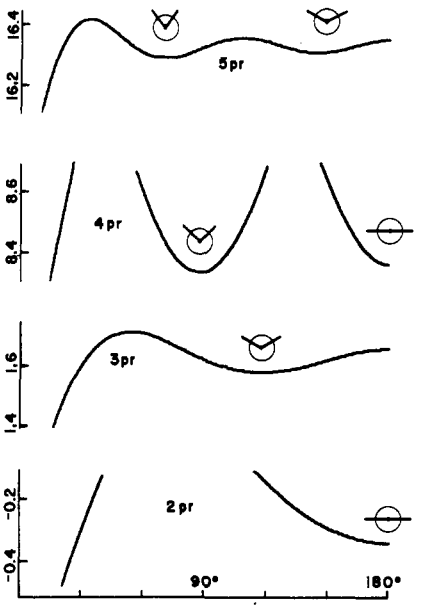

Figure 1. Energy vs. bond angle, for two-, three-, four-, and five-electron pair. The small figure above each minimum illustrates the corresponding predicted bond angle. The unit of energy is w_1^0 (see text).

angle 2α. In each case, minima appear at approximately $360°/N$ and multiples thereof, as would be predicted by a valence-shell-electron-pair-repulsion (VSEPR)[1] model. A second VSEPR prediction, that angles between two bonding pair are smaller than angles involving lone pair, is also borne out. The three-pair bond angle energy minimum is actually at 118°, the smaller four-pair minimum at 88°, and the smaller five-pair minimum at 71°. Further, if the variable λ is taken to represent the electronegativity of the ligand terminating the bond, each of these bond angles becomes smaller as electronegativity is increased. Thus for λ values of 0.5, 1, and 2 respectively, the three-pair bond angle is 118, 115, and 109°; the four-pair bond angle is 88, 86, and 82°; and the five-pair bond angle is 71, 69, and 66°. Finally, where two minima are found, as for instance in the five-pair case, the deeper minimum is that in which the bonding pairs are adjacent, with the second occurring at the geometry corresponding to a lone pair between the bonding pair. The position of the latter minimum is, as expected, nearly independent of the value of λ.

The abandonment of electrostatic valence-electron repulsions, and of two of the three spatial coordinates describing each electron, is, of course, the grossest of approximations, and can only be justified by success in mimicking nature as summarized by the VSEPR and similar models. A point worth noting is that the stereoactivity of the lone pair as here reflected arises from the Pauli exclusion principle alone.

The extension of this model to two angular coordinates, and to systems with three or more bonds, should be straightforward in principle.

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Novel Maytansinoids. Naturally Occurring and Synthetic Antileukemic Esters of Maytansinol[1-3]

Sir:

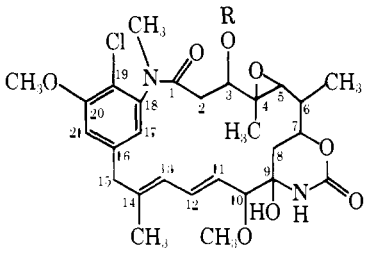
Maytansine (**1**),[4] a novel ansa macrolide isolated from several *Maytenus* species,[4-6] is an exceptionally interesting antitumor agent. It shows high inhibitory activity against several murine tumors, at the level of micrograms per kilogram body weight and over a wide dosage range.[7] The compound has undergone extensive preclinical toxicological studies and has recently been selected for clinical trial by the National Cancer Institute. Furthermore, recent biological studies have shown that maytansine is a highly active inhibitor of cell division[2,8] and of transformation of mouse cell cultures infected with murine sarcoma virus.[9]

We report herein the isolation, structural elucidation, and chemical interrelation of two new maytansinoids, maytanacine (**2**) and maytansinol (**3**), from *Putterlickia verrucosa* Szyszyl. (Celastraceae),[10] the richest reported source of maytansine (**1**) and related antileukemic esters. Maytanacine (**2**), which exhibits potent antileukemic activity,[11,12] is the first reported maytanside ester which does not bear an amino acid residue at C-3. Maytansinol (**3**), the parent alcohol of the potent maytanside esters, lacks antileukemic activity and shows ca. 1/100,000 the cytotoxicity of maytanacine (**2**).

The alcoholic extract of *P. verrucosa* stems was fractionated by the general procedure outlined earlier,[4] to yield maytansine (**1**, 12 mg/kg), maytanprine[5] (**4**, 8.5 mg/kg), and maytanbutine[5] (**5**, 4.5 mg/kg). Further separations by column and preparative layer chromatography gave maytanacine (**2**, 0.36 mg/kg) $C_{30}H_{39}ClN_2O_9$, mp 234-237°; $[\alpha]^{23}D$ −119° (*c* 0.100, $CHCl_3$); uv (EtOH) 233 (ϵ 3.03 × 10^4), 242 (sh, ϵ 2.8 × 10^4), 252 (ϵ 2.79 × 10^4), 281 (ϵ 5.36 × 10^3), 289 nm (ϵ 5.36 × 10^3); ir (KBr) 5.70, 5.80, 6.00, 6.34 μ; and maytansinol[13] (**3**, 0.025 mg/kg) $C_{28}H_{37}ClN_2O_8$, mp 173-174.5°, $[\alpha]^{23}D$ −309° (*c* 0.110, $CHCl_3$); uv (EtOH) 232 (ϵ 3.27 × 10^4), 244 (sh, ϵ 3.08 × 10^4), 252 (ϵ 3.16 × 10^4), 281 (ϵ 5.81 × 10^3), 288 nm (ϵ 5.70 × 10^3); ir (KBr) 5.85, 6.06, 6.35 μ.

The mass spectral characteristics of **2** (*m/e* 545.2180 ($M^+ - H_2O - HNCO$); *m/e* 485.1969) indicated that it was a maytanside ester similar to **1** except for differences in the R group of the ester side chain. The NMR spectrum of **2** contained an acetate methyl signal at τ 7.82 (3 H, s) and lacked the C(2′)H-CH_3 and N-CH_3 signals of the maytansine amino ester side chain.

Maytansinol (**3**) was prepared by $LiAlH_4$ treatment[14] of maytanbutine (**5**) in dry THF at −23° for 3 hr (40% yield). The NMR spectrum ($CDCl_3$) of **3** was identical with that of **1** or **2** except for the lack of the signals due to the ester moiety at C-3. The C-3 proton signal was shifted upfield and obscured by other peaks, and the C-3-O*H* proton signal appeared as a singlet at τ 6.56. Similar reductive cleavage of maytanacine (**2**) also gave maytansinol (**3**) and treat-



1, R = CO·CHCH$_3$·NCH$_3$·COCH$_3$
2, R = CO·CH$_3$
3, R = H
4, R = CO·CHCH$_3$·NCH$_3$·COCH$_2$CH$_3$
5, R = CO·CHCH$_3$·NCH$_3$·COCH(CH$_3$)$_2$
6, R = CO·CH$_2$CH$_3$
7, R = CO·CH$_2$Br
8, R = CO·CH=CHCH$_3$
9, R = CO·CF$_3$

ment of **3** with acetic anhydride-pyridine at 53° for 18 hr gave maytanacine (53% yield).

To evaluate the effects on biological activity of variations in structure of the ester moiety, several semisynthetic esters of maytansinol were prepared. The propionate (**6**, 38%, mp 187.2-188.6°, $[\alpha]^{22}D$ −119° (*c* 0.133, $CHCl_3$), ir (KBr) 5.71 μ), bromoacetate (**7**, 43%, mp >300°, $[\alpha]^{22}D$ −149° (*c* 0.107, $CHCl_3$), ir (KBr) 5.68 μ), crotonate (**8**, 13%, mp 214.1-216.0, $[\alpha]^{24}D$ −70° (*c* 0.037, $CHCl_3$), ir (KBr) 5.71 μ), and trifluoroacetate (**9**, 30%, mp 162.2-163.0°, $[\alpha]^{23}D$ −289° (*c* 0.080, $CHCl_3$), ir (KBr) 5.58 μ) esters of maytansinol were made by procedures involving either anhydride-pyridine (**6, 7, 8**)[15] or anhydride-acid (**9**)[16] treatment. The esters **2, 6, 7,** and **8** were found to show antileukemic activity[11] comparable to those of the naturally occurring substituted alanyl esters. The trifluoroacetate ester **9** showed no antileukemic activity, possibly because of ready solvolysis in vivo to inactive maytansides; cf. ref 6. Approaches to other synthetic modifications are in progress, to elucidate further the requirements for biological activity among the antileukemic maytansinoids.

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(12) Maytanacine (**2**) showed significant antileukemic activity against the P-388 lymphocytic leukemia over a 50–100-fold dosage range at the μg/kg level (with a T/C value of 230 at its optimal dose of 100 μg/kg), and cytotoxicity (ED_{50}) against KB cell culture at 10^{-5} μg/ml. Maytansinol (**3**) showed no antileukemic activity at doses up to 70 times the optimal dose (12.5 μg/kg; T/C 258) of maytansine (**1**).
(13) The isolation of maytanacine (**2**) and the other maytanside esters was guided by assays of fractions for antileukemic and cytotoxic activity. However, the isolation of the minor and biologically inactive constituent maytansinol (**3**) was effected by TLC and HPLC comparisons of fractions with a reference sample of semisynthetic origin. The isolation procedure omitted the acetylation step used earlier (ref 4).
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Carboxyl-Terminal Amino Acid Residue Analysis of Peptides. A New Method

Sir:

We wish to report a general and efficient method for the determination of the carboxyl-terminal amino acid residue of peptides. Efficient methods have been developed for both sequential and single residue determinations at the amino terminus;[1-4] however, the methods currently available for carboxyl terminal analysis suffer from limitations.[5-7]

Compound **5**, *O*-pivaloylhydroxylamine (OPHA), was found, of many derivatives surveyed,[8,9] to represent a sufficient compromise of stability, water solubility, and nucleophilicity to form *O*-pivaloylhydroxamic acids quantitatively from carbodiimide-activated carboxylic acids. Ionization of the hydroxamate **6** N-H bond (pK_a 6.4-7.4)[10] initiates on peptides a Lossen rearrangement[11,12] and eventual conversion of the carboxyl-terminal amino acid to an aldehyde, as shown in Scheme I. Such a scheme has appealed to others,[13] but could not be reduced to practice on a small scale because of the large number of reactions hitherto required to generate the O-acylated hydroxamic acid.

Table I. Results of the Carboxyl-Terminal Amino Acid Analysis

Peptide[a,d]	% degradation	Peptide[a,d]	% degradation
Ac·Gly·Asn	71	Ac·Ala·Leu·Gly	77
Ala·Ser	72	Ac·Gly·Leu·Tyr[d]	92
Ac·Ala·Glu	40(60)[b]	Ac·Pro·Phe·Gly·Lys(Ac)	94
Ac·Ala·Asp	40(50)[b]	Ac·Met·Arg·Phe·Ala	92
Pro·Gly	77	Ac·Phe·Asp·Ala·Ser·Val[c]	93
Gly·Met	81	Ac·Leu·Trp·Met·Arg·Phe[d]	94
Ac·Ala·Pro	82	Ac·γ-Glu·Cys(SO_3H)·Gly[c]	
Gly·Phe	87	(Oxidized acetylglutathione)	95 (Glu), 75 (Gly)
Ac·Gly·Leu	90		
Bz·Gly·Arg	93	Insulin A-chain[f]	
Gly·Trp[e]	86	(C-terminal Asn)	75
		Insulin B-chain[f]	
		(C-terminal Ala)	99

[a] Abbreviations: Ac = acetyl, Bz = benzoyl; see text for definition of degradation yield. [b] Yields in parentheses result from carrying out coupling step at pH 0.75; see text. [c] The side-chain of Asp is coupled to OPHA in this procedure, but does not rearrange; model studies to be reported also show that Glu side-chains behave similarly. Thus, these residues analyze for Asp and Glu, respectively, after degradation. γ-Glu, however, is readily lost in the degradation, because it presents a new C-terminus. The difference in α- and ω-linked residues is readily accounted for by the substantially different migrating groups in the two cases. [d] See Footnote 15. [e] The determination of Trp by amino acid analysis presented the usual difficulties. In order to carry out this degradation, chloride ion was avoided. Thus, free OPHA and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate were used in the degradation, and amino acid analyses were carried out by the procedure of Liu and Chang[20] using methanesulfonic acid. [f] The entire analysis was carried out in 8 *M* urea.