Two New Maytansinoids from *Maytenus buchananii*

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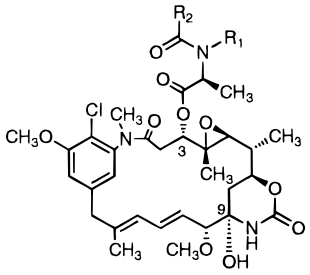
Reinvestigation of fractions derived from large-scale fractionation of *Maytenus buchananii* led to the isolation of two new maytansinoids. The structures of these principles were determined using electrospray MS, ^{1}H NMR, ^{13}C NMR, and 2D NMR techniques. One principle was found to be 2′-*N*-demethylmaytanbutine (**2**), while the other was found to be maytanbicyclinol (**3**), the first maytansinoid with two macrocyclic rings to be isolated from a *Maytenus* species.

Maytansine (**1**), the principal ansa macrolide of *Maytenus serrata* (Celastraceae) isolated and reported by Kupchan and co-workers in 1972, was one of the first antileukemic plant-derived natural products isolated by activity-guided fractionation to become a potential candidate for clinical trials under the auspices of the National Cancer Institute.[1,2] Because it was isolated only in quantities of approximately 0.2 mg/kg of plant material, examination of other *Maytenus* species was undertaken to find a better source of maytansine. Ultimately, the amounts of maytansine required for preclinical studies and initial clinical trials were obtained from large-scale collections of *Maytenus buchananii* (Loes.) R. Wilczek (Celastraceae).[2,3] During the isolation of maytansine from the collections of *M. buchananii*, a large number of homologues of maytansine was also isolated. Some of these were reported in the 1970s and 1980s,[3,4] but the structures of many of the homologues obtained in very small quantities were never confirmed or elucidated. The interest in maytansine and its homologues as potential antineoplastic agents also led to the isolation of homologues from other plant genera[5−8] as well as from fermentation of a *Nocardia* species.[9] However, with the withdrawal of maytansine from clinical trials, interest in the maytansinoids waned.

Reinvestigation of some of the unidentified homologues of maytansine isolated from *M. buchananii* was prompted by recent work on alkaloids from *M. buchananii* and *M. krukovii*.[10,11] During this work, the development of HPLC systems to separate the sesquiterpene nicotinoyl alkaloids common to *Maytenus* species was extended to development of better HPLC systems for the analysis of maytansinoids.[12] As the HPLC systems were developed, they were applied to analysis of fractions from the large-scale isolations of maytansine that were known to contain unidentified maytansinoids. Approximately 50 samples were analyzed and compared to samples of known maytansinoids. Fractions with similar HPLC or TLC chromatograms were pooled to give five samples, each containing a different unidentified maytansinoid as the principal component. Two of these pooled fractions contained sufficient quantities to warrant further isolation studies. Preparative TLC of these fractions resulted in the isolation of two new maytansinoids, which were identified from NMR and MS data as 2′-*N*-demethylmaytanbutine (**2**) and maytanbicyclinol (**3**).

2′-*N*-Demethylmaytanbutine (**2**) was isolated by preparative TLC of a fraction containing 23.1 mg of a mixture of three components. Two successive separations succeeded in giving 5.4 mg of an amorphous white solid. The structure of **2** was determined primarily through the use of 1D and 2D ^{1}H NMR data and electrospray MS. The high-resolution electrospray MS showed ions at *m*/*z* 728.2976 [M + Na$^+$] and *m*/*z* 706.3155 [M + H$^+$], indicating a molecular formula of $C_{35}H_{48}ClN_3O_{10}$. In the electrospray MS, loss of H_2O and HNCO from the parent ion, from fragmentation of the carbinolamide moiety, gave ions at *m*/*z* 687 [M$^+$ − 18] and *m*/*z* 644 [M$^+$ − 18−43], as is typical of maytansinoids.[3] Loss of the C-3 side chain (159 mass units) from the *m*/*z* 644 ion, again typical of the fragmentation pathway of maytansinoids,[3] resulted in an ion at *m*/*z* 485. This ion represents the macrocyclic ring of the usual maytansinoid skeleton[3] and indicates that **2** differs from **1** only in the side chain.

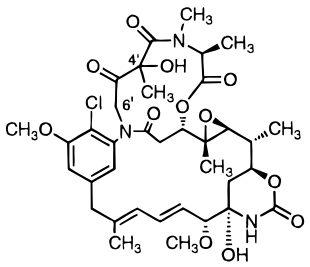
In the ^{1}H NMR spectrum of **2**, the first major difference from the spectrum of **1** was the absence of the 2′−$NCOCH_3$ methyl singlet at δ 2.2 and the appearance of two methyl doublets at δ 1.07 and 1.15 coupled to a septet at δ 2.33 [the couplings were determined from analysis of the homonuclear correlated (COSY) spectrum]. These resonances indicated that the *N*-acyl group was an isobutyroyl moiety, as in maytanbutine (**4**). The 2′−NCH_3 resonance



1 $R_1 = CH_3$; $R_2 = CH_3$

2 $R_1 = H$; $R_2 = CH(CH_3)_2$

4 $R_1 = CH_3$; $R_2 = CH(CH_3)_2$



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was absent in the spectrum of **2**, the 2′-CH quartet was shifted upfield to δ 4.82, and the 2′−CH_3 doublet was

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shifted downfield to δ 1.37, when compared with the spectrum of **4**. These data indicated that the 2′ nitrogen of **2** bears a proton rather than a methyl group and that the side chain was derived from *N*-isobutyroyl-L-alanine. This finding was consistent with the loss of 159 mass units ($C_7H_{13}NO_3$) in the mass spectrum. The resonances for all other protons found in the spectrum of **4** were present in the spectrum of **2**. These data, in conjunction with the MS data, correlated well with the data for demethyltrenudone,[8] another maytansinoid missing the side chain *N*-methyl, and confirmed that **2** was 2′-*N*-demethylmaytanbutine.

Maytanbicyclinol (**3**) was isolated during the isolation of **2**, as well as from a combined fraction containing 22.7 mg of material, as a white, amorphous solid. High-resolution electrospray MS gave an ion at *m*/*z* 770.2846 [M + Na^+], indicating a molecular formula of $C_{36}H_{46}ClN_3O_{12}$ (an index of hydrogen deficiency of 15). The fragmentation pattern, however, was different from that seen in other maytansinoids. There were ions at *m*/*z* 729 [$M^+ - 18$] and *m*/*z* 686 [$M^+ - 61$], corresponding to loss of H_2O followed by loss of HNCO from the carbinolamide. However, the typical ion at *m*/*z* 485 due to the macrocyclic ring was not present.

The ^{1}H NMR spectrum of **3** presented some significant differences from the usual maytansinoid spectrum. The 2′−$NCOCH_3$ and one of the NCH_3 singlets were absent. One aromatic proton (C-17−H) was shifted downfield almost 1 ppm to δ 7.84. Two broad, one-proton doublets (parts of an AB quartet) appeared at δ 3.1 and 4.61 and were coupled only to each other. These two protons were correlated to a methylene resonance in the ^{13}C NMR spectrum at δ 52.0, suggesting a N−CH_2−X moiety. The 2′−H was shifted downfield almost 1 ppm to δ 5.76, and the 3-H was shifted upfield 0.25 ppm to δ 4.38. In addition, there was a new methyl singlet at δ 1.64. These resonances pointed to major differences in the side chain. All of the other typical maytansinoid resonances were present, indicating that there was no modification of the macrocyclic ring. These data, taking into consideration the index of hydrogen deficiency, suggested the presence of an additional ring involving the side chain, in **3**. The basic ansa macrolide nucleus of a typical maytansinoid accounted for a partial formula of $C_{27}H_{34}ClN_2O_7$, leaving a partial formula of $C_9H_{12}NO_5$ for the ring involving the side chain.

Careful examination of the ^{13}C NMR data indicated the presence, in addition to the methylene resonance at δ 52.0 and the resonances due to an *N*-methyl-L-alanyl ester, of new resonances at δ 29.8 (CH_3) and 76.9 (quaternary). These resonances could only be assigned to the side-chain ring, accounting for seven of the nine carbons, 11 of the 12 hydrogens, the nitrogen, and two of the five oxygens. Comparison of the ^{13}C NMR data to data for other maytansinoids[6−8,13] indicated that only two resonances remained unassigned. These resonances could be assigned as two carbonyl moieties, one of which was a ketone (δ 203), leaving only one oxygen and one hydrogen to be accounted for as a hydroxyl group.

Starting with the probable structure of an *N*-methyl-L-alanyl ester at C-3, one of the carbonyl moieties was considered to be present as part of an *N*-acyl group. Working from the other end of the proposed ring, the N−CH_2 moiety could devolve from the C-18 *N*-methyl group found in typical maytansinoids. The NMR resonances attributed to this methylene are further downfield than would be expected if it were a typical alkyl methylene, indicating that a carbonyl group should be the other moiety on this methylene. The methyl group and hydroxyl group were placed on the quaternary carbon due to the ^{13}C chemical shifts of the methyl and the quaternary carbon. This moiety was proposed to connect the two carbonyl moieties of the other pieces, giving structure **3**.

One other structure, which could satisfy these data, is possible. This structure would have the quaternary carbon bearing the methyl group and hydroxyl moiety as C-6′, with the isolated methylene group as C-4′ joining the two carbonyl moieties. This structure was eliminated by examination of the MS fragmentation pattern for **3**. In the LRMS, ions at *m*/*z* 573 and 555 can be attributed to loss of the side chain through C-4′ followed by loss of H_2O. These ions support structure **3**. If the alternative structure were correct, these ions would be at *m*/*z* 603 and 585. No such ions were detected.

Maytansinoids with an additional macrocyclic ring have been previously isolated from *Euphorbia* species, including *N*-methyltrenudone, which differs from **3** by the presence of a methoxy at C-15. Comparison of the NMR data for **3** with the data for *N*-methyltrenudone[7] indicated that the two compounds were similar and served to confirm the structure of **3**.

This is the first report of maytansinoids lacking a 2′ *N*-methyl group or containing a second macrocyclic ring from *Maytenus* species. Although it was not possible to screen the compounds for biological activity, the major fraction that yielded **3** was essentially pure when originally isolated at the University of Virginia. Mass spectral data recorded at that time indicated that this pure fraction was indeed **3**.[14] This fraction demonstrated cytotoxic activity in vitro against the KB cell culture (ED_{50} $<10^{-5}$ μg/mL) and in vivo activity against the P-388 lymphocytic leukemia (T/C 140−195% at 0.1−1.6 μg/kg).[14]

Experimental Section

General Experimental Procedures. ^{1}H, ^{13}C, COSY, and HETCOR NMR spectra were acquired with a General Electric QE−300 NMR spectrometer using $CDCl_3$ as the solvent. IR data were obtained on a Perkin−Elmer 1600 series FTIR. HRMS were acquired using an IonSpec 4.7T Fourier transform ion cyclotron resonance mass spectrometer with an electrospray injector at VCU. Melting point values were measured on an Electrothermal Digital melting point apparatus and are uncorrected.

Plant Material and Initial Extraction. Wood and bark of *Maytenus buchananii* (Loes.) R. Wilczek (approximately 5900 kg) were collected in Kenya in 1972, and supplied to Monsanto Research Corporation, Dayton, Ohio, by the Medicinal Plant Resources Laboratory, USDA, Beltsville, MD, where voucher specimens were preserved. Extracts were prepared by Monsanto Research Corporation according to protocols indicated[1,3,4] and supplied to Dr. S. Morris Kupchan, Department of Chemistry, University of Virginia, Charlottesville, VA.

Fractionation of Extracts of *M. buchananii*. This work was performed at the University of Virginia in 1973.[14] Approximately 4.5 kg of extracts were obtained from Monsanto Corporation in four lots (nos. 73−06−07−02, 73−07−09−01, 73−08−08−01, and 73−08−08−02). These extracts were subjected, in ca. 900-g batches, to column chromatography over alumina eluted with CH_2Cl_2 containing increasing amounts of MeOH. Fractions eluting with MeOH−CH_2Cl_2 (5:95) were then subjected to column chromatography over silicAR CC-7 (Mallinckrodt) eluted with C_6H_6−EtOAc (50:50) followed by C_6H_6−EtOAc (33:67). Fractions obtained during the latter elution were subjected to preparative TLC over alumina three times, and similar fractions were combined to give 169 mg of material. This material was fractionated further by various combinations of preparative TLC over Si gel and partition chromatography to give numerous smaller fractions that were not examined further.

Isolation of 2′-*N*-demethylmaytanbutine (2). Two fractions from the sample collection of the Kupchan group, which were obtained during the isolation of other maytansinoids as described above and which gave similar TLC and HPLC patterns, were combined (23.1 mg) and subjected to preparative TLC on Si gel developed twice with MeOH−$CHCl_3$ (5:95). The lower of the two bands was removed and again subjected to preparative TLC on Si gel developed five times in MeCN−CH_2Cl_2 (25:75). The lower band was removed from the Si gel using MeOH−CH_2Cl_2 (20:80), filtered, and the filtrate evaporated to give **2** as an amorphous white solid, 5.4 mg. The upper band was removed in the same manner to give 2. 6 mg of **3** as an amorphous white solid.

Isolation of Maytanbicyclinol (3). Two other fractions from the original isolation were combined (22.7 mg) and subjected to preparative TLC on alumina developed with MeOH−EtOAc (5:95). The upper of two bands was removed from the alumina using MeOH−EtOAc (30:70), filtered, and the filtrate evaporated to give 12.0 mg of **3** as an amorphous white solid.

2′-*N*-Demethylmaytanbutine (2): mp 156−158 °C; IR (CH_2Cl_2) ν_{max} 1770, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.84 (3H, s, 4-CH_3), 1.07 (3H, d, J = 6.7 Hz, 4′-CH_3), 1.15 (3H, d, J = 6.7 Hz, 4′-CH_3), 1.23 (1H, m, 8-H), 1.29 (3H, d, J = 6.3 Hz, 6-CH_3), 1.37 (3H, d, J = 6.8 Hz, 2′-CH_3), 1.47 (1H, m, 6-H), 1.61 (1H, m, 8-H), 1.65 (3H, s,14-CH_3), 2.21 (1H, dd, J = 2.8, 14.1 Hz, 2-H), 2.33 (1H, septet, J = 6.7 Hz, 4′−H), 2.59 (1H, dd, J = 12, 14.1 Hz, 2-H), 2.83 (1H, d, J = 9.6 Hz, 5-H), 3.13 (1H, d, 12.7 Hz, 15-H), 3.15 (3H, s, 18-NCH_3), 3.35 (3H, s, 10-OCH_3), 3.52 (1H, d, J = 9 Hz, 10-H), 3.63 (1H, d, J = 12.7 Hz, 15-H), 3.98 (3H, s, 20-OCH_3), 4.27 (1H, m, 7-H), 4.83 (1H, m, 2-′H), 4.89 (1H, dd, J = 2.8, 12 Hz, 3-H), 5.60 (1H, dd, J = 9, 15 Hz, 11-H), 6.29 (1H, s, 9-NH), 6.45 (1H, dd, J = 11, 15 Hz, 12-H), 6.64 (1H, d, J = 11 Hz, 13-H), 6.75 (1H, br s, 17-H), 6.82 (1H, br s, 21-H); electrospray HRMS m/z 728.2976 (calcd for $C_{35}H_{48}ClN_3O_{10}$+Na: 728.2926), 706.3155 (calcd for $C_{35}H_{48}ClN_3O_{10}$+H: 706.3106); electrospray MS m/z 728 [M + Na^+], 706 [M^+ + 1], 687 [M^+ − H_2O], 644 [M^+ − H_2O − HNCO], 485 [M^+ − H_2O − HNCO − side chain].

Maytanbicyclinol (3): mp 185−190 °C; IR (CH_2Cl_2) ν_{max} 1770, 1637 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.75 (3H, s, 4-CH_3), 1.2 (1H, m, 8-H), 1.28 (3H, d, J = 6.3 Hz, 6-CH_3), 1.31 (3H, d, J = 7.1 Hz, 2′-CH_3), 1.4 (1H, m, 6-H), 1.46 (3H, s, 14-CH_3), 1.6 (1H, m, 8-H), 1.64 (3H, s, 4′-CH_3), 2.1 (1H, dd, J = 3.6, 14.5 Hz, 2-H), 2.54 (1H, dd, J = 12, 14.5 Hz, 2-H), 3.05 (1H, d, J = 14.2 Hz, 6′-CH_2), 3.08 (1H, d, J = 12.4 Hz, 15-H), 3.09 (1H, d, J = 9.6 Hz, 5-H), 3.17 (3H, s, 2′-NCH_3) 3.36 (3H, s, 10-OCH_3), 3.49 (1H, d, J = 9.0 Hz, 10-CH), 3.67 (1H, d, J = 12.4 Hz, 15-H), 3.98 (3H, s, 20-OCH_3), 4.26 (1H, m, 7-H), 4.38 (1H, dd, J = 3.6, 12 Hz, 3-H), 5.43 (1H, dd, J = 9, 14.2 Hz, 11-CH), 5.76 (1H, q, J = 7.1 Hz, 2′−H), 6.24 (1H, s, 9-NH), 6.40 (1H, d, J = 10.9 Hz, 13-H), 6.42 (1H, dd, J = 10.9, 14.2 Hz, 12-H), 6.82 (1H, d, J = 1.6 Hz, 21-H), 7.83 (1H, d, J = 1.6 Hz, 17-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.1, 12.4, 14.5, 16.0 (4-, 2′-, 6-, 14-CH_3), 29.8 (4′-CH_3), 30.3 (2′-NCH_3), 32.8 (C-2), 36.2 (C-8), 38.6 (C-6), 46.8 (C-15), 50.0 (C-2′), 52.0 (C-6′), 56.6 (10-OCH_3), 56.8 (20-OCH_3), 59.5 (C-4), 67.6 (C-5), 74.0 (C-7), 76.9 (C-4′), 79.4 (C-3), 80.9 (C-9), 88.9 (C-10), 112.7 (C-21), 118.0 (C-19), 124.5 (C-17), 125.1 (C-13), 126.8 (C-11), 133.7 (C-12), 140.0, 140.7, 142.8, 152.4, 155.5, 170.2, 171.9, 175.2, 203 (C-14, C-16, C-18, C-20, 5 × C═O); electrospray HRMS m/z 770.2846 (calcd for $C_{36}H_{46}ClN_3O_{12}$+Na: 770.2667); electrospray MS m/z 770 [M + Na^+], 748 [M^+ + 1], 729 [M^+ − H_2O], 686 [M^+ − H_2O − HNCO], 573 [M^+ − sidehain through C-4′], 555 [M^+ − side chain through C-4′ − H_2O].

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