STRUCTURE AND STEREOCHEMISTRY OF EMARGINATINE-A, A NOVEL CYTOTOXIC SESQUITERPENE PYRIDINE ALKALOID FROM MAYTENUS EMARGINATA: X-RAY CRYSTAL STRUCTURE OF EMARGINATINE-A MONOHYDRATE

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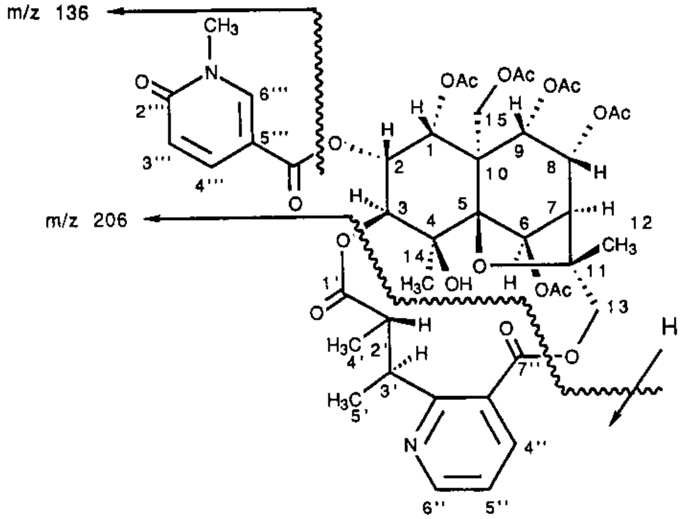
Abstract — The structure and stereochemistry of emarginatine-A (1), a novel cytotoxic sesquiterpene pyridine alkaloid isolated from Maytenus emarginata, have been established from spectral data in conjunction with a single-crystal X-ray analysis of its monohydrate.

In the course of our continuing search among Formosan plants for novel potential antitumor agents,[1] the methanolic extract of the stems and branches of Maytenus emarginata (Celastraceae) was found to display significant in vitro cytotoxicity against KB cell cultures and in vivo antitumor activity against P-388 lymphocytic leukemia. We report herein on the isolation and characterization of a novel sesquiterpene pyridine alkaloid, emarginatine-A, from M. emarginata.[2] Emarginatine-A (1) was isolated in 0.001% yield from the active chloroform fraction by silica gel column chromatography and preparative hplc guided by in vitro KB cell culture assay. Emarginatine-A {$C_{43}H_{50}N_2O_{19}$, m/z 898.3026 (M^+), mp 312-313 °C, $[\alpha]_D^{22}$ +70° (c 0.32, $CHCl_3$)} showed a uv band at 266 nm (pyridone) and ir absorptions at 3400 (OH), 1740 (ester), and 1660 (pyridone) cm^{-1}.

The ^{1}H nmr spectrum (400 MHz, $CDCl_3$) indicated the presence of five acetyl (δ 1.81 - 2.38), two methyl (δ 1.71, s, 12-H; 1.55, s, 14-H), and two methylene (δ 3.72 and 5.98, AX, J = 11.6 Hz, 13-H_a and 13-H_b; δ 4.16 and 5.54, AX, J = 13.5 Hz, 15-H_a and 15-H_b) groups. From spin-spin decoupling and COSY studies, seven methine protons [(δ 7.04, s, 6-H; AMX system for 7-H, 8-H, and 9-H at δ 2.38, 5.54, and 5.42 (J = 3.1 and 6.1 Hz) and for 1-H, 2-H, and 3-H at δ 5.67, 5.48, and 4.78 (J = 4.2 and 2.4 Hz)] were also identified.

The foregoing signals were suggestive of a dihydro-β-agarofuran-type sesquiterpene similar to that in euonymine[3] (2). In addition, the observation that two methyl doublets (δ 1.20, 4'-H and 1.39, 5'-H) were coupled with two methine protons [δ 2.57 (q, 2'-H, J = 7 Hz) and 4.67 (q, 3'-H, J = 6.8 Hz)], and an AMX system representing a 2,3-disubstituted pyridine [δ 8.08, d, 4"-H; 7.32, dd, 5"-H; 8.70, d, 6"-H (J = 1.8, 7.8, and 4.8 Hz, respectively)] occurred in the aromatic region, pointed to an evoninate macrocyclic diester linkage at C-3 and C-13. When taken together, all of these signals bear a striking resemblance to those reported for euonymine,[3] save for the fact that one acetyl group signal is missing and is replaced by those corresponding to a 5-carboxy-N-methylpyridone moiety {AMX system in the aromatic region [δ 6.59, d, 3'''-H; 7.90, dd, 4'''-H; 8.42, d, 6'''-H (J = 9.6 and 2.5 Hz)]} as well as an N-methyl group (δ 3.72, s). The downfield shift of the signal for the proton at C-2 in (1) vs. (2) suggested that the 5-carboxy-N-methylpyridonyl moiety was located at this position. Support for the presence of the pyridonyl ester and macrocyclic di-



1

2 OAc at C2

ester was derived from the EI mass spectral fragmentation pattern which contained ions at m/z 136 and 206 resulting from cleavage of the respective ester linkages. The ^{13}C nmr spectrum of (1)[4] was consistent with the proposed structure, comprising of signals from the euonymine-like skeleton with five acetyl groups in addition to signals for a 5-carboxy-N-methylpyridonyl moiety. The complete structure and relative stereochemistry of (1) were established unequivocally by X-ray analysis of a crystal of its monohydrate. Crystal data: $C_{43}H_{50}N_2O_{19}.H_2O$, M = 916.90, orthorhombic, space group $P2_12_12_1$, a = 18.867(2), b = 19.103(4), c = 12.786(6) Å, U = 4608.3 Å^3, Z = 4, D_c = 1.321 g cm^{-3}, μ(Cu-Kα radiation, λ = 1.5418 Å) = 8.5 cm^{-1}. Intensity data [(+h,+k,+ℓ); 4557 reflections] from a crystal of dimensions 0.14 x 0.14 x 0.16 mm were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-Kα radiation, incident-beam graphite monochromator; ω-2θ scans, $\theta_{max.}$ = 67°). The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-

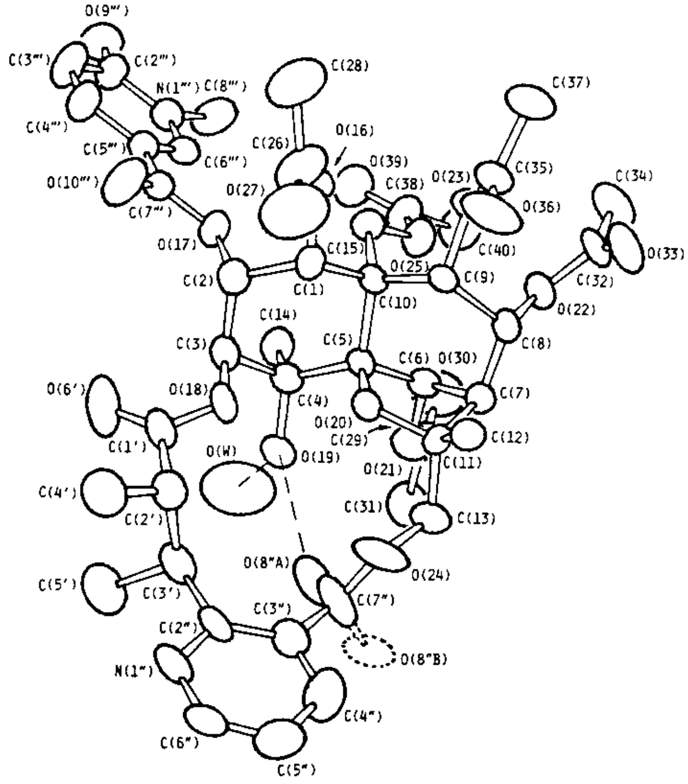


Figure 1. Structure and solid-state conformation of emarginatine-A (1) in crystals of the monohydrate; O(8") is disordered over two positions. Hydrogen atoms have been omitted for clarity.

squares refinement (Enraf-Nonius SDP) of atomic positional and thermal parameters (anisotropic C, N, O; fixed H contributions) converged at $\underline{R} = 0.059$ [$\underline{R}_w = 0.079$, $\underline{w} = 1/\sigma^2(|\underline{F}_o|)$] over 2349 reflections with $\underline{I} > 3.0\sigma(\underline{I})$.[5] A view of the structure is provided in Figure 1. One of the macrocyclic diester carbonyl oxygen atoms is disordered over two positions depending upon whether or not it is involved in an intramolecular O-19...O-8"A hydrogen bond. The absolute configuration could not be derived independently from the *X*-ray data but it is probably as represented by structure (**1**) since this has the same stereochemistry at all chiral centers in common with (**2**) and neoevonine.[6]

Compound (**1**) represents the first example of a euonymine-type polyester sesquiterpene alkaloid bearing a 5-carboxy-*N*-methylpyridonyl substituent. Bioassay of pure (**1**) determined its *in vitro* cytotoxicity against KB cells to be 4.0 μg/ml. Observation of this level of significant activity in this type of alkaloid clearly warrants further exploration.

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4. ^{13}C Nmr data (100 MHz, $CDCl_3$) for (**1**) (multiplicities determined by DEPT pulse sequence, and chemical shifts assigned by heteronuclear ^{1}H-^{13}C COSY spectra): δ 72.92(d, C-1), 69.18(d, C-2), 75.45(d, C-3), 70.25(s, C-4), 93.89(s, C-5), 73.55(d, C-6), 50.54(d, C-7), 68.75(d, C-8), 70.43 (d, C-9), 51.95(d, C-10), 84.22(s, C-11), 18.53(q, C-12), 69.82(t, C-13), 23.22(q, C-14), 60.32 (t, C-15), 173.82(s, C-1'), 44.93(d, C-2'), 36.34(d, C-3'), 9.67(q, C-4'), 11.79(q, C-5'), 165.52(s, C-2"), 124.93(s, C-3"), 137.75(d, C-4"), 121.11(d, C-5"), 151.53(d, C-6"), 168.92 (s, C-7"), 162.97(s, C-2"'), 119.80(d, C-3"'), 138.93(d, C-4"'), 108.15(s, C-5"'), 144.02(d, C-6"'), 162.45(s, C-7"'), 38.14(q, N-Me), 20.39, 20.48, 21.04, 21.29, 21.61 (5 x OCOMe), 162.47, 169.01 x 2, 169.97 and 170.98 (5 x C=O).
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