NORMAYTANSINE, A NEW ANTILEUKEMIC ANSA MACROLIDE FROM *MAYTENUS BUCHANANII*

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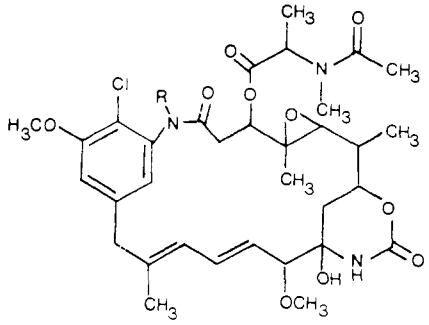
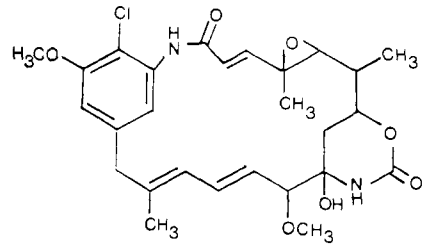
Maytansine **1** is a potent antileukemic ansa macrolide isolated in 1971 from *Maytenus serrata* (Celastraceae) which is currently undergoing Phase II clinical trials under the auspices of the National Cancer Institute (1, 2). A large number of homologous antileukemic ansa macrolides have been isolated from several *Maytenus* and *Putterlickia* species (Celastraceae), from *Colubrina texensis* (Rhamnaceae) and, more recently, from fermentation broths of *Nocardia* species No. C-15003(N-1) (1, 3, 4).

Approximately 15,000 kg of *Maytenus buchananii* were collected in Kenya in 1976 and sent to Monsanto Research Corporation for isolation of the maytansine. During the course of the isolation, several fractions were obtained which did not correspond to any known maytansinoids. When two of these fractions were combined and subjected to preparative



1 R = CH_3

2 R = H



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thin layer chromatography (ptlc) on silica gel 60 (EM Labs) two separate times a white solid was obtained which was crystallized from dichloromethane-hexanes to yield 9 mg of normaytansine **2**, mp 166–167°.[1]

The ultraviolet spectrum of **2** was indicative of a maytansinoid with absorptions at 240, 250, 281, and 288 nm, and the infrared spectrum had bands at 1740, 1710, and 1660 cm^{-1} which suggested that **2** was a maytanside ester. The mass spectrum was typical of a maytanside ester with peaks at *m/e* 616 corresponding to $M^+-H_2O-HNCO$,[2] *m/e* 471 resulting from subsequent loss of the side-chain ester at C–3, *m/e* 456 due to loss of a methyl group from the *m/e* 471 ion, and *m/e* 436 due to loss of a chlorine from the *m/e* 471 ion. The pattern of ions at *m/e* 471, 456, and 436 was typical of a maytansinoid with a demethyl C–1 amide such as normaysine, and indicated that **2** had the same macrocyclic ring system as normaysine **3** (1). The loss of 145 mass units from *m/e* 616 to *m/e* 471 suggested that the C–3 side chain ester was the same as in maytansine **1**, an ester of *N*-acetyl-*N*-methyl-L-alanine (1). Peaks at *m/e* 128 and *m/e* 100 corresponded to loss of –OH and –COOH, respectively, from *N*-acetyl-*N*-methyl-L-alanine.

Comparison of the proton magnetic resonance (pmr) spectrum of **2** with the pmr spectrum of maytansine **1** confirmed the structure of **2**. The major difference between the two spectra was the absence of the three proton singlet assigned to the C–1 amide methyl in the spectrum of **2**. Identification of the C–3 side-chain ester as *N*-acetyl-*N*-methyl-L-alanine was confirmed by the characteristic signals at δ 1.33 (3H, d, $J=7$ Hz, $C_2'-CH_3$), δ 1.98 (3H, s, $C_2'-NCOCH_3$), δ 2.85 (3H, s, $C_2'-NCH_3$), and δ 5.29 (1H, q, $J=7$ Hz, $C_2'-H$). In addition, all the peaks expected for a maytanside ring identical to maytansine were present, and normaytansine was assigned structure **2**.[2]

In order to confirm the maytanside ring structure, **2** was treated with sodium carbonate in methanol to eliminate the C–3 ester (1). The product obtained was identical by mixed tlc in several systems with normaysine **3**.

Normaytansine **2** was found to have significant *in vivo* activity against the P388 lymphocytic leukemia in mice at doses comparable to maytansine (e.g., *T/C* 181 at 100 μg/kg) and *in vitro* activity against the KB cell culture (ED_{50} 10^{-3} μg/ml) (5).[3] Thus the amide methyl at C–1 did not seem to affect the antileukemic activity of the maytansinoids.

EXPERIMENTAL[4]

ISOLATION OF FRACTIONS 207640–16 & 19.—Large scale extractions and isolation of maytansine from 15,000 kgs of *Maytenus buchananii* (PR–46541, stemwood from Kenya collected app. January and February

[1]Normaytansine is relatively unstable in solvent and decomposes upon standing in solvent at room temperature.

[2]The parent ion of a maytansinoid is usually not detectable in the mass spectrum due to the facile loss of H_2O and HNCO from the C9 carbinolamide. Thus, the highest peak detectable is the M^+-61 peak (1).

[3]Normaytansine **2** was apparently isolated from an earlier large-scale preparation of maytansine by the group at the University of Virginia under the direction of the late Dr. S. Morris Kupchan but was never reported.

[4]All mps are uncorrected. The nmr spectra were recorded on a Bruker WP–80 spectrometer with TMS as an internal standard. The ir spectra were measured on a Perkin-Elmer model 283 instrument and the uv spectra were measured on a Beckman Acta MVII recording spectrophotometer. The low resolution mass spectra were measured on a Finnigan model 4000 spectrometer. The 9KB cytotoxicity assays were performed at Arthur D. Little, Inc., Cambridge, Massachusetts, and the P–388 *in vivo* assays were performed at Raltech Associates, Madison, Wisconsin.

1976)[5] were performed by Monsanto Research Corporation in Dayton, Ohio. Four separate lots of maytansine were produced under conditions elaborated in Reference 6. One lot of maytansine, when analyzed on a Partisil PXS 10/25 silica gel column with a mobile phase of methylene chloride-methanol-diethylamine-*n*-hexane (75:75:2:1000) and an ultraviolet detector at 254 nm, showed a slow eluting impurity (normaytansine) of approximately 10% total area. To isolate the maytansine from the normaytansine, laboratory preparative chromatography was deemed necessary.

The sample of maytansine containing the normaytansine was divided into two fractions. One portion dissolved in methylene chloride and chromatographed with a Partisil 10/50 silica gel column (Whatman) with 7% isopropyl alcohol in methylene chloride as mobile phase. Elution was monitored by an ultraviolet detector at 254 nm. Maytansine fractions were recovered and combined. The remaining fraction contained approximately 70–80% normaytansine. On sitting in solvent, however, the water-clear sample was seen to turn yellow. On hplc analysis, additional faster eluting components were seen at the expense of the normaytansine. The sample was vacuum dried at 40° and labeled 207640-16.

The second portion (5.2 gms) of maytansine containing normaytansine dissolved in methylene chloride was chromatographed on a 2.54 cm by 1 meter preparative hplc column, developed by Monsanto Research Corporation specifically for natural products purification, slurry packed with 230–400 mesh silica gel (E. M. Darmstadt) and eluted under the following program: Two hundred mls of methylene chloride followed by 500 mls of a 3% isopropyl alcohol in methylene chloride finished off with 4% isopropyl alcohol in methylene chloride to completion. Fractions (10 mls each) were analyzed by hplc using a Partisil PXS 10/25 silica gel column and a mobile phase of methylene chloride-methanol-diethylamine-*n*-hexane (75:75:2:1000) and a uv detector at 254 nm. Fractions containing maytansine plus normaytansine were combined, dried *in vacuo*, and re-chromatographed on a Partisil M9 10/50 silica gel prep column with methylene chloride-methanol-diethylamine-*n*-hexane (75:75:2:1000) as mobile phase. Fractions were collected and analyzed by hplc. Normaytansine-containing fractions were combined, dried *in vacuo*, and labeled 207640-19.

ISOLATION OF NORMAYTANSINE **2**.—Fractions 207640-16 & 19 were combined and subjected to ptlc on silica gel 60 (EM Labs) developed with 5% methanol-chloroform (3X). Elution of the major band gave a yellowish solid which was again subjected to ptlc on silica gel 60 developed with 5% methanol-chloroform. Elution of the major band gave a white solid which was crystallized from dichloromethane-hexanes to yield 9 mg **2**: mp 166–167°; uv max (EtOH) 240 nm (ε 34100), 250 (25,800), 281 (4100), 288 (3900); ir (KBr) 3440, 2940, 1740, 1710, 1660, 1590, 1460, 1430, 1400, 1310, 1290, 1100, 1085 cm^{-1}; nmr ($CDCl_3$) δ 1.04 (3H, s, C4-CH_3), 1.30 (3H, d, J=6 Hz, C6-CH_3), 1.33 (3H, d, J=7 Hz, C2'-CH_3), 1.67 (3H, s, C14-CH_3), 1.98 (3H, s, C2'-$NCOCH_3$), 2.85 (3H, s, C2'-NCH_3), 3.07 (1H, d, $J_{5,6}$=9 Hz, C5-H), 3.13 (1H, d, $J_{15,15}$=13 Hz, C15-H), 3.38 (3H, s, C10-OCH_3), 3.55 (1H, d, $J_{10,11}$=9 Hz, C10-H), 3.59 (1H,s, C9-OH), 3.61 (1H, d, $J_{15,15}$=13 Hz, C15-H), 3.96 (3H, s, C20-OCH_3), 4.35 (1H, m, C7-H), 4.89 (1H, dd, $J_{2,3}$=11,3 Hz, C3-H), 5.29 (1H, q, J=7 Hz, C2'-H), 5.74 (1H, dd, $J_{10,11}$=9 Hz, $J_{11,12}$=14 Hz, C11-H), 6.60, 6.75 (2H, 2s, C17-H, C21-H), 1.0–2.5 (5H, C2-H_2, C6-H, C8-H_2), 6.20–7.0 (4H, 2NH, C12-H, C13-H); mass spectrum *m/e* 616, 471, 456, 436, 248, 222, 187, 170, 149, 128, 100; high resolution mass spectrum *m/e* 617.2556, $C_{32}H_{41}N_2O_5Cl+H^+$ (M^+-61+H^+)=617.2629.

HYDROLYSIS OF **2**.—1.3 mg of **2** was dissolved in 0.5 ml of methanol-water-tetrahydrofuran (1:1:0.1) and 1.6 mg of anhydrous Na_2CO_3 was added. The mixture was stirred at 25° for two hours, then cooled in an ice bath and acidified with 3 drops of 1N HCl. The aqueous solution was extracted with ethyl acetate (3×5 ml), and the combined extracts were dried over anhydrous Na_2SO_4. The ethyl acetate was removed on a rotary evaporator to yield 2.2 mg of a yellow oil. Ptlc on silica gel 60 developed with 5% methanol in chloroform yielded 0.5 mg normaysine **3**, identical with authentic material in three different tlc systems.

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[5]Plant material was supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, through which voucher specimens are preserved.

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