ip or sc against P388 leukemias.

To be able to compare our results with the literature, we also experimented with the ip mode. Barnett et al.[4] claimed VDS in terms of its "collective activity" to be superior to VLB, deacetylvinblastine, or N-substituted VDS analogues. According to our results we come to the conclusion that, in the one-injection schedule, VDS indeed is superior to other parent alkaloids; however, derivative **17** is by far the best compound, with a very good increase in lifespan and a high percentage of long-term survivors. It is also to be noticed that in the nine daily doses schedule, compound **17** and VCR look as the best derivatives.

Todd et al.[19] studied the comparative acute iv toxicity of VLB, VCR, and VDS in mice and rats. The LD_{50} values in these species found for VDS, 6.3 and 2.6 mg/kg, respectively, are between those for VLB (10.0 and 2.9 mg/kg) and VCR (2.1 and 1 mg/kg). They found VDS was less toxic than VLB and more toxic than VCR. Table VI shows that the presence of a tryptophan at the C-23-oyl moiety diminishes the toxicity (derivative **17**).

Although no direct relationship between the lipophilicity and the degree of neurotoxicity of the small number of dimeric alkaloid agents now in use is evident at this time, the regional increase in lipophilicity associated with the conversion of VLB to compound **17** appears as a desirable feature.

The highest activity of the tryptophan derivative might also be the result of its chemical structural similarity to vinca alkaloids. Incidentally, Perkin and Robinson[20] were the first to suggest that the aromatic portion present in the indole alkaloids is derived from tryptophan that has undergone decarboxylation to tryptamine.

Studies of the disposition and tissue levels of compound **17** in animals hopefully may lead to some understanding of factors affecting toxicity and efficiency.

Among the congeners tested, compounds **15** and **17** emerge as having optimum activity against the tumor models used. Both agents are worth being further studied. Currently, derivative **17** is undergoing phase I and II clinical trials[21] at European Organization for Research on Treatment of Cancer (EORTC) and Cliniques St. Luc, UCL, Belgium.

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Registry No. **4**, 55383-37-4; **5**, 55324-86-2; **7**, 81571-37-1; **8**, 81571-26-8; **9**, 81600-04-6; **10**, 81571-25-7; **11**, 81571-29-1; **12**, 81571-30-4; **13**, 96806-26-7; **14**, 81571-27-9; **15**, 81571-28-0; **16**,

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Decomposition Reactions of (Hydroxyalkyl) nitrosoureas and Related Compounds: Possible Relationship to Carcinogenicity

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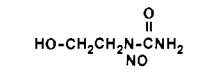
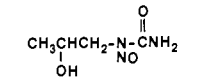
(Hydroxyalkyl)nitrosoureas and the related cyclic carbamates *N*-nitrosooxazolidones are potent carcinogens.[1] The decompositions of four such compounds, 1-nitroso-1-(2-hydroxyethyl)urea (I), 3-nitrosooxazolid-2-one (II), 1-nitroso-1-(2-hydroxypropyl)urea (III), and 5-methyl-3-nitrosooxazolid-2-one (IV), in aqueous buffers at physiological pH were studied to determine if any obvious differences in decomposition pathways could account for the variety of tumors obtained from these four compounds. The products predicted by the literature mechanisms for nitrosourea and nitrosooxazolidone decompositions (which were derived from experiments at pH 10–12) were indeed the products formed, including glycols, active carbonyl compounds, epoxides, and, from the oxazolidones, cyclic carbonates. Furthermore, it was shown that in pH 6.4–7.4 buffer epoxides were stable reaction products. However, in the presence of hepatocytes, most of the epoxide was converted to glycol. The analytical methods developed were then applied to the analysis of the decomposition products of some related dialkylnitrosoureas, and similar results were obtained. The formation of chemically reactive secondary products and the possible relevance of these results to carcinogenesis studies are discussed.

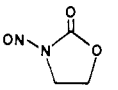
Nitrosoureas have long been of interest both as chemical carcinogens and also as cancer chemotherapeutic agents. Various nitrosoureas have been shown to cause a wide variety of tumors in test animals. Lijinsky and Reuber[1] recently reported the results of carcinogenicity studies on a group of four related nitroso compounds, 1-nitroso-1-(2-hydroxyethyl)urea (I, NHEU), 3-nitrosooxazolid-2-one (II, NOX), 1-nitroso-1-(2-hydroxypropyl)urea (III, NHPU), and 5-methyl-3-nitrosooxazolid-2-one (IV, NMOX). Compound I caused tumors in a wide variety of organs, with the most frequent tumors occurring in the lung and forestomach, although several other organs were affected. The other nitrosourea (III) caused mainly lymphomas and leukemias and forestomach tumors with a scattering of tumors in other organs. The nitrosooxazolidones, in contrast, caused forestomach tumors almost exclusively. We have undertaken a study of the decomposition reactions of these four compounds in aqueous buffers at physiological pH, seeking patterns of product formation that might be related to carcinogenesis. These reactions are also compared with those of some dialkylnitrosoureas that are chemically closely related to I and III and are currently being tested for carcinogenicity.



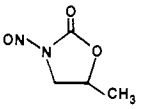
I, NHEU



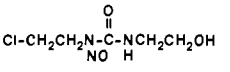
III, NHPU



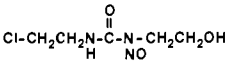
II, NOX



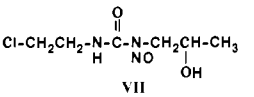
IV, NMOX



V



VI



VII

Results

According to the literature decomposition pathways (determined at pH 10–12) for nitrosoureas[2] and nitrosooxazolidones,[3] one may anticipate the formation of certain products (Schemes I and II). NHEU (I) could give acetaldehyde, ethylene glycol, and ethylene oxide, II could give these compounds and ethylene carbonate, III should give acetone, propylene glycol, propionaldehyde and propylene oxide, while IV would be expected to give these same products plus propylene carbonate. Different methods of analysis were required for the different classes of compounds. Glycols are particularly difficult to analyze and are not readily extracted from aqueous solutions. Direct GC analysis of glycols is possible, but quantitation is difficult since the glycols are apt to adhere to the column packing and elute as "ghost peaks" in later injections particularly when aqueous solutions are employed.[4] Direct GC analysis of the cyclic carbonates is, however, readily achieved.

The test compounds were dissolved in pH 7.4, 0.067 or 0.1 M phosphate buffer at 37 °C. Several different methods of analysis were employed. The reactions were monitored for disappearance of starting material by HPLC,

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