ANTITUMOR AGENTS 60.[1] MAYTANSINE, AN ANTILEUKEMIC PRINCIPLE FROM *MAYTENUS DIVERSIFOLIA*

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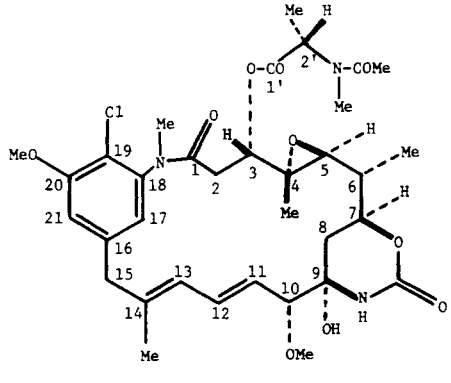
The genus *Maytenus* is known as the source of novel ansa macrolides including the potent tumor inhibitor maytansine (**1**) (1,2). Maytansine is currently undergoing Phase II clinical trials as an anticancer agent by the National Cancer Institute (3). The extreme scarcity (0.00002% yield) of maytansine from plant sources, coupled with its high (at the level of μg/kg) and wide (from 50–100-fold dosage) range of activity against mouse P-388 lymphocytic leukemia and other tumor systems (2) prompted our examination of a hitherto uninvestigated Formosan *Maytenus diversifolia* (4) for a possible better source of maytansine as well as other potent new antileukemic constituents of unusual structure.

The spinescent shrub *M. diversifolia* (Gray) Hou [=*Gymnosporia diversifolia* (Gray) Maxim., or *Catha diversifolia* A. Gray ex Maxim., or *Celastrus diversifolia* (Gray) Hemsl., or *Celastrus wallichianus* Sensu Hance] (Celastraceae) is known as "Pak-Tiong (Pei-Chung)" or "Tzu-Lou-Shih" (5) in Taiwan. The methanolic extract of the stems of *M. diversifolia* was found to show significant inhibitory activity *in vivo* against the P-388 lymphocytic leukemia growth in BDF_1 mice (T/C=180%) at 50 mg/kg/day, I.P. Subsequent bioassay-directed fractionation in P-388 *in vivo* (6) led to the conclusion that the most active component of this extract was maytansine (**1**). Maytansine was isolated in a better yield of 0.0000374% of the dried plant material compared to that reported previously (1).



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EXPERIMENTAL

PLANT MATERIAL.—The stems of *Maytenus diversifolia* (Celastraceae) used was from a collection made in December 1979 in Mt. Li-Long, Ping-tong Shen. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

[1]For part 59 see I. H. Hall, Y. F. Liou, K. H. Lee, S. G. Chaney and W. Willingham, Jr., *J. Pharm. Sci.*, submitted.

BIOASSAY-DIRECTED ISOLATION AND CHARACTERIZATION OF MAYTANSINE.—The ground air-dried stems of *M. diversifolia* (9.09 kg) were extracted with methanol. Removal of the methanol gave a syrup [*in vivo* P-388 assay (6): T/C=164% at 25 mg/kg/day] which was dissolved in methanol-water (3:1) and extracted successively with hexane and chloroform. The chloroform residue (230 g, T/C=178% at 12.5 mg/kg/day was chromatographed on silica gel (1.7 kg) and eluted with benzene, benzene-chloroform, chloroform, chloroform-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol. From the ethyl acetate fraction (T/C=228% at 6.25 mg/kg/day) after purification by preparative tlc [silica gel; chloroform-methanol (9.5:0.5) and then benzene-methanol (9.8:0.2)], 3.4 mg of **1** was obtained as colorless crystals. The identity of **1** with an authentic sample of maytansine was confirmed by comparative mp, tlc [chloroform-methanol (10:1)], $[\alpha]_D$ and superimposable ir (KBr) and nmr spectra.[2,3]

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[2]The 250 MHz nmr spectrum ($CDCl_3$) of **1** showed different chemical shifts compared to those obtained from a 100 MHz instrument for maytansine (1) at δ 0.79 (3H, s, Me-4), 1.26 (3H, d, J=6.0 Hz, Me-6), 1.31 (3H, d, J=7.0 Hz, Me-2'), 1.56 (3H, br. s, Me-14), 2.11 (3H, s, C-2'-NCO*Me*), 2.18 (1H, dd, $J_{2,2}$=15.0 Hz, $J_{2,3}$=3.0 Hz, H-2), 2.61 (1H, dd, $J_{2,2}$=15.0 Hz, $J_{2,3}$=12.0 Hz, H-2), 2.85 (3H, s, C-2'-N-*Me*), 3.02 (1H, d, J=9.0 Hz, H-5), 3.11 (1H, d, J=13.0 Hz, H-15), 3.19 (3H, s, C-1-N-*Me*), 3.35 (3H, s, OMe-10), 3.47 (1H, s, OH-9), 3.49 (1H, d, $J_{10,11}$=9.0 Hz, H-10), 3.64 (1H, d, $J_{15,15}$=13.0 Hz, H-15), 3.97 (3H, s, OMe-20), 4.27 (1H, m, H-7), 4.75 (1H, dd, $J_{2,3}$=12.0 and 3.0 Hz, H-3), 5.32 (1H, q, J=7.0 Hz, H-2'), 5.65 (1H, dd, $J_{10,11}$=9.0 Hz, $J_{11,12}$=15.0 Hz, H-11), 6.21 (1H, br. s, C-9-NH), 6.41 (1H, dd, $J_{11,12}$=15.0 Hz, $J_{12,13}$=11.0 Hz, H-12), 6.67 (1H, br. d, $J_{12,13}$=11.0 Hz, H-13), 6.72 (1H, d, $J_{17,21}$=1.5 Hz, H-17) and 6.81 (1H, d, $J_{17,21}$=1.5 Hz, H-21).

[3]Full details of the isolation and identification can be made available to the reader on request to the senior author.