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ANTITUMOR AGENTS, 116.[1] CYTOTOXIC TRITERPENES FROM *MAYTENUS DIVERSIFOLIA*

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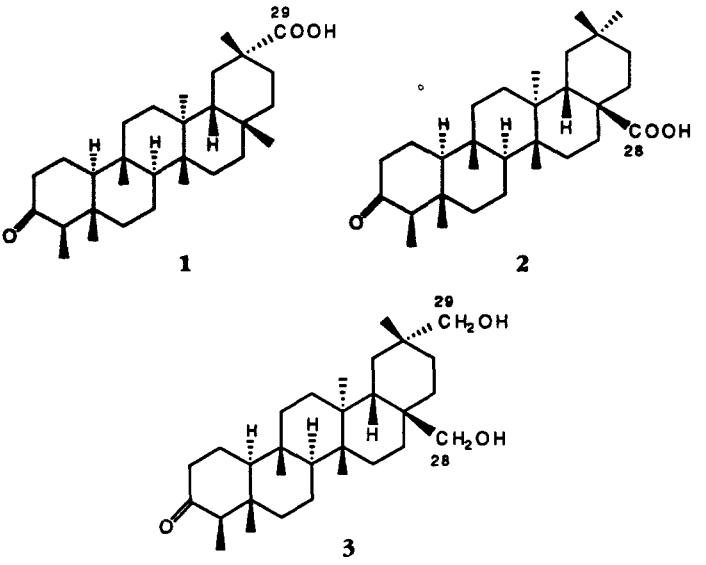
ABSTRACT.—The known triterpenes 3-oxofriedelan-29-oic aicd [**1**], 3-oxofriedelan-28-oic acid [**2**], and 28,29-dihydroxyfriedelan-3-one [**3**] have been isolated from *Maytenus diversifolia*. Compounds **1–3** demonstrated cytotoxicity against the A-549 lung carcinoma cells with ED_{50} values of 0.21, 1.18, and 0.64 μg/ml, respectively.

We reported previously on the isolation of new triterpenes, maytenfolic acid and maytenfoliol, together with maytansine and sitosterol-β-D-glucoside, as antileukemic principles from the $CHCl_3$ extract of *Maytenus diversifolia* (Gray) Hou (Celastraceae) (1–3). Further investigation on the MeOH extract of this same plant, which showed potent in vitro cytotoxicity against A-549 lung carcinoma cells, has now led to the isolation and characterization of three known triterpenes, 3-oxofriedelan-29-oic acid (polpunonic acid) [**1**] (4,5), 3-oxofriedelan-28-oic acid [**2**] (6), and 28,29-dihydroxyfriedelan-3-one [**3**] (7), as the cytotoxic principles. Compounds **1–3** all demonstrated cytotoxicity against the A-549 lung carcinoma cells with ED_{50} values of 0.21, 1.18, and 0.64 μg/ml, respectively. Compound **2** was also cytotoxic against both L-1210 (ED_{50} = 2.95 μg/ml) and KB (ED_{50} = 3.70 μg/ml) tumor cells. Compound **2** was previ-



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ously isolated only from *Euonymus revolutus* (Celastraceae) (8). Other triterpenes that are cytotoxic against A-549, L-1210, and KB tumor cells, include ursolic acid and its derivatives (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Thomas Hoover melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. ^{1}H-nmr spectra were recorded on a Bruker WM-250 Fourier Transform spectrometer and are given in ppm (δ) downfield from an internal TMS standard. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV using a direct inlet system. Si gel for cc refers to Merck Si gel 60 (70–230 mesh). Si gel for preparative tlc refers to Analtech Si gel G (1000 μm). Compounds were visualized by uv light or spraying with 1% $Ce(SO_4)_2$/10% H_2SO_4 solution followed by heating.

PLANT MATERIAL.—The stems of *M. diversifolia* (10) were procured at Mt. Lilong, Pingtong Shen, Taiwan. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

BIOASSAY-DIRECTED ISOLATION AND CHARACTERIZATION OF 3-OXOFRIEDELAN-29-OIC ACID [**1**], 3-OXOFRIEDELAN-28-OIC ACID [**2**], AND 28,29-DIHYDROXYFRIEDELAN-3-ONE [**3**].—The active MeOH extract (400 g) of *M. diversifolia* was partitioned to a *n*-hexane-soluble portion (24.5 g) and an MeOH-soluble portion. Repeated cc (Si gel) of the *n*-hexane portion yielded active fractions, and from fraction 4, of 3-oxofriedelan-29-oic aicd [**1**] (23.7 mg) and of 3-oxofriedelan-28-oic acid [**2**] (18.7 mg) were isolated.

The MeOH portion was further concentrated and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ yielded a residue (42.3 g), which was chromatographed on Si gel and eluted with a gradient of C_6H_6, C_6H_6/$CHCl_3$, $CHCl_3$, $CHCl_3$/EtOAc, EtOAc, EtOAc/MeOH, and MeOH. From the EtOAc fraction after purification by preparative tlc [Si gel; $CHCl_3$-EtOAc (8:1)] and recrystallization [$CHCl_3$-MeOH (9:1)], 28,29-dihydroxyfriedelan-3-one [**3**] (41.5 mg) was obtained as colorless crystals.

The identities of **1** and **3** as 3-oxofriedelan-29-oic acid and 28,29-dihydroxyfriedelan-3-one, respectively, were established by comparing mp, [α]D, and superimposable ir and nmr spectra with those of their corresponding authentic samples. The characterization of **2** as 3-oxofriedelan-28-oic acid was achieved by a direct comparison with the acid obtained by Jones oxidation of canophyllol. Canophyllol was isolated previously from this same plant (3).

Compound **1**.—Mp 274–275° [lit. (5) mp 261–262°]; [α]D −33.6° (*c* = 0.7, $CHCl_3$) [lit. (5) [α]D −41.6° (*c* = 1.5, $CHCl_3$)]; ir ($CHCl_3$) 3300–2500, 2932, 1700 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.72, 0.87, 0.88, 1.00, 1.09 and 1.25 (each 3H, s), 0.87 (3H, d, *J* = 6.2 Hz); ^{13}C nmr ($CDCl_3$) δ 6.18 (q, C-23), 14.64 (q, C-24), 16.27 (q, C-27), 18.04 (q, C-25), 18.22 (t, C-7), 18.41 (q, C-26), 22.26 (t, C-1), 29.45 (t, C-21), 29.51 (t, C-19), 29.69 (t, C-15), 30.09 (s, C-17), 30.24 (t, C-12), 31.55 (q, C-30), 31.79 (q, C-28), 35.27 (t, C-11), 36.13 (t, C-16), 36.57 (t, C-22), 37.41 (s, C-9), 39.13 (s, C-13), 39.22 (s, C-14), 40.42 (s, C-20), 41.30 (t, C-6), 41.49 (t, C-2), 42.05 (s, C-5), 44.23 (d, C-18), 50.65 (d, C-8), 58.24 (d, C-4), 59.75 (d, C-10), 184.52 (s, C-29), 213.32 (s, C-3) [These data are, in general, in accord with those reported earlier by Ramaiah *et al.* (5) except for the differences in the decimal places of each carbon atom]; ms *m/z* $[M]^+$ 456 (40.4%), 441 (10.8), 273 (49.0), 250 (26.5), 235 (14.6), 155 (14.6), 109 (100).

Compound **3**.—Mp 286–289°; [α]D −10.4° (*c* = 0.3, $CHCl_3$); ir (KBr) 3670–3070, 1712, 1055, 998 cm^{-1}; ^{1}H nmr (C_5D_5N) δ 0.65, 0.75, 0.97, 1.21, 1.28 (each 3H, s), 0.94 (3H, d, *J* = 6.7 Hz), 3.56, 3.68 (each 1H, ABq, *J* = 10.1), 3.98, 4.05 (each 1H, ABq, *J* = 10.7); ^{13}C nmr (C_5D_5N) δ 7.22 (q, C-23), 14.68 (q, C-24), 18.04 (q, C-25), 18.44 (t, C-7), 18.97 (q, C-27), 20.07 (q, C-26), 22.41 (t, C-1), 27.47 (t, C-30), 29.18 (t, C-21), 29.50 (t, C-15), 29.77 (t, C-19), 30.47 (t, C-12), 32.60 (t, C-16), 33.04 (t, C-22), 33.62 (s, C-20), 35.74 (t, C-11), 36.62 (s, C-17), 37.54 (s, C-9), 38.25 (s, C-14), 39.01 (s, C-18), 40.11 (s, C-13), 41.17 (t, C-6), 41.58 (t, C-2), 42.06 (s, C-5), 53.16 (d, C-8), 57.95 (d, C-4), 59.24 (d, C-10), 67.11 (t, C-28), 73.62 (t, C-29), 211.75 (s, C-3); ms *m/z* $[M - H_2O]^+$ 440 (24%), 427 (100), 409 (34.0), 273 (68.1), 109 (97.0).

Compound **2**.—Mp 307–309°; [α]D −17.1° (*c* = 1.1, $CHCl_3$); ir ($CHCl_3$) 3300–2450, 1700 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.72, 0.81, 0.86, 0.94 (each 3H, s), 1.04 (6H, s), 0.87 (3H, d, *J* = 6.6 Hz); ^{13}C nmr ($CDCl_3$) δ 6.79 (q, C-23), 14.62 (q, C-24), 17.47 (q, C-25), 18.04 (t, C-7), 18.52 (q, C-27), 20.56 (q, C-26), 22.23 (t, C-1), 28.39 (s, C-20), 29.31 (t, C-15), 29.69 (q, C-29), 31.01 (t, C-12), 32.56 (t, C-16), 32.60 (t, C-22), 34.46 (q, C-30), 34.79 (t, C-21), 35.41 (t, C-19), 35.87 (t, C-11), 37.61 (d, C-18), 37.64 (s, C-17), 37.75 (s, C-9), 38.85 (s, C-14), 41.05 (s, C-13), 41.47 (t, C-6), 42.05 (t, C-2), 44.74 (s, C-5), 52.95 (d, C-8), 58.16 (d, C-4), 59.21 (d, C-10), 184.99 (s, C-28), 213.26 (s, C-3); ms *m/z* $[M]^+$ 456 (30.0%), 273 (36.2), 191 (51.4).

OXIDATION OF CANOPHYLLOL.—One drop of Jones reagent was added to a solution of canophyllol (11.3 mg) in Me_2CO (3 ml). After the mixture was allowed to stand at room temperature for 3 h, it was diluted with H_2O and the product was extracted with $CHCl_3$. The $CHCl_3$ extract was washed, dried, and evaporated in vacuo. The crude product was purified by cc to give an acid **2** (5.6 mg) and an aldehyde (7.1 mg). The latter was identical to an authentic sample of canophyllal (3-oxofriedelan-3-al): mp 260–262°; $[\alpha]D -12.8°$ ($c=0.5$, $CHCl_3$); ir (KBr) 2790, 1710, 1700, 1450, 1379 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.66, 0.72, 0.84, 0.95, 0.98, 1.08 (each 3H, s), 0.86 (3H, d, $J=6.5$ Hz), 9.44 (1H, s); ms *m/z* $[M]^+$ 440 (23.0%), 411 (100), 355 (11.2), 273 (58.3).

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