THE JOURNAL OF Organic Chemistry®

VOLUME 42, NUMBER 14

JULY 8, 1977

The Maytansinoids. Isolation, Structural Elucidation, and Chemical Interrelation of Novel Ansa Macrolides[1a,2]

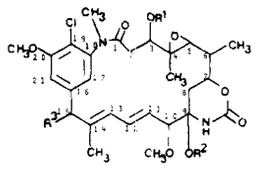
S. M. Kupchan,[1b] Yasuo Komoda, Alan R. Branfman, Albert T. Sneden,* William A. Court, Gareth J. Thomas, H. P. J. Hintz, Roger M. Smith, Aziz Karim, Gary A. Howie, Ashok K. Verma, Yoshimitsu Nagao, Richard G. Dailey, Jr., Virginia A. Zimmerly, and William C. Sumner, Jr.

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901

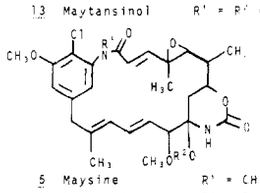
Received January 27, 1977

The details of the isolation and structural elucidation of the potent antileukemic ansa macrolide principles maytansine (**1**), maytanprine (**2**), maytanbutine (**3**), maytanvaline (**4**), and maytanacine (**12**), and the companion maytansides, maysine (**5**), normaysine (**6**), maysenine (**7**), and maytansinol (**13**), are reported. The isolation and characterization of a new antileukemic principle, maytanbutacine (**8**), is also reported. **1**, **2**, **3**, and **4** were shown to be *N*-acyl amino acid esters of **13**, and **12** was found to be the acetate ester of **13**. Reductive cleavage of **3** and **12** afforded **13** as well. **8** was found to contain two acyl ester groups, a C-3 isobutyrate ester and a C-15 acetate ester. **5**, **6**, and **7** all lack the C-3 ester moiety, but retain the ansa macrolide ring system.

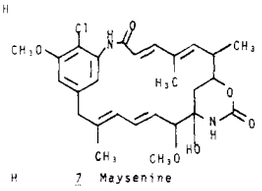
In the course of a continuing search for tumor inhibitors from plant sources, we found that an alcoholic extract of *Maytenus serrata* (Hochst. ex A. Rich.) R. Wilczek[3a,b] showed significant inhibitory activity in vitro against cells derived from human carcinoma of the nasopharynx (KB) and in vivo against five standard animal tumor systems.[4] Our preliminary communications[5-7] described the isolation and structural elucidation of the potent antileukemic (PS) maytanside esters, maytansine (**1**), maytanprine (**2**), maytanbutine (**3**), and maytanvaline (**4**), as well as the maytansides, maysine (**5**), normaysine (**6**), and maysenine (**7**). Chemical[8,9] and biological[10-17] interest in the maytansinoids continues and maytansine is currently undergoing clinical trials under the auspices of the National Cancer Institute. In this paper we present in detail the isolation and structural elucidation of the maytansinoids, and, in addition, the characterization of a new maytanside diester, maytanbutacine (**8**), is described.



1	Maytansine	R^1 = COCH(CH$_3$)N(CH$_3$)COCH$_3$; R^2 = R^3 = H
2	Maytanprine	R^1 = COCH(CH$_3$)N(CH$_3$)COCH$_2$CH$_3$; R^2 = R^3 = H
3	Maytanbutine	R^1 = COCH(CH$_3$)N(CH$_3$)COCH(CH$_3$)$_2$; R^2 = R^3 = H
4	Maytanvaline	R^1 = COCH(CH$_3$)N(CH$_3$)COCH$_2$CH(CH$_3$)$_2$; R^2 = R^3 = H
8	Maytanbutacine	R^1 = COCH(CH$_3$)$_2$; R^2 = H; R^3 = OCOCH$_3$
9	Desacetylmaytanbutacine	R^1 = COCH(CH$_3$)$_2$; R^2 = H; R^3 = OH
10	Colubrinol acetate	R^1 = COCH(CH$_3$)N(CH$_3$)COCH(CH$_3$)$_2$; R^2 = H; R^3 = OCOCH$_3$
11	Maytansine bromopropyl ether	R^1 = COCH(CH$_3$)N(CH$_3$)COCH$_3$; R^2 = CH$_2$CH$_2$CH$_2$Br; R^3 = H
12	Maytanacine	R^1 = COCH$_3$; R^2 = R^3 = H
13	Maytansinol	R^1 = R^2 = R^3 = H



5	Maysine	R^1 = CH$_3$; R^2 = H
6	Normaysine	R^1 = R^2 = H
14	Maysine methyl ether	R^1 = R^2 = CH$_3$



7 Maysenine

Fractionation (Chart I) of the ethanolic extract, guided by assay against KB tissue culture and PS leukemia in mice, revealed that the inhibitory activity was concentrated, successively, in the ethyl acetate layer of an ethyl acetate–water partition and in the methanol layer of a 10% aqueous methanol–petroleum ether partition. Column chromatography of the aqueous methanol solubles on SilicAR CC-7 was followed by treatment of the 5% methanol–chloroform eluent with acetic anhydride–pyridine,[18] and the resulting residue was subjected to extensive column chromatography first on SilicAR and then on alumina. The fraction eluted with 30% methanol–chloroform from the alumina column was then subjected to preparative thin layer chromatography (PTLC) on alumina to give fraction D. Further purification of fraction D by PTLC on silica gel yielded fraction F (high R_f) and fraction E (low R_f), both of which showed high biological activity. PTLC of fraction E on ChromAR 7GF afforded a highly enriched concentrate (fraction G, 1 mg/kg of plant) as a solid residue which was homogeneous by both silica gel and alumina TLC yet resisted all attempts at crystallization.

Elemental analysis of fraction G indicated the presence of three nitrogen atoms. Partitioning between 2 N hydrochloric acid and ether, with the active principle remaining in the ether, indicated that none of the nitrogen atoms was strongly basic. Attempts to prepare a quaternary salt derivative from fraction G revealed that a common crystalline product, apparently a methyl derivative, was formed in methanolic solution in low yield (<1%). Similar experiments in ethanolic

Chart I. Fractionation of the Active Extract from *Maytenus serrata*

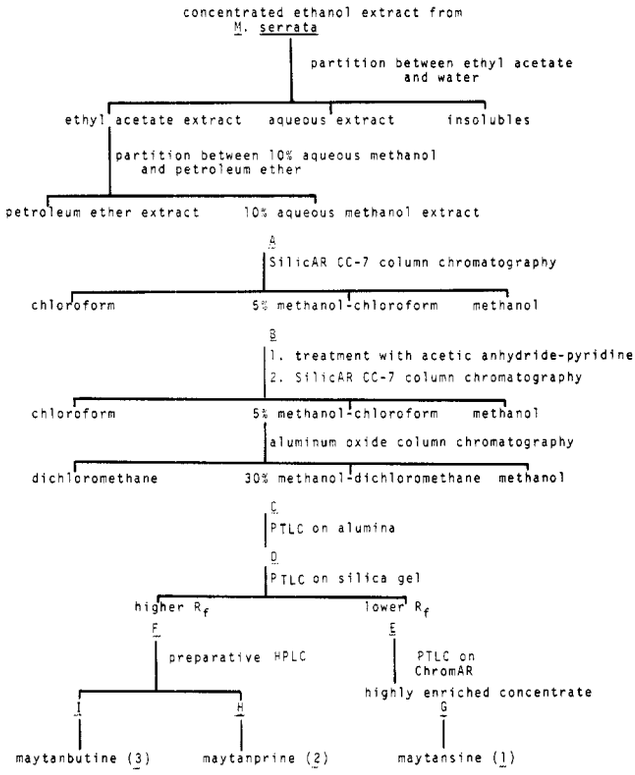
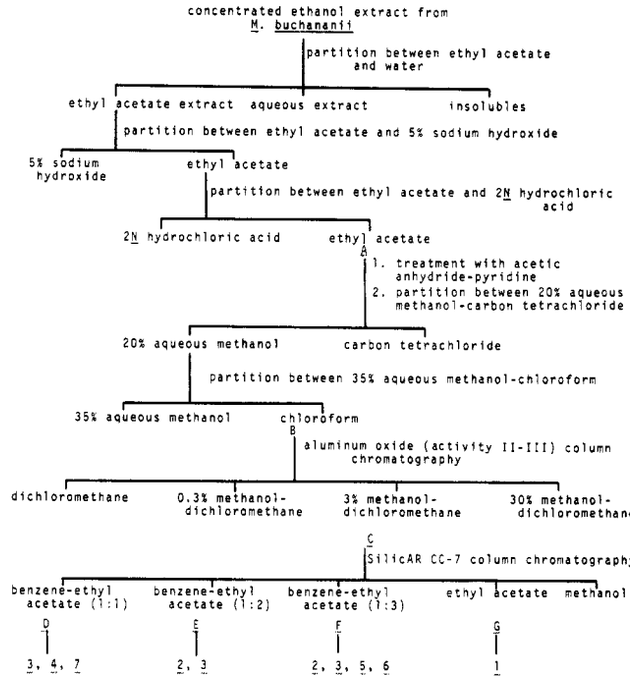


Chart II. Fractionation of the Active Extract from *Maytenus buchananii*



solution afforded a common crystalline ethyl derivative also in low yield. Accordingly, when fraction G was treated with 3-bromopropanol and *p*-toluenesulfonic acid in dichloromethane at room temperature, the crystalline 3-bromopropyl derivative, **11**, $C_{37}H_{51}BrClN_3O_{10}$, was obtained in 10% yield. Treatment of **11** with 2 N hydrochloric acid in aqueous methanol afforded a crystalline hydrolysis product which was used to seed a solution of fraction G and yield crystalline maytansine (**1**, 0.2 mg/kg of plant, 0.00002%). On the basis of elemental analysis and high-resolution mass spectrometry, maytansine was assigned the molecular formula $C_{34}H_{46}ClN_3O_{10}$ [mass spectrum *m/e* 630.2680, $C_{33}H_{43}ClN_2O_8$ [M − 61 (H_2O + HNCO)][19] = 630.2708].

Owing to the extremely small quantity of maytansine obtained and the reversible interrelation of maytansine (**1**) and 3-bromopropyl derivative **11**, the latter compound was an attractive target for x-ray crystallographic analysis. The structure of **11** was solved by the heavy-atom method,[5,20] and led to structural assignment **1** for maytansine. The absolute configurations of **11** were found to be 3*S*, 4*S*, 5*S*, 6*R*, 7*S*, 9*S*, 10*R*, and 2′*S*.

Fraction F (Chart I) was purified further by preparative high-pressure liquid chromatography (HPLC) to give fractions H and I. These fractions were crystallized by seeding each with the crystalline hydrolysis product obtained from their respective alkyl ether derivatives, to give maytanprine (**2**, 0.031 mg/kg) and maytanbutine (**3**, 0.036 mg/kg).

A search for a better source of maytansine revealed that *Maytenus buchananii* (Loes.) R. Wilczek, collected in Kenya in 1970 and 1972[3a] and fractionated by the same procedure as *M. serrata*, gave higher yields of maytanprine (1.2 mg/kg) and maytanbutine (0.9 mg/kg) as well as maytansine (1.5 mg/kg).

Additional extracts of *Maytenus buchananii* were fractionated by an improved procedure as shown in Chart II. The active principles were concentrated, successively, in the ethyl acetate layers of an ethyl acetate–water partition, an ethyl acetate–5% sodium hydroxide partition, and an ethyl acetate–2 N hydrochloric acid partition to give fraction A. After treatment of fraction A with acetic anhydride–pyridine,[18] the active components were further concentrated in the aqueous methanol layer of a 20% aqueous methanol–carbon tetrachloride partition and in the chloroform layer of a 35% aqueous methanol–chloroform partition to afford fraction B. Column chromatography of fraction B over alumina (activity II–III) concentrated the activity in fraction C (3% methanol–dichloromethane) which was then subjected to column chromatography over SilicAR CC-7 to yield fractions D–G. PTLC in several systems gave maytansine (**1**) from fraction G, maytanprine (**2**) from fractions E and F, maytanbutine (**3**) from fractions D, E, and F, maytanvaline (**4**) from fraction D, maysine (**5**) and normaysine (**6**) from fraction F, and maysenine (**7**) from fraction D.

The relationship of compounds **2–7** to maytansine (**1**) was established from the spectral and analytical data. The ultraviolet (UV) spectra of all the compounds were almost identical with that of maytansine, with characteristic absorptions at 233, 243, 254, 282, and 290 nm. The infrared (IR) spectra of **2–4** were also virtually identical with that of maytansine, and **5–7** differed primarily in the disappearance of the ester carbonyl absorption at 5.75 μ and the appearance of a carbonyl band in the 6.12–6.21-μ region. The respective empirical formulas were assigned based on microanalyses and high-resolution mass spectral measurements. The mass spectral fragmentation patterns (Table I) also gave valuable structural information.

The four maytanside esters (**1–4**) possess the same mass spectral peaks at *m/e* 485, 470, and 450. The ion at *m/e* 485 results from the initial loss of H_2O and HNCO from the carbinolamide moiety (a) and subsequent elimination of the ester side chains as carboxylic acids (b). The ion at *m/e* 470 is *m/e* 485 minus a methyl group and the ion at *m/e* 450 is *m/e* 485 minus the chlorine atom. The principal peaks derived from side chain cleavage (Table I) correspond to (b) − (OH) and (b) − (COOH), and between each of the side chain acids of compounds **1–4** there is one methylene group difference, respectively. Each compound also has major ions at *m/e* 58 and 44 derived from the (b) − (COOH) fragment which correspond to $C_3H_8N^+$ and $C_2H_6N^+$, respectively. These mass spectral

Table I[a]

Compd	$M^+ - (a)$	$M^+ - (a + b)$	$485 - (CH_3)$	$485 - (Cl)$	$b - (OH)$	$b - (COOH)$
1	630	485	470	450	128	100
2	644	485	470	450	142	114
3	658	485	470	450	156	128
4	672	485	470	450	170	142
12	545	485	470	450		
13	503	485	470	450		

Compd	$M^+ - (a)$	$M^+ - (a + CH_3)$	$M^+ - (a + Cl)$
5	485	470	450
6	471	456	436
7	455	440	420

Compd	$M^+ - (a)$	$M^+ - (a + c)$	$M^+ - (a + c + b)$	$483 - (CH_3)$	$483 - (Cl)$
8	631	571	483	468	448
9	589	571	483	468	448

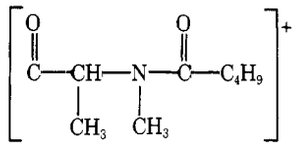
[a] (a) = H_2O + HNCO; (b) = R'OH; (c) = R^3H.

characteristics indicated that **2–4** have ansa macrolide structures similar to **1** except for differences in the *N*-acyl group of the ester side chains.

The chemical relationships among compounds **1–4** were confirmed by comparison of their nuclear magnetic resonance (NMR) spectra. The NMR spectra of **2–4** differed from that of **1** solely in the signals attributed to the terminal *N*-acyl group, as expected from the mass spectral fragmentation patterns. The NMR signals for the *N*-acyl group of maytanprine (**2**) [δ 1.18 (3 H, t, J = 7 Hz), 2.37 (1 H, m), 2.41 (1 H, m)] indicated a $-CH_2CH_3$ group with nonequivalent methylene protons. This was confirmed by spin-decoupling studies.[21,22]

The NMR signals for the *N*-acyl group of maytanbutine (**3**) [δ 1.12 (3 H, d, J = 7 Hz), 1.19 (3 H, d, J = 7 Hz), 2.80 (1 H, m)] suggested a $-CH(CH_3)_2$ moiety with two nonequivalent methyl groups. This was supported by a combination of spin decoupling experiments and solvent shift studies. The two signals for the C-2′ N-CH_3 group [δ 2.87 (0.75 H, s) and 2.92 (2.25 H, s)] in the NMR of **3** indicated that the rate of rotation about the carbonyl to nitrogen bond was reduced by steric interaction of the isopropyl group and the aromatic ring.[21,22]

The mass spectral data for maytanvaline (**4**) suggested a molecular weight of 170 for the ester fragment, consistent with structure A. Hydrolysis of **4** with sodium carbonate in



A

50% aqueous methanol at room temperature yielded maysine (**5**) and *N*-isovaleryl-*N*-methyl-L-alanine, characterized as its methyl ester by comparison with a synthetic sample prepared by acylation of *N*-methyl-L-alanine methyl ester with isovaleryl chloride.[23]

The mass spectral characteristics of maytansides **5–7** (Table I) indicated that these compounds have ansa macrolide structures similar to **1–4** but lack the ester side chains. The NMR spectrum of maysine (**5**) showed the presence of a trans α,β-unsaturated amide [δ 5.65 (1 H, d, J = 16 Hz), 6.37 (1 H, d, J = 16 Hz)] with no proton in the γ position. Treatment of maytansine with sodium carbonate in 50% aqueous methanol at room temperature gave one major product which was identical with maysine in all respects. This information, along with the disappearance of the carbonyl IR absorptions of the C_3 ester, established structure **5** for maysine.

The mass spectral fragmentation pattern of normaysine (**6**) with $M^+ - (a)$ at *m/e* 471, $M^+ - (a + CH_3)$ at *m/e* 456, and $M^+ - (a + Cl)$ at *m/e* 436 indicated that normaysine is the *N*-demethyl homologue of maysine. The NMR spectrum of **6** showed a signal corresponding to the proton on C-1 nitrogen [δ 7.38 (1 H, br s), exchangeable with D_2O] and lacked the NCH_3 signal of maysine.

The mass spectrum of maysenine (**7**) showed that **7** is a deoxy derivative of **6**. The NMR spectrum of **7** showed signals for a vinyl methyl group [δ 1.56 (3 H, br s)] and vinyl proton [δ 5.50 (1 H, br d, J = 10 Hz)] instead of the signals for the 4-methyl and 5-H protons of the 4,5-epoxide system of **6** and a downfield shift of the C-2 and C-3 protons relative to **6**. The structure of **7** was supported also by the bathochromic shift of its UV and IR carbonyl absorption bands in comparison with those of **6**. Chemical interrelation was effected by reductive elimination of the epoxide of **6** with chromous chloride in acetic acid to give maysenine (**7**).[24]

The relationships established for maytansinoids **1–7** aided the structural elucidation of a previously unreported active maytansinoid from *M. serrata*, maytanbutacine (**8**). The plant material was fractionated as in Chart I to give fraction E which was separated into two bands by PTLC on alumina. PTLC of the lower R_f band on silica gel again gave two bands, and HPLC of the higher R_f band gave a fraction enriched in maytanbutacine (**8**). Further purification of this fraction by PTLC on ChromAR and then crystallization from dichloromethane–diethyl ether yielded maytanbutacine (**8**) (0.115 mg/kg plant, 0.0000115%).

The structure of maytanbutacine (**8**) differs from that of colubrinol acetate (**10**)[25] only in the C-3 side chain ester. The UV spectrum was typical of a maytanside ester. The IR spectrum was also similar to those of maytanside esters **1–4**, but the band at 5.73 μ attributed to the ester carbonyl was more intense than in the spectra of **1–4**.

The mass spectral data (Table I) provided an important indication of the structure. A weak parent ion was observed at *m/e* 692, and a strong ion at *m/e* 631 resulted from the typical loss of H_2O and HNCO from the carbinolamide moiety of the parent compound. The next major ion expected for a normal maytanside ester would be at *m/e* 485 (loss of the side chain acid). However, the next major ion observed was at *m/e*

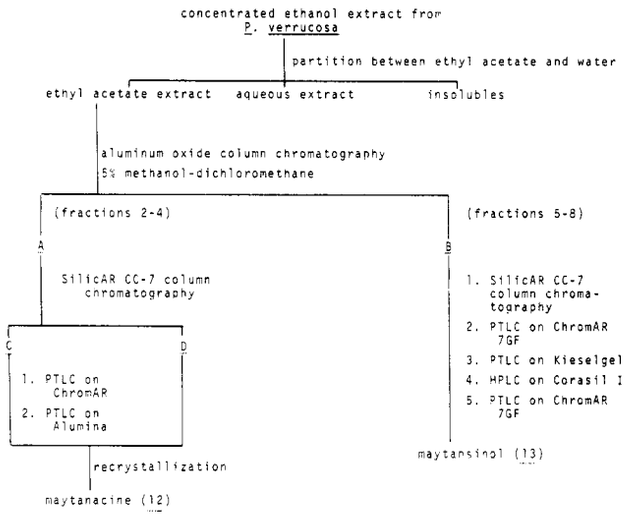
571, a loss of 60 mass units and typical of the loss of CH_3COOH. A further loss of 88 mass units, corresponding to loss of $C_4H_8O_2$, gave a major ion at *m/e* 483. The next two major ions, *m/e* 468 and 448, resulted from the loss of CH_3 and Cl, respectively, from the *m/e* 483 ion. The latter three ions thus fit the typical fragmentation pattern of maytansinoids but each ion was two mass units less than expected. This, taken together with the ions at *m/e* 631 and 571, indicated that there were two side chain esters, one of which was an acetate.

The relative positions of the two esters and the identity of the second ester were established by NMR and chemical means. The NMR spectrum revealed that there was no *N*-methyl in the side chain ester. There was, however, a six-proton doublet centered at δ 1.28 and multiple signals from δ 2.6 to 2.0 containing three protons, two which could be assigned to the C-2 protons and one which could be assigned to the second ester. These data suggested that the second ester was an isobutyrate. In addition, the two doublets corresponding to the C-15 protons which should appear at δ 3.7 and 3.1 were absent. Instead signals were observed for an acetate methyl singlet at δ 2.23 and a one-proton singlet at δ 6.21. Mild hydrolysis of maytanbutacine with sodium bicarbonate in aqueous methanol removed the acetate, and in the NMR spectrum the one-proton singlet at δ 6.21 shifted upfield to δ 5.37. This behavior was similar to that observed in colubrinol acetate[25] and indicated that the isobutyrate ester was at C-3, since it was not affected by the hydrolysis. The acetate was, therefore, at C-15. This latter fact was confirmed by oxidation of the deacetyl compound (**9**) with Jones reagent to form a conjugated enone system, as indicated by UV.

The biological activity of the extracts of both *M. serrata* and *M. buchananii* prompted a search of other related Celastraceae plants as potential sources of the maytansinoids. Thus, *Putterlickia verrucosa* Szyszyl. was found to be the best source of maytansine to date.[8] A 1-kg ethanolic extract of *P. verrucosa*[26] was fractionated by a modification of the procedure developed for *M. serrata* (Chart I) guided both by assay against PS and KB and by analytical TLC using authentic materials as references. The ethanolic extract was partitioned between ethyl acetate and water, and the ethyl acetate soluble material was subjected to column chromatography over SilicAR CC-7. The fraction which was eluted with 5% methanol–chloroform was treated with acetic anhydride–pyridine, and the residue from this reaction was subjected to column chromatography over SilicAR CC-7. The fraction which was eluted with 5% methanol–chloroform from this column was then subjected to PTLC first over alumina and then over ChromAR to give three homogeneous solids which upon crystallization yielded maytansine (**1**, 12.3 mg/kg), maytanbutine (**3**, 4.5 mg/kg), and maytanprine (**2**, 8.5 mg/kg).[27] PTLC of a higher R_f band on ChromAR and subsequent crystallization yielded a new maytansinoid, maytanacine (**12**, ~0.2 mg/kg).

The high yield of maytansine and the isolation of maytanacine, apparently an acetyl derivative, prompted fractionation of *P. verrucosa* on a larger scale. To eliminate the possibility that maytanacine was an artifact arising from the acetylation step, an alternative procedure was employed. A 10-kg ethanolic extract of *P. verrucosa* (Chart III) was fractionated by a modification of the procedure developed for *M. buchananii*, again guided by biological assays and by analytical TLC using authentic materials for references. The ethanolic extract was first partitioned between ethyl acetate and water. The active ethyl acetate solubles were then subjected to column chromatography over deactivated alumina (activity II–III). The fractions which were eluted with 5% methanol–chloroform were examined by HPLC, and similar fractions were combined to give fractions A (fractions 2–4) and B (fractions 5–8).

Chart III. Fractionation of the Active Extract from *Putterlickia verrucosa*



Fraction A was subjected to column chromatography over SilicAR CC-7 with increasing amounts of ethyl acetate in benzene as eluent. The fractions which were eluted with 66% ethyl acetate in benzene were examined by HPLC, and similar fractions were combined to give fractions C and D, both of which contained maytanacine (**12**). PTLC of each fraction on ChromAR with 5% methanol–chloroform followed by PTLC of the band corresponding to **12** on alumina with 10% methanol–ethyl acetate gave pure **12**. Crystallization from dichloromethane–hexanes yielded a total of 0.18 mg/kg of maytanacine (**12**).

The presence of such a variety of C-3 esters prompted an effort to isolate a possible common precursor, the C-3 alcohol. To aid in the isolation, synthetic maytansinol (**13**) was prepared by reductive cleavage of maytanbutine (**3**). Treatment of **3** with lithium aluminum hydride in dry tetrahydrofuran[28] gave, after extensive PTLC of the products, maytansinol (**13**) in 40% yield. This synthetic material was then used as a reference in the isolation of the naturally occurring maytansinol.

Fraction B was subjected to column chromatography over SilicAR CC-7 and eluted with increasing amounts of methanol in chloroform. The material eluted with 5% methanol–chloroform was then chromatographed on ChromAR developed with 5% methanol–ethyl acetate. The band corresponding to synthetic maytansinol was isolated and subjected to preparative TLC on Kieselgel plates developed with 15% ethanol–ether. Further preparative HPLC and TLC gave pure maytansinol (**13**) (0.025 mg/kg), identical in every respect with synthetic **13**.

To confirm the chemical relationship between maytanacine and maytansinol, maytanacine (**12**) was treated with lithium aluminum hydride to give maytansinol (**13**). This maytansinol was converted back to maytanacine (53%) by treatment with acetic anhydride and pyridine[29] and was identical with the natural product in all respects.

The structures of maytanacine and maytansinol were confirmed by their spectra. The UV spectra of both **12** and **13** were typical of maytansinoids, with maxima at 233, 242, 252, 281, and 289 nm. The IR spectra confirmed the principal difference between **12** and **13**; **12** had the absorptions (5.70, 5.80, 6.00 μ) expected for a maytanside ester, while **13** had only two carbonyl bands (5.85 and 6.06 μ). The absence of the band at 5.70 μ indicated that **13** was missing the side chain ester moiety at C-3.

The mass spectral data (Table I) corroborated differences

between **12** and **13**. The mass spectrum of **12** had fragments at *m/e* 545 [$M^+ - (H_2O + HNCO)$] and 485 [$M^+ - (H_2O + HNCO)$ − side chain acid]. The loss of 60 mass units, which corresponded to loss of the side chain acid, indicated that the ester was an acetate. The fragmentation pattern of **13** showed ions at *m/e* 503 [$M^+ - (H_2O + HNCO)$] and 485 [$M^+ - (H_2O + HNCO)$ − side chain acid]. Loss of 18 mass units as the "side chain acid" corresponded to loss of water, as would be expected for **13**.

The NMR spectrum of maytanacine lacked signals for the C-2′ H, C-2′ CH_3, and NCH_3 of the amino ester side chain, but had an acetate methyl singlet at δ 2.18. The NMR spectrum of maytansinol lacked signals due to a side chain ester; the C-3 proton signal was shifted upfield and obscured by other peaks, and the C-3 hydroxyl proton appeared as a singlet at δ 3.44.

The spectral evidence taken in conjunction with the chemical evidence provided by the lithium aluminum hydride reductive cleavage and subsequent acylation thus established the structure of maytanacine as **12** and that of maytansinol as **13**.

We would like to acknowledge with thanks the preparation of many large scale extracts of the plant material by Mr. Barry R. Sickles.

Experimental Section

Melting points were determined on a Mettler FP2 melting point apparatus. Ultraviolet absorption spectra were determined on Beckman Model DK-2A and Coleman Hitachi Model EPS-3T recording spectrophotometers. Infrared spectra were determined on a Perkin-Elmer Model 257 recording spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian HA-100 spectrometer or a JEOL PS-100 pulsed FT NMR spectrometer interfaced to a Texas Instrument JEOL 980A computer, with tetramethylsilane as the internal standard. Mass spectra were determined on Hitachi Perkin-Elmer Model RMU-6E and AEI Model MS902 spectrometers at the University of Virginia. Additional mass spectra were obtained at the Mass Spectrometry Laboratories of Battelle Memorial Institute and Research Triangle Institute. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Analytical high-pressure liquid chromatography was performed on Waters Associates Models ALC-100 and ALC-202 employing a Corasil II column (4 ft × 0.125 in.) with 1.5% methanol–dichloromethane as the eluent and a flow rate of 1 mL/min. Gas–liquid chromatography was carried out on a Varian Model 1860-1 aerograph moduline gas chromatograph. Petroleum ether refers to the fraction with bp 60–68 °C. All thin layer chromatography was carried out on prepared plates (E. Merck and Mallinckrodt).

Maytenus serrata. The ground dried fruit (10 kg) of *M. serrata* was extracted in Soxhlet extractors with 80 L of 95% ethanol for 6 h. The plant material was extracted again with 80 L of fresh 95% ethanol for 15 h. After a third extraction of 24 h, the extracts were combined and concentrated at 40–50 °C in vacuo to give a dark gum (1.35 kg). The concentrated alcoholic extract was partitioned between ethyl acetate (3 L) and water (1.5 L) by stirring vigorously with a mechanical stirrer for 12 h. The suspension was filtered and the insoluble material was treated two more times with ethyl acetate (1 L) and water (0.5 L), followed by filtration. The aqueous layers were combined and washed with ethyl acetate (2 × 0.5 L). The combined ethyl acetate layers were washed with water (0.5 L) and concentrated to a dark gum (220 g). This residue was partitioned between 10% aqueous methanol (1.5 L) and petroleum ether (1 × 1.5 L, 2 × 0.5 L). The combined petroleum ether layers were washed with 10% aqueous methanol (300 mL) and concentrated to a dark green oil (60 g). Concentration of the aqueous methanol layers gave a dark green gum (A, 160 g).

Fraction A was dissolved in 250 mL of 30% methanol–chloroform and adsorbed on 320 g of SilicAR. The mixture was thoroughly dried and placed on top of a column prepared from 1.28 kg of SilicAR in chloroform, and 3.2-L fractions were collected. Fractions 1–5 were eluted with chloroform, fractions 6–15 with 5% methanol–chloroform, and fractions 16–18 with methanol. The PS and KB active fractions 7 and 8 were combined (B, 18 g). A solution of fraction B in 108 g of pyridine–acetic anhydride (1:2) was stirred at room temperature for 15 h, then concentrated to a dark oil.

The dark oil was chromatographed on a column of SilicAR (100 g), collecting 200-mL fractions. Fractions 1–5 were eluted with chloroform, fractions 6–10 with 5% methanol–chloroform, and fractions 11–13 with methanol. The PS and KB active fractions 7–10 were concentrated, then chromatographed on a column of alumina (Woelm, activity I) collected 18-mL fractions. Fractions 1–3 were eluted with dichloromethane, fractions 4–8 with 30% methanol–chloroform, and fractions 9–11 with methanol. The PS and KB active fractions 5–8 were combined (C, 720 mg).

PTLC of fraction C on alumina developed with 7% methanol–ethyl acetate gave a band (R_f 0.5) in which most of the activity was concentrated (D, 120 mg). PTLC of fraction D on silica gel developed with 3% methanol–ethyl acetate gave a band (R_f 0.2) corresponding to fraction E (19 mg) and all bands of higher R_f than fraction E were combined to afford fraction F (57 mg). Fraction E was further purified by PTLC on ChromAR developed with 5% methanol–ethyl acetate to give a highly enriched concentrate of maytansine (**1**) (R_f 0.25, fraction G, 10 mg).

Maytansine Bromopropyl Ether (11). Method A. A mixture of fraction G (20.2 mg), 3-bromopropanol (234 mg), and *p*-toluenesulfonic acid (0.6 mg) in 1.0 mL of dichloromethane was allowed to stand at room temperature for 7 h, then quenched with 3 mL of 5% sodium bicarbonate solution and 10 mL of dichloromethane. The water layer was extracted with dichloromethane (2 × 5 mL) and the combined dichloromethane layers were washed with water and concentrated to dryness. PTLC of this residue on ChromAR developed with ethyl acetate gave a product (11.6 mg) which crystallized from a mixture of dichloromethane–hexane. Two recrystallizations gave 3.7 mg (~15%) of maytansine 3-bromopropyl ether (**11**): mp 176–178 °C; IR (KBr) 5.76, 6.01, 6.34, 8.42, 9.29 μ. Recrystallization of **11** from methanol by slow evaporation gave crystals suitable for x-ray analysis.

Method B. A 1-dram vial containing maytansine (4.8 mg), 3-bromopropanol (60 μL, Aldrich 98%, purified by PTLC on silica gel 60 F-254 using ethyl acetate as the eluent and visualization with iodine), benzene (300 μL, dried over 3 Å molecular sieves), and trifluoroacetic acid (4 drops) was allowed to stand for 3 days under nitrogen in a sealed 1-oz jar containing 3 Å molecular sieves. PTLC on silica gel 60 F254 plates developed with ethyl acetate followed by crystallization (twice) from methylene chloride–ether–hexanes gave **11** (3.1 mg, 55%, 71% based on maytansine recovered).

Acid Hydrolysis of Maytansine Bromopropyl Ether (11). A solution of **11** in 10% aqueous methanol (0.1 mL) and 1 drop of 2 N hydrochloric acid was allowed to stand at room temperature for 2.5 h, then quenched with 1 mL of 5% sodium bicarbonate solution and 5 mL of dichloromethane. The water layer was extracted with dichloromethane (2 × 2 mL) and the combined organic layer was purified by PTLC on ChromAR developed with ethyl acetate. Crystallization twice from dichloromethane–hexane gave the hydrolysis product (**1**).

Maytansine (1). A solution of fraction G (54.9 mg) in dichloromethane–hexane was seeded with the crystalline hydrolysis product of **11** to induce crystallization. Several recrystallizations from this system and finally from dichloromethane–ether gave maytansine (**1**, 11.0 mg, 0.2 mg/kg of plant): mp 171–172 °C; $[\alpha]^{26}_D$ − 145° (*c* 0.055, $CHCl_3$); UV max (EtOH) 233 nm (ε 29 800), 243 (sh, 27 100), 254 (27 200), 282 (5690), 290 (5520); IR (KBr) 5.75, 5.80, 6.02, 6.34, 8.42, 9.26 μ; NMR ($CDCl_3$) δ 0.87 (3 H, s, C-4 CH_3), 1.34 (3 H, d, J = 6 Hz, C-6 CH_3), 1.37 (3 H, d, J = 7 Hz, C-2′ CH_3), 1.69 (3 H, br s, C-14 CH_3), 2.15 (3 H, s, C-2′ $NCOCH_3$), 2.21 (1 H, dd, $J_{2,2}$ = 15, $J_{2,3}$ = 3 Hz, C-2 H), 2.65 (1 H, dd, $J_{2,2}$ = 15, $J_{2,3}$ = 12 Hz, C-2 H), 2.89 (3 H, s, C-2′ NCH_3), 3.04 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.13 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.22 (3 H, s, C-1 NCH_3), 3.38 (3 H, s, C-10 Och_3), 3.50 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.53 (1 H, s, C-9 OH), 3.67 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.99 (3 H, s, C-20 OCH_3), 4.28 (1 H, m, C-7 H), 4.79 (1 H, dd, $J_{2,3}$ = 12, 3 Hz, C-3 H), 5.35 (1 H, q, J = 7 Hz, C-2′ H), 5.66 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.24 (1 H, br s, C-9 NH), 6.42 (1 H, dd, $J_{11,12}$ = 15, $J_{12,13}$ = 11 Hz, C-12 H), 6.70 (1 H, br d, $J_{12,13}$ = 11 Hz, C-13 H), 6.75, 6.84 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 0.80–2.50 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 630.2680, $C_{33}H_{43}ClN_2O_8$ [$M - 61 (H_2O + HNCO)$] = 630.2708.

Anal. Calcd for $C_{34}H_{46}ClN_3O_{10}\cdot H_2O$: C, 57.50; H, 6.98; Cl, 4.99; N, 5.91. Found: C, 57.34; H, 7.42; Cl, 4.81; N, 6.38.

Isolation of Fraction F from *Maytenus buchananii.* Fraction D (296 mg) was obtained from stem barks (5.6 kg) and roots (4.0 kg) of *M. buchananii* (Loes.) R. Wilczek collected in Kenya in 1970. PTLC of fraction D on silica gel developed with ethyl acetate followed by 3% methanol–ethyl acetate gave fraction E (72 mg) which yielded 13.5 mg of maytansine (0.00015%) after further purification, and fraction F (110 mg). Preparative HPLC (Corasil II, 3 ft × 0.375 in., 2% methanol–dichloromethane, 1.4 mL/min) of fraction F gave fractions H (16 mg) and I (20 mg).

Maytanprine (2). Fraction H was crystallized by seeding with the crystalline hydrolysis product of the ethyl ether derivative of fraction H, and recrystallized three times from dichloromethane–ether to afford maytanprine (**2**, 0.00012%): mp 169–170 °C; $[\alpha]^{30}_D$ − 125° (*c* 0.056, $CHCl_3$); UV max (EtOH) 234 nm (ε 30 700), 243 (sh, 28 200), 254 (27 800), 282 (5870), 290 (5800); IR (KBr) 5.73, 5.80, 6.03, 6.33, 8.43, 9.26 μ; NMR ($CDCl_3$) δ 0.87 (3 H, s, C-4 CH_3), 1.18 (3 H, t, *J* = 7 Hz, C-4′ CH_3), 1.35 (3 H, d, *J* = 7 Hz, C-6 CH_3), 1.36 (3 H, d, *J* = 7 Hz, C-2′ CH_3), 1.70 (3 H, br s, C-14 CH_3), 2.19 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 3 Hz, C-2 H), 2.37 (1 H, m, C-4′ H), 2.41 (1 H, m, C-4′ H), 2.64 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 12 Hz, C-2 H), 2.86 (3 H, s, C-2′ NCH_3), 3.04 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.12 (1 H, d, $J_{15,15}$ = 12 Hz, C-15 H), 3.21 (3 H, s, C-1 NCH_3), 3.38 (3 H, s, C-10 OCH_3), 3.50 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.55 (1 H, s, C-9 OH), 3.68 (1 H, d, $J_{15,15}$ = 12 Hz, C-15 H), 4.00 (3 H, s, C-20 OCH_3), 4.28 (1 H, m, C-7 H), 4.79 (1 H, dd, $J_{2,3}$ = 12, 2.5 Hz, C-3 H), 5.40 (1 H, q, *J* = 7 Hz, C-2′ H), 5.67 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.26 (1 H, br s, C-9 NH), 6.42 (1 H, dd, $J_{11,12}$ = 15, $J_{12,13}$ = 11 Hz, C-12 H), 6.76 (1 H, br d, $J_{12,13}$ = 11 Hz, C-13 H), 6.66, 6.82 (2 h, d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 0.80–2.00 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 644.2810, $C_{34}H_{45}ClN_2O_8$ [M − 61 (H_2O + HNCO)] = 644.2864.

Anal. Calcd for $C_{35}H_{48}ClN_3O_{10}$: C, 59.52; H, 6.85; N, 5.95. Found: C, 59.31; H, 6.78; N, 5.89.

Maytanbutine (3). Fraction I was crystallized by seeding with the crystalline hydrolysis product of the 3-bromopropyl ether derivative of fraction I, and recrystallized three times from dichloromethane–ether to afford maytanbutine (**3**, 0.00009%): mp 170–171 °C; $[\alpha]^{30}_D$ − 122° (*c* 0.049, $CHCl_3$); UV max (EtOH) 234 nm (ε 33 100), 243 (sh, 30 400), 254 (30 500), 282 (6430), 290 (6380); IR (KBr) 5.72, 5.79, 6.04, 6.06, 6.34, 8.44, 9.28 μ; NMR ($CDCl_3$) δ 0.88 (3 H, s, C-4 CH_3), 1.12 (3 H, d, *J* = 7 Hz, C-4′ CH_3), 1.19 (3 H, d, *J* = 7 Hz, C-4′ CH_3), 1.35 (3 H, d, *J* = 6 Hz, C-6 CH_3), 1.36 (3 H, d, *J* = 6 Hz, C-2′ CH_3), 1.70 (3 H, br s, C-14 CH_3), 2.20 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 3 Hz, C-2 H), 2.65 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 12 Hz, C-2 H), 2.80 (1 H, m, C-4′ H), 2.87 (0.75 H, s) and 2.92 (2.25 H, s, C-2′ NCH_3), 3.04 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.12 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.20 (3 H, s, C-1 NCH_3), 3.37 (3 H, s, C-10 OCH_3), 3.51 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.52 (1 H, s, C-9 OH), 3.68 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 4.00 (3 H, s, C-20 OCH_3), 4.29 (1 H, m, C-7 H), 4.78 (1 H, dd, $J_{2,3}$ = 12, 3 Hz), 5.39 (1 H, q, *J* = 7 Hz, C-2′ H), 5.64 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.25 (1 H, br s, C-9 NH), 6.43 (1 H, dd, $J_{11,12}$ = 15, $J_{12,13}$ = 11 Hz, C-12 H), 6.79 (1 H, br d, $J_{12,13}$ = 11 Hz, C-13 H), 6.66, 6.82 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 0.80–2.00 (3 H, C-6 H, C-8 H_2); NMR ($CDCl_3$ + C_6D_6) δ 1.17 (3 H, d, *J* = 7 Hz, C-4′ CH_3), 1.22 (3 H, d, *J* = 7 Hz, C-4′ CH_3); mass spectrum *m/e* 658.3030, $C_{35}H_{47}ClN_2O_8$ [M − 61 (H_2O + HNCO)] = 658.3021.

Anal. Calcd for $C_{36}H_{50}ClN_3O_{10}$: C, 60.03; H, 7.00; N, 5.83. Found: C, 59.87; H, 7.11; N, 5.87.

***Maytenus buchananii*, Improved Fractionation Procedure.** The ground dried stems and bark (19.8 kg) of *M. buchananii* were extracted in three batches in a Soxhlet extractor with 8 L of 95% ethanol for 6 h for each batch. The plant material was extracted again with 8 L of fresh 95% ethanol for 15 h. After a third extraction of 24 h, the extracts were combined and concentrated at 40–50 °C in vacuo to give a dark gum (963 g). The concentrated alcoholic extract was partitioned between ethyl acetate (4 L) and water (3 L) by stirring vigorously with a mechanical stirrer for 12 h. The suspension was filtered and the insoluble material was treated two more times with ethyl acetate (2 L) and water (1 L), followed by filtration. The aqueous layers were combined and washed with ethyl acetate (2 × 1 L).

The combined ethyl acetate layer was partitioned with a cold 5% sodium hydroxide solution (1 × 4 L, 4 × 1 L). The combined aqueous layer was washed with ethyl acetate (5 × 600 mL), acidified with 3 N hydrochloric acid, and extracted with ethyl acetate to give the acidic fraction which was devoid of biological activity. The combined ethyl acetate layer was then partitioned with cold 2 N hydrochloric acid (5 × 600 mL). The combined aqueous layer was washed with ethyl acetate (3 × 600 mL), basified with sodium bicarbonate, and extracted with ethyl acetate to give the alkaloidal fraction which was devoid of biological activity. The combined ethyl acetate layer was washed with water until neutral pH and concentrated to afford fraction A (135 g).

A solution of fraction A in 400 mL of pyridine–acetic anhydride (1:1) was stirred at room temperature for 15 h, then concentrated to a dark oil. The dark oil was partitioned between aqueous methanol (50 mL) and carbon tetrachloride (1 × 50 mL, 4 × 20 mL). The combined carbon tetrachloride layer was extracted with 20% aqueous methanol (7 × 20 mL) and concentrated to dryness. Water was added to the combined aqueous methanol layer to give 35% aqueous methanol layer which was partitioned with chloroform (1 × 50 mL, 4 × 20 mL). The combined chloroform layer was dried over sodium sulfate and concentrated to give fraction B (20.8 g).

Fraction B was chromatographed on a column of alumina (600 g, activity II–III), collecting 3-L fractions. Fractions 1–3 were eluted with dichloromethane, fractions 4–6 with 0.3% methanol–dichloromethane, fractions 7–10 with 3% methanol–dichloromethane, and fraction 11 with 30% methanol–dichloromethane. The PS and KB active fractions 7–11 were combined to give fraction C (4.9 g) which was chromatographed on a column of SilicAR, collecting 1-L fractions. Fractions 1–41 were eluted with benzene–ethyl acetate (1:1), fractions 42–49 with ethyl acetate, and fraction 50 with methanol. The eluent was monitored by analytical HPLC. Fractions 6–10 were combined to give fraction D (514 mg), fractions 11–17 to give fraction E (246 mg), fractions 18–41 to give fraction F (723 mg), and fractions 42–49 to give fraction G (666 mg).

Maytanvaline (4). PTLC of the appropriate portion of fraction D on alumina, developed with 5% methanol–ethyl acetate, gave crude 4 which was separated from a higher R_f maytansinoid by PTLC on ChromAR developed with 20% benzene–ethyl acetate × 2. Partition chromatography (20% aqueous methanol/20% chloroform–heptane) over Celite, followed by PTLC on ChromAR, developed with 5% methanol–chloroform, gave homogeneous material which was crystallized from dichloromethane–ether to give 4 (6.9 mg, 0.0000035%): mp 175–176.5 °C; $[\alpha]^{26}_D$ −135° (*c* 0.950, $CHCl_3$); UV (EtOH) 233 nm (ε 29 100), 243 (sh, 26 400), 254 (26 800), 281 (5300), 288 (5360); IR (KBr) 5.72, 5.80, 6.02, 6.34, 8.48, 9.27 μ; NMR ($CDCl_3$) δ 0.81 (3 H, s, C-4 CH_3), 0.92 (3 H, d, *J* = 7 Hz, C-5′ CH_3), 0.95 (3 H, d, *J* = 7 Hz, C-5′ CH_3), 1.29 (3 H, d, *J* = 6 Hz, C-6 CH_3), 1.32 (3 H, d, *J* = 7 Hz, C-2′ CH_3), 1.65 (3 H, s, C-14 CH_3), 2.14 (2 H, d, *J* = 7 Hz, C-4′ H_2), 2.24 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 3 Hz, C-2 H), 2.68 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 11 Hz, C-2 H), 2.86 (3 H, s, C-2′ NCH_3), 3.03 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.12 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.20 (3 H, s, C-1 NCH_3), 3.35 (3 H, s, C-10 OCH_3), 3.49 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.52 (1 H, s, C-9 OH), 3.67 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.98 (3 H, s, C-20 OCH_3), 4.30 (1 H, m, C-7 H), 4.78 (1 H, dd, $J_{2,3}$ = 11, 3 Hz, C-3 H), 5.34 (1 H, q, *J* = 7 Hz, C-2′ H), 5.66 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.24 (1 H, s, C-9 NH), 6.48 (1 H, dd, $J_{11,12}$ = 15, $J_{12,13}$ = 10 Hz, C-12 H), 6.71 (1 H, d, $J_{12,13}$ = 10 Hz, C-13 H), 6.70, 6.83 (2 H, 2 d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 1.0–2.0 (4 H, C-6 H, C-8 H_2, C-5′ H).

Anal. Calcd for $C_{37}H_{52}ClN_3O_{10}$: C, 60.51; H, 7.14; N, 5.72. Found: C, 60.43; H, 7.20; N, 5.71.

Conversion of Maytanvaline to Maysine and Isolation of *N*-Isovaleryl-*N*-methyl-L-alanine Methyl Ester. A mixture of maytanvaline (19.8 mg) and sodium carbonate (15 mg) in 2 mL of 50% aqueous methanol containing 0.2 mL of tetrahydrofuran was allowed to stir at room temperature for 3 h. The reaction mixture was acidified in the cold and extracted with ethyl acetate (3 × 5 mL) to give 22.2 mg of yellow solid. This solid was purified by PTLC on ChromAR, developed with ethyl acetate, to give a fraction corresponding in R_f to maysine (3.8 mg) and 4.2 mg of recovered maytanvaline. All fractions (separations made based on UV detection) of higher R_f than maysine were dissolved separately in methanol and each one was treated with ethereal diazomethane. Preparative GLC of the appropriate fractions gave 1.2 mg of *N*-isovaleryl-*N*-methyl-L-alanine methyl ester characterized by mixture GLC (E307 and 3% SE-30), IR, NMR, and mass spectrum with an authentic sample of the synthetically prepared amino acid ester. The maysine isolated was further purified by PTLC on alumina, developed with 10% methanol–ethyl acetate, to give 1.4 mg of maysine which was identical with an authentic sample by mixture TLC and HPLC, IR, NMR, and mass spectrum.

***N*-Isovaleryl-*N*-methyl-L-alanine Methyl Ester.** A methanolic solution of *N*-methyl-L-alanine (103 mg, 1 mmol) was treated with ethereal diazomethane at 0 °C. The reaction mixture was allowed to warm to room temperature over a 2-h period and most of the solvent was removed under a stream of nitrogen gas. A mixture of the crude methyl ester and potassium carbonate (500 mg) in chloroform–water (4 mL, 1:1) was cooled to 0 °C and an excess of isovaleryl chloride (prepared from isovaleric acid and benzoyl chloride)[30] was added. The mixture was allowed to warm to room temperature and stirred vigorously for 18 h. Water was added and the aqueous layer was thoroughly extracted with chloroform. Preparative GLC (E307 Chromosorb W 60/80 glass column, He 40 mL/min, column temperature 105 °C, injection port 187 °C, t_R 6.24 min) gave 75 mg (37%) of *N*-isovaleryl-*N*-methyl-L-alanine methyl ester as a colorless liquid: IR (neat) 3.36, 3.48, 5.72, 6.07 μ; NMR ($CDCl_3$) δ 0.95 (6 H, d, *J* = 6 Hz, 2 CH_3), 1.36 (3 H, d, *J* = 7.6 Hz, $CHCH_3$), 2.16 (1 H, m, CH_2CH), 2.17 (2 H, d, *J* = 2 Hz, CH_2CH), 2.77, 2.89 (0.5 H, 2.5 H, NCH_3), 3.62 (3 H, s, $COOCH_3$), 5.16 (1 H, q, *J* = 7.6 Hz, $CHCH_3$); mass spectrum *m/e* 202.1434, $C_{10}H_{19}NO_3$ (M + H^+) = 202.1438.

Maysine (5). PTLC of fraction F on alumina, with 5% methanol–ethyl acetate × 3 as eluent, gave **5** (9.9 mg, 0.000005%): mp 137–141 °C; $[\alpha]^{30}_D$ −173° (*c* 0.023, EtOH); UV (EtOH) 226 nm (ε 29 100), 241 (sh, 23 300), 252 (sh, 17 500), 280 (4280), 289 (sh, 3900); IR (KBr) 5.85, 6.01, 6.14, 6.34, 9.19 μ; NMR ($CDCl_3$) δ 1.06 (3 H, s, C-4 CH_3), 1.30 (3 H, d, J = 6 Hz, C-6 CH_3), 1.68 (3 H, br s, C-14 CH_3), 2.62 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.02 (1 H, d, $J_{15,15}$ = 12 Hz, C-15 H), 3.22 (3 H, s, C-1 NCH_3), 3.28 (3 H, s, C-10 OCH_3), 3.39 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.42 (1 H, d, $J_{15,15}$ = 12 Hz, C-15 H), 3.92 (3 H, s, C-20 OCH_3), 4.24 (1 H, m, C-7 H), 5.43 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 5.65 (1 H, d, $J_{2,3}$ = 16 Hz, C-2 H), 6.02 (1 H, br d, $J_{12,13}$ = 11 Hz, C-13 H), 6.29 (1 H, s, C-9 NH), 6.34 (1 H, dd, $J_{12,13}$ = 11, $J_{11,12}$ = 15 Hz, C-12 H), 6.37 (1 H, d, $J_{2,3}$ = 16 Hz, C-3 H), 6.62, 6.74 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 3.20–3.50 (1 H, C-9 OH), 0.70–2.50 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 485.1974, $C_{27}H_{32}ClNO_5$ [M − 61 (H_2O + HNCO)] = 485.1969.

Normaysine (6). The PTLC which gave maysine also gave normaysine (**6**, 13.8 mg, 7 × 10^{-6}%): mp 187–188 °C (acetone); $[\alpha]^{30}_D$ −217° (*c* 0.051, EtOH); UV (EtOH) 229 nm (ε 44 500), 242 (sh, 36 400), 252 (sh, 27 300), 280 (sh, 5770), 290 (sh, 5200); IR (KBr) 5.92, 6.01, 6.12, 6.34, 9.24 μ; NMR ($CDCl_3$) δ 1.21 (3 H, s, C-4 CH_3), 1.32 (3 H, d, J = 6 Hz, C-6 CH_3), 1.69 (3 H, br s, C-14 CH_3), 2.67 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.05 (1 H, d, $J_{15,15}$ = 12 Hz, C-15 H), 3.20 (3 H, s, C-10 OCH_3), 3.43 (1 H, d, $J_{15,15}$ = 12 Hz, C-15 H), 3.44 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.58 (1 H, s, C-9 OH), 3.88 (3 H, s, C-20 OCH_3), 4.28 (1 H, m, C-7 H), 5.48 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.03 (1 H, br d, $J_{12,13}$ = 11 Hz, C-13 H), 6.05 (1 H, d, $J_{2,3}$ = 16 Hz, C-2 H), 6.35 (1 H, s, C-9 NH), 6.41 (1 H, dd, $J_{12,13}$ = 11, $J_{11,12}$ = 15 Hz, C-12 H), 6.52 (1 H, d, $J_{2,3}$ = 16 Hz, C-3 H), 6.58, 6.63 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 7.38 (1 H, s, C-1 NH), 1.00–2.00 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 471.1807, $C_{26}H_{30}ClNO_5$ [M − 61 (H_2O + HNCO)] = 471.1812.

Anal. Calcd for $C_{27}H_{33}ClN_2O_7 \cdot \frac{1}{2}H_2O$: C, 59.83; H, 6.32; N, 5.17. Found: C, 59.24; H, 6.25; N, 5.22.

Maysenine (7). PTLC of fraction D on alumina developed with 5% methanol–ethyl acetate gave a major component which was submitted to PTLC on ChromAR, with 10% benzene–ethyl acetate × 2 as the eluent, to afford maysenine (**7**, 5.1 mg, 0.0000026%): mp 184–185 °C (acetone); $[\alpha]^{30}_D$ −57° (*c* 0.056, EtOH); UV max (EtOH) 234 nm (sh, ε 44 000), 243 (53 400), 252 (sh, 41 400), 271 (23 500), 300 nm (sh, 9470); IR (KBr) 5.87, 6.01, 6.21, 6.31, 9.26 μ; NMR ($CDCl_3$) δ 1.25 (3 H, d, J = 6 Hz, C-6 CH_3), 1.56 (3 H, br s, C-4 CH_3), 1.65 (3 H, br s, C-14 CH_3), 3.07 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.10 (1 H, s, C-9 OH), 3.29 (3 H, s, C-10 OCH_3), 3.40 (1 H, d, $J_{15,15}$ = 13 Hz, C-15 H), 3.45 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.88 (3 H, s, C-20 OCH_3), 4.11 (1 H, m, C-7 H), 5.42 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 14 Hz, C-11 H), 5.50 (1 H, br d, $J_{5,6}$ = 10 Hz, C-5 H), 5.82 (1 H, d, $J_{2,3}$ = 16 Hz, C-2 H), 6.00 (1 H, br d, $J_{12,13}$ = 10 Hz, C-13 H), 6.16 (1 H, s, C-9 NH), 6.37 (1 H, dd, $J_{12,13}$ = 10, $J_{11,12}$ = 14 Hz, C-12 H), 6.51, 6.57 (2 H, d, $J_{17,21}$ = 1 Hz, C-17 H, C-21 H), 7.15 (1 H, s, C-1 NH), 7.28 (1 H, d, $J_{2,3}$ = 16 Hz, C-3 H), 1.00–2.00 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 455.1844, $C_{26}H_{30}ClNO_4$ [M − 61 (H_2O + HNCO)] = 455.1863.

Anal. Calcd for $C_{27}H_{33}ClN_2O_6 \cdot \frac{1}{2}H_2O$: C, 61.65; H, 6.51; N, 5.33. Found: C, 61.29; H, 6.74; N, 5.45.

Methyl Maysine (14). A mixture of crude maysine (16.7 mg) and *p*-toluenesulfonic acid (one small crystal) in dry methanol (0.5 mL) was allowed to stand at room temperature for 17 h. The reaction mixture was quenched by the addition of 1 drop of 5% sodium bicarbonate and purified by PTLC on ChromAR, developed with ethyl acetate to give 13.0 mg of crystalline product which was recrystallized from dichloromethane–ether–hexane to give methyl maysine (2.3 mg, 13%): mp 178–179 °C; IR (KBr) 5.80, 6.00, 6.13, 6.33, 9.20 μ; NMR ($CDCl_3$) δ 3.21 (6 H, s, C-1 NCH_3, C-9 OCH_3); mass spectrum *m/e* 528.2031, $C_{28}H_{33}ClN_2O_6$ (M − CH_3OH) = 528.2027.

Hydrolysis of Methyl Maysine. A mixture of methyl maysine (2.0 mg), 50% aqueous methanol (0.2 mL), and 2 N hydrochloric acid (1 drop) was allowed to stand at room temperature for 1 h. The reaction mixture was quenched by the addition of 1 drop of 5% sodium bicarbonate and purified by PTLC on alumina, developed with 10% methanol–ethyl acetate, to give **5** which was identical with natural maysine in all respects.

Conversion of Maytansine to Maysine. A mixture of maytansine (18.0 mg) and sodium carbonate (20 mg) in 2 mL of 50% aqueous methanol was allowed to stir at room temperature for 4 h. The reaction mixture was extracted with dichloromethane (3 × 5 mL) to give a solid residue which was purified by PTLC on alumina, developed with 10% methanol–ethyl acetate. In addition to the major product (2.3 mg), which was identical with natural maysine in all respects, 1.8 mg of maytansine was recovered. The product was further characterized by preparation of the methyl ether derivative which was identical with an authentic sample by IR and mixture melting point.

Conversion of Normaysine (6) to Maysenine (7). A mixture of normaysine (3.8 mg), chromous chloride (2 drops), and acetic acid (3 drops) was allowed to stand at room temperature for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane (three times). The organic layer was washed with water (twice), 5% sodium bicarbonate, and again with water (three times). PTLC on alumina, developed with 10% methanol–ethyl acetate × 2, gave 1.2 mg of an unknown product and 1.2 mg of maysenine, mp 184–185 °C, identical with an authentic sample by UV, IR, and mixture melting point.

Maytanbutacine (8). PTLC of fraction E from *Maytenus serrata* on alumina with 5% methanol–ethyl acetate gave two bands. The lower R_f band was separated and subjected to PTLC on silica gel in ethyl acetate. Again two bands resulted and the higher R_f band was isolated. Preparative HPLC of this band on a Corasil II column (3 ft × 0.375 in., 1 mL/min, 1 faction/2 min) using 1% methanol–dichloromethane gave fraction H (fractions 74–94). PTLC of fraction H on SilicAR in ethyl acetate (twice) gave a concentrated band of maytanbutacine (R_f 0.67). Repeated crystallization from dichloromethane–ether afforded crystals of maytanbutacine (0.115 mg/kg plant, 0.0000115%): mp 253–255 °C; $[\alpha]^{33}_D$ −90° (*c* 0.055, EtOH); UV (EtOH) 233 (ε 27 200), 253 (24 200), 282 (5050), 290 nm (5080); IR (KBr) 5.73, 5.82, 6.00, 6.32, 8.16, 9.17 μ; NMR ($CDCl_3$) δ 0.79 (3 H, s, C-4 CH_3), 1.20 (3 H, d, J = 7 Hz, C-2′ CH_3), 1.28 (3 H, d, J = 7 Hz, C-2′ CH_3), 1.28 (3 H, d, J = 7 Hz, C-6 CH_3), 1.67 (3 H, s, C-14 CH_3), 2.23 (3 H, s, C-15 $OCOCH_3$), 2.43 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 11 Hz, C-2 H), 2.47 (1 H, dd, $J_{2,2}$ = 14, $J_{2,3}$ = 3 Hz, C-2 H), 2.95 (1 H, d, $J_{5,6}$ = 8.5 Hz, C-5 H), 3.16 (3 H, s, C-1 NCH_3), 3.35 (3 H, s, C-10 OCH_3), 3.51 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 4.02 (3 H, s, C-20 OCH_3), 4.26 (1 H, m, C-7 H), 4.79 (1 H, dd, $J_{2,3}$ = 11, 3 Hz, C-3 H), 5.60 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 14 Hz, C-11 H), 6.21 (1 H, s, C-15 H), 6.30 (1 H, dd, $J_{12,13}$ = 10 Hz, C-12 H), 6.39 (1 H, s, C-9 NH), 6.44 (1 H, d, $J_{12,13}$ = 10 Hz, C-13 H), 6.85 (1 H, d, $J_{17,21}$ = 1.5 Hz, C-21 H), 7.09 (1 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H), 1.0–2.0 (3 H, C-6 H, C-8 H_2), 2.0–2.6 (1 H, m, C-2′ H); mass spectrum *m/e* 631.2540, $C_{33}H_{42}ClNO_9$ [M − 61 (H_2O + HNCO)] = 631.2548, *m/e* 571.2331, $C_{31}H_{38}ClNO_7$ [M − 61 − 60(CH_3COOH)] = 571.2337.

Anal. Calcd for $C_{34}H_{45}ClN_2O_{11}$: C, 58.91; H, 6.54; N, 4.04. Found: C, 58.90; H, 6.67; N, 3.92.

Deacetylmaytanbutacine (9). Maytanbutacine (**8**, 17 mg) was treated with 80 mg of sodium bicarbonate in 4 mL of methanol–water (1:1) at room temperature for 43 h. The solvent was evaporated, and PTLC of the residue on ChromAR developed with ethyl acetate (twice) gave two major bands, one corresponding to **8**. Isolation of the lower band (R_f 0.38) gave 8.3 mg of homogeneous material. Crystallization of this material from dichloromethane–ether–*n*-hexane gave 4.9 mg of **9** (30%): mp 227–228 °; $[\alpha]^{25}_D$ −94° (*c* 0.053, EtOH); UV (EtOH) 233 nm (ε 24 700), 252 (21 700), 281 (4540), 289 (4540); IR (KBr) 5.76, 5.89, 6.02, 6.35, 9.22 μ; NMR ($CDCl_3$ + acetone-d_6) δ 0.88 (3 H, s, C-4 CH_3), 1.19 (3 H, d, J = 7 Hz, C-2′ CH_3), 1.21 (3 H, d, J = 7 Hz, C-2′ CH_3), 1.27 (3 H, d, J = 7 Hz, C-6 CH_3), 1.68 (3 H, s, C-14 CH_3), 2.1–2.3 (1 H, m, C-2′ H), 2.40 (1 H, dd, $J_{2,2}$ = 15, $J_{2,3}$ = 11 Hz, C-2 H), 2.67 (1 H, dd, $J_{2,2}$ = 15, $J_{2,3}$ = 3 Hz, C-2 H), 2.88 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.15 (3 H, s, C-1 NCH_3), 3.37 (3 H, s, C-10 OCH_3), 3.47 (1 H, d, J = 7 Hz, C-15 H), 3.57 (1 H, s, C-9 OH), 3.59 (1 H, d, J = 9 Hz, C-10 H), 4.03 (3 H, s, C-20 OCH_3), 4.29 (1 H, m, C-7 H), 4.80 (1 H, dd, $J_{2,3}$ = 11, 3 Hz, C-3 H), 5.37 (1 H, s, C-15 H), 5.63 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 5.82 (1 H, d, $J_{12,13}$ = 11 Hz, C-13 H), 6.40 (1 H, s, C-9 NH), 6.63 (1 H, dd, $J_{11,12}$ = 15, $J_{12,13}$ = 11 Hz, C-12 H), 6.89 (1 H, d, $J_{17,21}$ = 1.5 Hz, C-21 H), 7.49 (1 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H), 1.0–2.0 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 589 [M − 61 (H_2O + HNCO)], 571 [M − 61 − 18 (H_2O)], 554 [M − 61 − 35 (Cl)], 501 [M − 61 − 88 ($C_4H_8O_2$)], 486 [M − 61 − 88 − 15 (CH_3)], 466 [M − 61 − 88 − 35 (Cl)].

Oxidation of Deacetylmaytanbutacine. Deacetylmaytanbutacine (0.7 mg) was dissolved in 3 drops of acetone and treated with 1 drop of Jones reagent at room temperature for 3 min. Three drops of water were added, the mixture was extracted with ethyl acetate, and the ethyl acetate was evaporated. PTLC of the residue on alumina in 10% methanol–ethyl acetate gave three bands. Isolation of the band with R_f 0.31 gave an unsaturated ketone: UV (MeOH) 285 nm; mass spectrum *m/e* 587 [M − 61 (H_2O + HNCO)], 562 [M − 61 − 15 (CH_3)], 552 [M − 61 − 35 (Cl)], 499 [M − 61 − 88 ($C_4H_8O_2$)], 484 [M − 61 − 88 − 15 (CH_3)], 464 [M − 61 − 88 − 35 (Cl)].

***Putterlickia verrucosa*. Procedure A.** The ground dried stem wood and bark (1 kg) of *P. verrucosa* was extracted in Soxhlet extractors with 8 L of 95% ethanol for 6 h. The plant material was extracted two additional times with 8 L of fresh 95% ethanol for 15 and 24 h. The extracts were combined and concentrated at 40–50 °C in

vacuo to give a dark gum which was partitioned between ethyl acetate (300 mL) and water (150 mL). The insoluble material was removed by filtration and washed again with ethyl acetate (2 × 200 mL) and water (2 × 100 mL). The combined aqueous layers were washed with an additional 200 mL of ethyl acetate and the combined ethyl acetate layers were then concentrated in vacuo to give 12.4 g of a dark gum. This material was subjected to column chromatography over SilicAR CC-7 (62 g) packed in chloroform. The column was eluted with chloroform (140 mL), 5% methanol–chloroform (1600 mL), and methanol (280 mL). The fraction which was eluted with 5% methanol–chloroform (10.1 g) was treated with 60 mL of acetic anhydride–pyridine (1:1) at room temperature for 18 h. The residue from the acetylation step (11.5 g), after removal of the excess acetic anhydride–pyridine, was subjected to column chromatography over SilicAR CC-7 (57 g) eluted first with chloroform (550 mL) and then with 5% methanol–chloroform (900 mL). The 5% methanol–chloroform eluate (1.8 g) was subjected to PTLC on alumina developed with 5% methanol–ethyl acetate. The bands corresponding to the maytansinoids were isolated (60 mg) and again subjected to PTLC using the same conditions. The bands corresponding to the maytansinoids (R_f 0.3–0.6) were isolated (47 mg), and subjected to PTLC on ChromAR developed with ethyl acetate (twice). Isolation and crystallization of the appropriate bands gave maytansine (**1**, 12.3 mg, 0.0012%), maytanprine (**2**, 8.5 mg, 0.00085%), and maytanbutine (**3**, 4.5 mg, 0.00045%).

PTLC of a band with R_f 0.55 from the previous step on ChromAR developed with 10% benzene–ethyl acetate gave <200 μg of a crystalline maytansinoid identical with maytanacine (**12**) isolated from a large-scale extraction (procedure B).

Procedure B. The ground dried wood stems and stem bark (10.0 kg) of *P. verrucosa* were extracted in Soxhlet extractors with 80 L of 95% ethanol for 6 h. The plant material was extracted again with 80 L of fresh 95% ethanol for 15 h. After a third extraction of 24 h, the extracts were combined and concentrated at 40–50 °C in vacuo to give a dark gum (277 g). The concentrated alcoholic extract was shaken between ethyl acetate (1 L) and water (500 mL). The suspension was filtered and the insoluble material was treated two more times with ethyl acetate (250 mL), followed by filtration. The aqueous layer was washed with an additional 250 mL of ethyl acetate.

The combined ethyl acetate layers (93 g) were chromatographed on a column of alumina (1 kg, activity II–III), packed in dichloromethane. Beginning with 5% methanol–dichloromethane, 250-mL fractions were collected and analyzed by HPLC for maytansinoid content. Fractions 2–4 were combined to give fraction A (9.3 g) and fractions 5–8 were combined to give fraction B (1.7 g).

Maytanacine (12). Fraction A was chromatographed on a column of SilicAR CC-7 (1 kg), packed in 50% ethyl acetate–benzene, with each 1-L fraction being analyzed by HPLC. Elution with 66% ethyl acetate–benzene gave fractions C (100 mg) and D (124 mg). PTLC of fraction D on ChromAR, developed with 5% methanol–chloroform, gave a band (7.4 mg) corresponding in R_f to the maytanacine standard. Further PTLC of this material on alumina, developed with 10% methanol–ethyl acetate, gave 1.5 mg of crystalline material. Identical purification of fraction C gave an additional 0.3 mg of crystalline isolate. The combined crystalline material (1.8 mg) was found to be identical by mixture HPLC, TLC, and mass spectrum with the synthetic sample prepared from maytansinol.

Maytansinol (13). Fraction B was chromatographed on a column of SilicAR CC-7 (170 g), packed in chloroform, and eluted with increasing amounts of methanol in chloroform. Elution with 5% methanol–chloroform gave 240 mg of material which was submitted to PTLC on ChromAR, developed with 5% methanol–ethyl acetate. The band (46 mg) corresponding to maytansinol was chromatographed further on Kieselgel plates, developed with 15% ethanol–ether, to give 5.7 mg of material with the same R_f as maytansinol. Preparative HPLC, collecting the component with the proper retention time, followed by PTLC on ChromAR, developed with 5% methanol–chloroform × 2, yielded 0.25 mg of isolate. This material was found to be identical by mixture HPLC, TLC, and mass spectrum with an authentic sample of maytansinol prepared by lithium aluminum hydride treatment of maytanbutine.

Maytansinol (13). A mixture of maytanbutine (**3**, 40 mg, 0.0556 mmol) and excess lithium aluminum hydride was stirred in dry tetrahydrofuran (4 mL) at −23 °C (carbon tetrachloride–dry ice bath) for 3 h. Ethyl acetate (10 mL) was added, followed by 10 mL of pH 6.8 phosphate buffer,[31] and the mixture was further extracted with ethyl acetate (4 × 10 mL). The extracts were combined, dried over sodium sulfate, and brought to dryness. The residue (45 mg) was submitted to PTLC on ChromAR, developed with 5% methanol–chloroform × 2, to give 21 mg of material which was further purified by PTLC on ChromAR, developed with 3% isopropyl alcohol–ethyl acetate × 2. The major band (17.2 mg) was chromatographed over a very short column of aluminum oxide (activity II–III), packed in dichloromethane with the product eluted with 5% methanol–dichloromethane, to give 16.0 mg of maytansinol. Precipitation from dichloromethane–hexane afforded white, solid **13** (12.5 mg, 40%): mp 173–174.5 °C; $[\alpha]^{23}_D$ −309° (*c* 0.110, $CHCl_3$); UV (EtOH) 232 nm (ε 32 700), 244 (sh, 30 800), 252 (31 600), 281 (5810), 288 (5700); IR (KBr) 5.85, 6.06, 6.35 μ; NMR ($CDCl_3$) δ 0.84 (3 H, s, C-4 CH_3), 1.32 (3 H, d, J = 6 Hz, C-6 CH_3), 1.68 (3 H, s, C-14 CH_3), 3.20 (3 H, s, C-1 NCH_3), 3.36 (3 H, s, C-10 OCH_3), 3.44 (1 H, br s, C-3 OH), 3.64 (1 H, br s, C-9 OH), 3.98 (3 H, s, C-20 OCH_3), 4.36 (1 H, m, C-7 H), 5.53 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.19–6.39 (3 H, C-12 H, C-13 H, C-9 NH), 6.81, 7.05 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17 H, C-21 H), 1.30–3.55 (10 H, C-2 H_2, C-3 H, C-5 H, C-6 H, C-8 H_2, C-10 H, C-15 H_2); mass spectrum *m/e* 503.2075 ($M^+ - H_2O$ − HNCO) (calcd, 503.2074).

Maytanacine (12). Maytansinol (**13**, 1.5 mg, 0.0027 mmol) prepared from maytanacine under the same conditions used for maytanbutine was treated with 1 mL of acetic anhydride–pyridine (1:1) at 53 °C for 18 h. The reaction mixture was brought to dryness and the residue was chromatographed on ChromAR developed with 5% methanol–chloroform. The major band was removed, eluted with 10% methanol in ethyl acetate, and evaporated to a white solid. Crystallization from dichloromethane–hexanes gave **12** (0.8 mg, 48%): mp 234–237 °C; $[\alpha]^{23}_D$ −119° (*c* 0.100, $CHCl_3$); UV (EtOH) 233 nm (ε 30 300), 242 (sh, 28 000), 252 (27 900), 281 (5360), 289 (5360); IR (KBr) 5.70, 5.80, 6.00, 6.34 μ; NMR ($CDCl_3$) δ 0.84 (3 H, s, C-4 CH_3), 1.28 (3 H, d, J = 6 Hz, C-6 CH_3), 1.69 (3 H, s, C-14 CH_3), 2.18 (3 H, s, C-3 $OCOCH_3$), 2.05–2.30 (1 H, C-2 H), 2.46 (1 H, dd, $J_{2,3}$ = 12, $J_{2,2}$ = 14 Hz, C-2 H), 2.89 (1 H, d, $J_{5,6}$ = 9 Hz, C-5 H), 3.18)3 H, s, C-1 NCH_3), 3.36 (3 H, s, C-10 OCH_3), 3.52 (1 H, d, $J_{10,11}$ = 9 Hz, C-10 H), 3.10–3.60 (3 H, C-9 OH, C-15 H_2), 3.99 (3 H, s, C-20 OCH_3), 4.16 (1 H, m, C-7 H), 4.92 (1 H, dd, $J_{2,3}$ = 3, 12 Hz, C-3 H), 5.48 (1 H, dd, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11 H), 6.10–6.59 (3 H, C-9 NH, C-12 H, C-13 H), 6.84, 6.76 (2 H, s, C-17 H, C-20 H), 0.80–2.50 (3 H, C-6 H, C-8 H_2); mass spectrum *m/e* 545.2180 ($M^+ - H_2O$ − HNCO) (calcd, 545.2180), 485.1969 ($M^+ - H_2O$ − HNCO − CH_3COOH) (calcd, 485.1969).

Anal. Calcd for $C_{30}H_{39}ClN_2O_9$: C, 59.35; H, 6.48; N, 4.61. Found: C, 59.19; H, 6.39; N, 4.69.

Registry No.—**1**, 35846-53-8; **2**, 38997-09-0; **3**, 38997-10-3; **4**, 52978-27-5; **5**, 52978-28-6; **6**, 52978-29-7; **7**, 52978-30-0; **8**, 62414-95-3; **9**, 62414-96-4; **11**, 36482-96-9; **12**, 57103-69-2; **13**, 57103-68-1; **14**, 62414-97-5; 3-bromopropanol, 627-18-9; *N*-isovaleryl-*N*-methyl-L-alanine methyl ester, 62414-98-6; *N*-methyl-L-alanine, 3913-67-5; isovaleryl chloride, 108-12-3; unsaturated ketone, 62414-99-7.

References and Notes

(1) (a) Tumor Inhibitors. 122. Part 121: S. M. Kupchan, I. Uchida, K. Shimada, B. Y. Fei, D. M. Stevens, A. T. Sneden, R. W. Miller, and R. F. Bryan, *J. Chem. Soc., Chem. Commun.*, in press. (b) Deceased October 19, 1976.

(2) Supported by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (T-275, T-541, and CI-102K) and a contract with the Division of Cancer Treatment, National Cancer Institute (NIH-71-2099, N01-CM-12099, N01-CM-67002). A.R.B. was a NIH Postdoctoral Fellow, 1972–1975.

(3) (a) We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material in accordance with the program developed by the National Cancer Institute. (b) Fruits were collected in Ethiopia in Jan 1962. Roots and the wood of stems from Ethiopia and Kenya also yielded active extracts.

(4) Activity was noted against sarcoma 180, Lewis lung carcinoma, and L-1210 and P-388 leukemias in the mouse and Walker 256 intramuscular carcinosarcoma in the rat. Cytotoxicity and in vivo activity were assayed as in *Cancer Chemother. Rep.*, **25**, 1 (1962), and by the procedures described by R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

(5) S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger, and R. F. Bryan, *J. Am. Chem. Soc.*, **94**, 1354 (1972).

(6) S. M. Kupchan, Y. Komoda, G. J. Thomas, and H. P. J. Hintz, *J. Chem. Soc., Chem. Commun.*, **1065** (1972).

(7) S. M. Kupchan, Y. Komoda, A. R. Branfman, R. G. Dailey, Jr., and V. A. Zimmerly, *J. Am. Chem. Soc.*, **96**, 3706 (1974).

(8) S. M. Kupchan, A. R. Branfman, A. T. Sneden, A. K. Verma, R. G. Dailey, Jr., Y. Komoda, and Y. Nagao, *J. Am. Chem. Soc.*, **97**, 5294 (1975).

(9) For recent studies on synthetic approaches to various model compounds related to segments of the maytansine structure, see (a) W. J. Elliott and J. Fried, *J. Org. Chem.*, **41**, 2469 (1976); (b) E. J. Corey and M. G. Bock, *Tetrahedron Lett.*, 2643 (1975); (c) A. I. Meyers, C. C. Shaw, D. Horne, L. M. Trefonas, and R. J. Majeste, *ibid.*, 1745 (1975); (d) A. I. Meyers and R. S. Brinkmeyer, *ibid.*, 1749 (1975); (e) A. I. Meyers and C. C. Shaw, *ibid.*, 717 (1974).

(10) S. Remillard, L. I. Rebhun, G. A. Howie, and S. M. Kupchan, *Science*, **189**, 1002 (1975).

(11) M. K. Wolpert-DeFilippes, R. H. Adamson, R. L. Cysyk, and D. G. Johns, *Biochem. Pharmacol.*, **24**, 751 (1975).
(12) T. E. O'Connor, C. Aldrich, A. Hadidi, N. Lomex, P. Okano, S. Sethi, and H. B. Wood, *Proc. Am. Assoc. Cancer Res.*, **16**, Abstr. 114 (1975).
(13) M. K. Wolpert-DeFilippes, V. H. Bono, Jr., R. L. Dion, and D. G. Johns, *Biochem. Pharmacol.*, **24**, 1735 (1975).
(14) F. Mandelbaum-Shavit, M. K. Wolpert-DeFilippes, and D. G. Johns, *Biochem. Biophys. Res. Commun.*, **72**, 47 (1976).
(15) R. H. Adamson, S. M. Sieber, J. Whang-Peng, and H. B. Wood, *Proc. Am. Assoc. Cancer Res.*, **17**, Abstr. 165 (1976).
(16) C. D Aldrich and T. E. O'Connor, *Proc. Am. Assoc. Cancer Res.*, **17**, Abstr. 339 (1976).
(17) S. M. Sieber, M. K. Wolpert, R. H. Adamson, R. L. Cysyk, V. H. Bono, and D. G. Johns, *Bibl. Haematol. (Basel)*, *495* (1976); *Chem. Abstr.*, **85**, 104123p (1976).
(18) Pilot experiments indicated that treatment with acetic anhydride–pyridine facilitated the subsequent separation without affecting the active principles.
(19) K. Sesaki, K. L. Rinehart, Jr., G. Slomp, M. F. Grostic, and E. C. Olson, *J. Am. Chem. Soc.*, **92**, 7591 (1970).
(20) R. F. Bryan, C. J. Gilmore, and R. C. Haltiwanger, *J. Chem. Soc., Perkin Trans. 2*, 897 (1973).
(21) T. H. Siddall and W. E. Stewart, *Progr. Nucl. Magn. Reson. Spectrosc.*, **5**, 33 (1969).
(22) L. M. Jackman and S. Sternhill, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, Elmsford, N.Y., 1969, p 368.
(23) H. Krebs and W. Schumacher, *Chem. Ber.*, **99**, 1341 (1966).
(24) W. Cole and P. L. Julian, *J. Org. Chem.*, **19**, 131 (1954).
(25) M. C. Wani, H. L. Taylor, and M. E. Wall, *J. Chem. Soc., Chem. Commun.*, 390 (1973).
(26) Stem wood and stem bark were collected in South Africa in 1972. We again acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr.
(27) Later collections of *P. verrucosa* have also been assayed for maytansine yield and have been found to average 8–9 mg/kg.
(28) We thank Professor a. I. Meyers, Colorado State University, for unpublished information concerning $LiAlH_4$ reductive cleavage of esters in synthetic model compounds.
(29) M. P. Mertes, P. E. Hanna, and A. A. Ramsey, *J. Med. Chem.*, **13**, 125 (1969).
(30) H. C. Brown, *J. Am. Chem. Soc.*, **60**, 1325 (1938).
(31) I. M. Kolthoff and C. Rosenblum, "Acid-Base Indicators", Macmillan, New York, N.Y., 1937, p 249.

Studies of Resin Acids. 10. Approaches to the Synthesis of Podocarpic and Dehydroabietic Acids†[1]

J. W. Huffman* and P. G. Harris[2]

Department of Chemistry and Geology, Clemson University, Clemson, South Carolina 29631

Received January 21, 1977

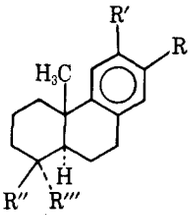
In a new stereoselective synthesis of the tricyclic nucleus of the resin acids, 2-(2-phenylethyl)cyclohexane-1,3-dione is cyclized to a tricyclic enone (**5**). Conjugate addition of lithium dimethylcuprate gives a mixture of the 5α and 5β isomers of 18,19-dinorpodocarpa-8,11,13-triene (**6**), which reacts with methylenetriphenylphosphorane to give as a major product olefin **7**, which is also prepared from podocarpic acid (**2**). A new stereoselective synthesis of dehydroabietic acid (**1**) from the dinorketone **9** via the sequence methylenetriphenylphosphorane to olefin **10**, conversion of **10** to aldehyde **17**, alkylation with allyl bromide to **22**, is presented. Wolff–Kishner reduction of **22** followed by oxidation affords homodehydroabietic acid (**24**), which has been converted previously to acid **1**.

Although a number of syntheses of diterpenoid acids, such as dehydroabietic acid (abieta-8,11,13-trien-18-oic acid, **1**) and podocarpic acid (12-hydroxypodocarpa-8,11,13-trien-19-oic acid, **2**) have been described,[3] all of these syntheses are rather lengthy and many are nonstereoselective. Also, in none of these syntheses could a single intermediate well along the synthetic route be used to obtain stereoselectively both epimeric C-4 carboxylic acids. Either the reaction sequence gave a mixture of epimers at this center, or the synthesis was designed in such a way that it provided only one epimer from the outset.

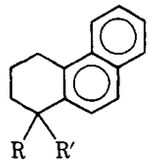
In an effort to overcome both of these shortcomings a new approach to the total synthesis of these diterpenoid acids has been devised which is a modification of an earlier synthesis, reported from this laboratory, which resulted in a short, stereoselective synthesis of eudesmol and several related sesquiterpenes.[4] The modified synthetic sequence as applied to the diterpene acids is shown in Scheme I. In order to utilize readily available starting materials, this approach was to be applied to the syntheses of podocarpa-8,11,13-trien-18-oic (**3**) and -19-oic (**4**) acids, both of which have been converted to naturally occurring compounds.[3e,5]

The key steps of the synthesis were first, the conjugate addition of lithium dimethylcuprate to enone **5**, and second, the reaction of methylenetriphenylphosphorane with ketone **6** to give selectively the 5α olefin (**7**). Olefin **7** could easily be transformed to aldehyde **8**, which then could, hopefully, be utilized to synthesize acids **3** and **4**.

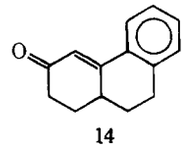
† Dedicated to Professor R. B. Woodward on the occasion of his 60th birthday.



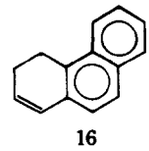
1, R = $CH(CH_3)_2$; R′ = H; R″ = CH_3; R‴ = CO_2H
2, R = H; R′ = OH; R″ = CO_2H; R‴ = CH_3
3, R,R′ = H; R″ = CH_3; R‴ = CO_2H
4, R,R′ = H; R″ = CO_2H; R‴ = CH_3
11, R = $CH(CH_3)_2$; R′,R‴ = H; R″ = CH_3
18, R = $CH(CH_3)_2$; R′ = H; R″ = CO_2H; R‴ = CH_3
20, R = $CH(CH_3)_2$; R′ = H; R″ = CHO; R‴ = CH_3
21, R = $CH(CH_3)_2$; R′ = H; R″,R‴ = CH_3
22, R = $CH(CH_3)_2$; R′ = H; R″ = CHO; R‴ = $CH_2CH{=}CH_2$
23, R = $CH(CH_3)_2$; R′ = H; R″ = CH_3; R‴ = $CH_2CH{=}CH_2$
24, R = $CH(CH_3)_2$; R′ = H; R″ = CH_3; R‴ = CH_2CO_2H



12, R,R′ = O
13, R,R′ = H
15, R = OH; R′ = H



14



16

In order to ascertain the feasibility of the trans-selective Wittig reaction which had worked well in other cases,[4,6] the