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ANTITUMOUR TRITERPENES OF *MAYTENUS DIVERSIFOLIA**

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Key Word Index—*Maytenus diversifolia*; Celastraceae; cytotoxic and antileukemic activity; maytenfoliol; maytenfolic acid; maytensifolins -A and -B; canophyllal; canophyllol; pachysonol; 29-hydroxyfriedelan-3-one; 30-hydroxyfriedelan-3-one; maytansine.

Abstract—The known compounds maytansine and sitosterol-β-D-glucoside as well as the new triterpenes maytenfoliol and maytenfolic acid were isolated as antileukemic agents from *Maytenus diversifolia*. Other triterpenes isolated included the new maytensifolin-A and -B as well as the known friedelin, canophyllal, β-amyrin, canophyllol, pachysonol, 29-hydroxyfriedelan-3-one and 30-hydroxyfriedelan-3-one.

INTRODUCTION

The spinescent shrub *Maytenus diversifolia* (Gray) Hou [= *Gymnosporia diversifolia* (Gray) Maxim.] is known as 'Pak-Tiong (Pei-Chung)' or 'Tzu-Lou-Shih' [2, 3] in Taiwan. In the course of a continuing search for potential plant antitumour agents, the methanolic extract of the stem of a hitherto uninvestigated *M. diversifolia* was found to show significant inhibitory activity *in vivo* against the P-388 lymphocytic leukemia growth in BDF_1 mide (T/C = 180%) at 50 mg/kg/day.IP.‡ Preliminary reports described the isolation of maytansine as the most active component of this extract [5] as well as the structural determination of the additional new antileukemic triterpenes maytenfoliol (**1**) and maytenfolic acid (**2**) [6], and a novel hydroperoxy-nortriterpene maytensifolin-A [7]. The present report fully describes the isolation and structural elucidation of the triterpenes as well as the companion compounds which include the new maytensifolin-B (**4**) and the known friedelin (**5**), canophyllal (**6**), β-amyrin (**7**), canophyllol (**8**), pachysonol (**9**) 29-hydroxyfriedelan-3-one (**10**) and 30-hydroxyfriedelan-3-one (**11**) and β-sitosterol-β-D-glucoside.

RESULTS AND DISCUSSION

The active methanolic extract of the ground air-dried stems of *M. diversifolia* were dissolved in methanol–water (3:1) and extracted successively with hexane and chloroform. Chromatography of the chloroform extract resulted in the isolation of the active principles maytansine (0.0000374%, T/C = 228% at 6.25 mg/kg/day in P-388) [5, 8], maytenfoliol (**1**, 0.00165%, ED_{50} = 4.56 μg/ml in KB, T/C = 120% at 10 mg/kg/day in P-388) [6], maytenfolic acid (**2**, 0.00114%, T/C = 148% at 6.25 mg/kg/day in P-388) [6] and β-sitosterol-β-D-glucoside (0.00121%, T/C = 132% at 0.25 mg/kg/day in P-388) [9] together with maytensifolin-A (**3**, 0.00013%) [7], maytensifolin-B (**4**, 0.00142%) [10], friedelin (**5**, 0.00144%) [11], canophyllal (**6**, 0.00008%) [12], β-amyrin (**7**, 0.00124%) [13], canophyllol (**8**, 0.00263%) [12], pachysonol (**9**, 0.00046%) [10], 29-hydroxyfriedelan-3-one (**10**, 0.00013%) [14] and 30-hydroxyfriedelan-3-one (**11**, 0.00085%) [15]. Maytenfoliol (**1**, $C_{30}H_{50}O_3$) was a triterpene alcohol of the friedelin type bearing one secondary and five tertiary methyl groups and three protons α to a six-membered ring ketone [ν1700 cm^{-1} and δ212.94(s)] (Table 1). The presence of two primary hydroxyl groups was confirmed by the formation of diacetate [**1a**; $C_{34}H_{54}O_5$; ν1724 cm^{-1}; δ2.06 and 2.09 (each 3H, *s*)]. A characteristic peak at *m/z* 273 arising from the cleavage of C-13–C-18 and C-14–C-15 bonds in addition to a base peak at *m/z* 427 resulting from loss of an angular CH_2OH group (e.g. CH_2OH–C-17) as seen in a typical friedelin [16] fragmentation pattern (Scheme 1), led to the conclusion that one of the CH_2OH groups was located at C-17 and the other was at C-20. Further evidence for the assignment of the CH_2OH group at C-17 was obtained by reduction of maytenfoliol ditosylate (**16**) to a 3β-hydroxy monotosylate (**1c**), which was found to be identical with the reduction product of canophyllol monotosylate derived from canophyllol (**8**) under similar conditions. The stereochemistry at C-20 was unambiguously elucidated by single-crystal X-ray analysis as reported before [6].

Maytenfolic acid (**2**, $C_{30}H_{48}O_5$) contained a carboxylic acid and two hydroxyl groups, seven tertiary methyl moieties and a trisubstituted double bond, indicating the presence of an olean-12-ene system [17]. These data, when considered in conjunction with the occurrence of a pair of

*Part 73 in the series "Antitumour Agents". For Part 72 see ref. [1].

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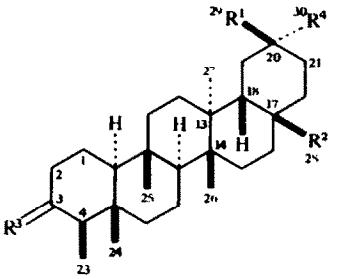
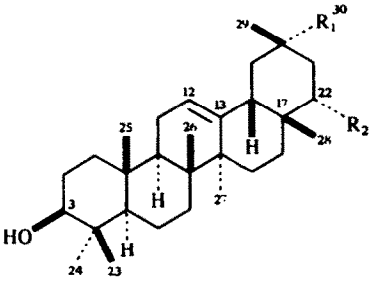
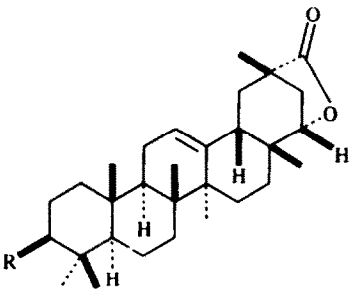
‡*In vivo* (in P-388 lymphocytic leukemia in BDF_1 mice) and *in vitro* (in KB cell culture) assays were carried out by Dr. Y. C. Cheng and Mr. M. Fisher, Cancer Research Center, and by Dr. I. H. Hall, Division of Medicinal Chemistry and Natural Products by a literature method [4].

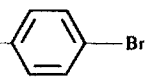


1 $R^1 = R^2 = CH_2OH$, $R^3 = O$, $R^4 = Me$
1a $R^1 = R^2 = CH_2OAc$, $R^3 = O$, $R^4 = Me$
1b $R^1 = R^2 = CH_2OTs$, $R^3 = O$, $R^4 = Me$
1c $R^1 = R^4 = Me$, $R^2 = CH_2OTs$, $R^3 = \beta\text{-}OH, \alpha\text{-}H$
5 $R^1 = R^2 = R^4 = Me$, $R^3 = O$
6 $R^1 = R^4 = Me$, $R^2 = CHO$, $R^3 = O$
8 $R^1 = R^4 = Me$, $R^2 = CH_2OH$, $R^3 = O$
10 $R^1 = CH_2OH$, $R^2 = R^4 = Me$, $R^3 = O$
11 $R^1 = R^2 = Me$, $R^3 = O$, $R^4 = CH_2OH$



2 $R^1 = COOH$, $R^2 = OH$
7 $R^1 = Me$, $R^2 = H$



2a R = OAc
2b R = OH
2c R = OCO—  —Br

diagnostically important mass spectal peaks (*m/z* 264 and 207) (Scheme 1) associated with a *retro*-Diels–Alder fragmentation in triterpene ring-C of members of the Δ^{12}-β-amyrin class [18], led to the conclusion that **2** was a new member of the olean-12-en-χ-oic acid series having a C-17- or C-20-carboxy-group and with one of the two secondary hydroxyl groups located adjacent to a carbon atom bearing two hydrogen atoms in ring D or E. The group was assigned at a site γ to the carboxy-function because upon acetylation of **2** with acetic anhydride in pyridine at room temperature, it formed unexpectedly a monoacetyl γ-lactone [**2a**; $C_{32}H_{48}O_4$; ν1720 and 1762 cm^{-1}; δ2.05 (3H, *s*, OAc-3, 4.48 (1H, *dd*, J = 6.0 and 10.0 Hz, H-3) and 4.14 (1H, *d*, J = 5.0 Hz, H-22)]. Acid treatment of **2** with sulphuric acid in ethanol under reflux for 2 hr yielded a 3-hydroxy-γ-lactone [**2b**; $C_{30}H_{46}O_3$; *m/z* 246 and 207 (Scheme 1)] which was further converted into its *p*-bromobenzoate (**2c**, $C_{37}H_{49}O_4Br$) and acetate. The acetate was identical to the monoacetyl-γ-lactone (**2a**) obtained from acetylation of **2** as mentioned above. Further confirmation of the structure and stereochemistries of **2** and **2b*** were achieved by single-crystal X-ray analyses as described previously [6]. It is noteworthy that immediately after our first report [6] on **2**, Chang *et al.* [19] reported the isolation of abruslactone-A as a new triterpene sapogenin from the roots and vines of *Abrus precatorius*. Abruslactone-A is structurally identical to **2b**. However, since abruslactone-A was obtained by acid hydrolysis of a methanolic extract with dilute sulphuric acid in aqueous methanol [19, 20], it might be an artifact of **2b**.

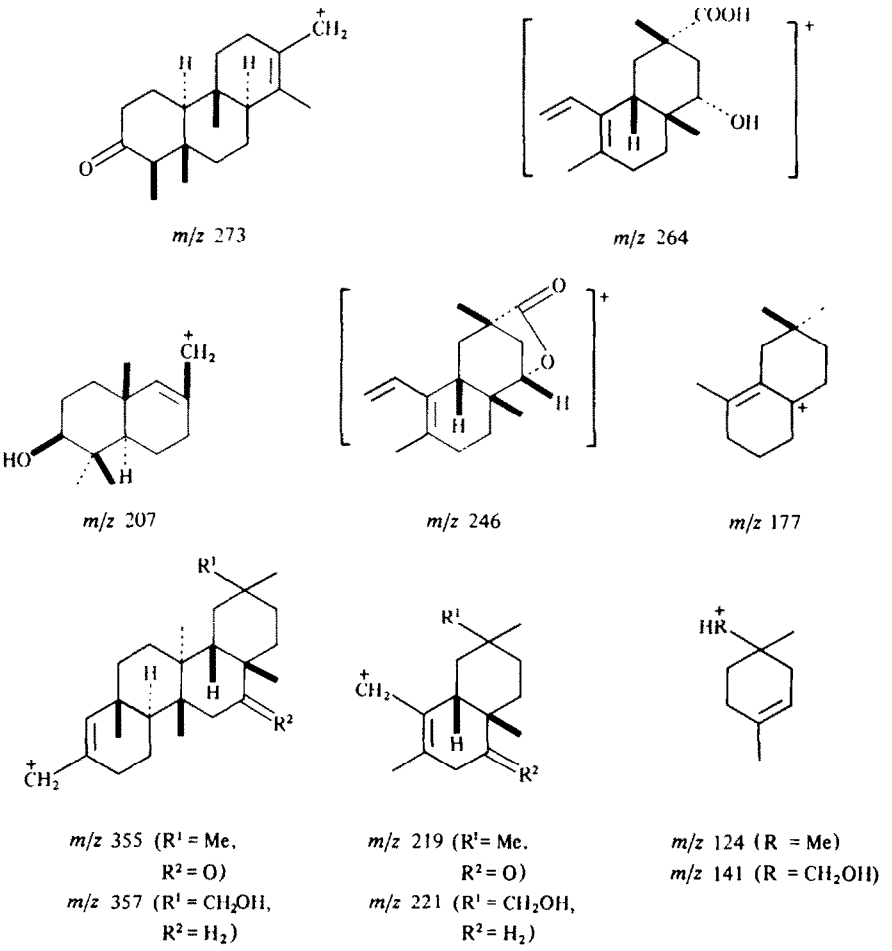
Maytensifolin-A (**3**) formed readily a monoacetate [**3a**, $C_{31}H_{50}O_4$ (*m/z* 486.3728, $[M]^+$)] with acetic anhydride in pyridine, confirming that the mass peak at *m/z* 426.3497 ($C_{29}H_{46}O_2$) in **3** was $[M-H_2O]^+$. Maytensifolin-A possessed a carbonyl [ν1696 cm^{-1}; δ212.875 (*s*)] and a hydroperoxy [ν3350 (O–OH), 880 and 883 (–O–O–) cm^{-1} moiety; δ6.83 (1H, *s*, O–OH as it disappeared upon addition of D_2O and δ203.715 (*s*, quaternary carbon bonded to oxygen function)] group. The assignment of this hydroperoxy group at C-17 of friedelan-3-one was based on the observation of a diagnostically

*Unpublished data of Professor A. T. McPhail, Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27706, U.S.A.

Table 1. ^{1}H NMR spectral data* for triterpenes isolated from *M. diversifolia*

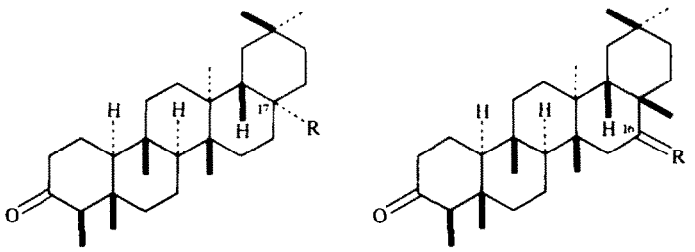
Compound	H-2	H-4	H-3	H-12	Me-4	Me-5, 9, 13, 14 (17), 20 (20)	Me-4, 4, 8, 10, 14, 17, 20 (20)	CH_2OH-20	CH_2OH-17	Misc.
1	2.10–2.50 (*m*)	2.10–2.50 (*m*)			0.87 (*d*, 6.0)	0.72, 0.86, 0.90, 1.00, 1.14, (each 3H, *s*)		3.30, 3.35 (each 1H, *d*, 10.0)	3.54, 3.63 (each 1H, *d*, 10.5)	
2†			3.45 (*dd*; 6.0, 9.5)	5.38 (*br s*)			1.00, 1.09, 1.09, 1.25, 1.30 1.38, 1.61 (each 3H, *s*)			4.04 (*dd*; 4.0, 12.5, H-22)
3	2.20–2.50 (*m*)	2.20–2.50 (*m*)			0.87 (*d*, 6.0)	0.70, 0.89, 0.92, 0.92, 0.96, 1.05 (each 3H, *s*)				6.83 (*s*, OOH-17)
4	2.20–2.50 (*m*)	2.20–2.50 (*m*)			0.88 (*d*, 6.0)	0.74, 0.90, 0.91, 0.96, 1.05, 1.20, 1.30 (each 3H, *s*)				2.08, 2.40 (each 1H, AB*d*, 19.0, H-15)
5	2.10–2.50 (*m*)	2.10–2.50 (*m*)			0.87 (*d*, 6.0)	0.72, 0.86, 0.94, 0.99, 1.00, 1.04, 1.17 (each 3H, *s*)				
6					0.86 (*d*, 6.0)	0.66, 0.72, 0.84, 0.95, 0.98, 1.08 (each 3H, *s*)				9.44 (*s*, CHO-17)
7			3.21 (*m*)	5.18 (*t*-like, 3.0)			0.79, 0.83, 0.87, 0.87, 0.94, 0.96, 1.00, 1.13 (each 3H, *s*)			
8	2.20–2.50 (*m*)	2.20–2.50 (*m*)			0.87 (*d*, 6.0)	0.72, 0.87, 0.91, 0.98, 0.99, 1.14 (each 3H, *s*)			3.62 (*s*)	
9					0.88 (*d*, 6.0)	0.72, 0.87, 0.97, 1.01, 1.03, 1.08, 1.19 (each 3H, *s*)				4.02, (*t*, 9.0, H-16)
10	2.20–2.50 (*m*)	2.20–2.50 (*m*)			0.87 (*d*, 6.0)	0.71, 0.86, 0.98, 0.99, 1.06, 1.14 (each 3H, *s*)		3.34, 3.42 (each 1H, *d*, 10.0)		
11	2.20–2.50 (*m*)	2.20–2.50 (*m*)			0.87 (*d*, 6.0)	0.72, 0.86, 1.03, 1.03, 1.04, 1.21 (each 3H, *s*)		3.25 (*br s*)		

*Run in $CDCl_3$ at 250 MHz. Values are in δ (ppm). Coupling constants (J in Hz) are given in parentheses.
†Measured in pyridine-d_5.



Scheme 1. Diagnostic mass spectral fragments from triterpenes of *Maytenus diversifolia*.

important mass peak at *m/z* 273 and a base peak at *m/z* 177 (Scheme 1), formed most likely *via* the pathway described previously [7], which was observed in the 28-nor-$\Delta^{17(18)}$-oleanenes [18]. The possibility of placing the hydroperoxy group at C-18 was ruled out by judging from its more hindered nature due to a 1,3-diaxial interaction with the methyl groups at C-14 and C-20. The ready formation of an acetate (**3a**) at C-17 also supported this consideration. The foregoing evidence suggested that **3** was 17-perhydroxy-28-norfriedelan-3-one which appeared to be the first example of a triterpene bearing a hydroperoxy moiety. The complete structure and stereochemistry of **3** were established unequivocally by a single-crystal X-ray analysis as reported before [7]. It should be noted that the C-17–C-18 *trans*-fusion in **3** contrasts with the *cis*-decalin D/E system usually present in friedelin type triterpenes and allows all of the six-membered rings to adopt chain conformations in a fully extended form, in



3 R = OOH
3a R = OOAc

4 R = O
9 R = β-OH, α-H

which the hydroperoxy group at C-17 is in an axial disposition. Thus, the conformation of **3** is a novel example different from two kinds of stable conformation (S and F) which have been observed in friedelin-class triterpenes [21]. Recently, elaeodendrol was isolated as a new nor-triterpene from *Elaeodendron glaucum*, and established as 17β-hydroxy-28-norfriedelan-3-one [22]. Elaeodendrol has a normal D/E-*cis* boat and boat conformation with a β-hydroxyl group at C-17. Such a difference of conformation between elaeodendrol and **3** is very interesting in view of the biosynthesis of nor-friedelan type triterpenes.

Maytensifolin-B (**4**; $C_{30}H_{48}O_2$) is a pentacyclic triterpene with two carbonyl groups. The ^{1}H NMR spectrum of **4** displayed one secondary methyl, seven tertiary methyls and two active methylene groups, one of them [δ 2.08 and 2.40 (each 1H, *d*, *J* = 19.0 Hz, H-15)] was attached to a quarternary carbon atom. The presence of diagnostically important mass peaks at *m/z* 273, 355, 219 and 124 (Scheme 1)], suggested that one of the two carbonyl groups adjacent to the foregoing active methylenes to be placed either at C-15 or C-16 in ring-D. The identity of **4** as 16-oxofriedelan-3-one was established by a direct comparison of **4** with a product obtained by Jones oxidation of 16-hydroxyfriedelan-3-one (**9**, i.e. pachysonol), co-occurring in this plant. 16-Oxofriedelan-3-one was isolated for the first time from the plant, despite the fact that it was previously prepared from pachysonol by Kikuchi *et al.* [10].

Identification of known compounds isolated from *M. diversifolia* was achieved by spectroscopic evidences as well as by direct comparison with authentic samples. These known compounds include friedelin (**5**), canophyllal (**6**), β-amyrin (**7**), canophyllol (**8**), pachysonol (**9**), 29-hydroxyfriedelan-3-one (**10**) and 30-hydroxyfriedelan-3-one (**11**). Compound **10** demonstrated physical and spectral data comparable to those of 29-hydroxyfriedelan-3-one [14] except for a minor difference in the coupling pattern of the hydroxymethylene signal in their ^{1}H NMR spectra, in which **10** had two AB doublets at δ3.34 and 3.42 (each 1H, *J* = 10.0 Hz) whereas 29-hydroxyfriedelan showed only a singlet at δ3.42 (2H) [14]. The former is similar to those [δ 3.30 and 3.35 (each 1H, *d*, *J* = 10.0 Hz)] observed for maytenfoliol (**1**) which has a β-hydroxymethylene group at C-20.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 250 MHz and 62.89 MHz, respectively. MS were determined at 70 eV using a direct inlet system. Silica gel (Merk silica gel 60, 70–230 mesh) was used for CC and precoated silica gel (Merk silica gel 60 F-254) for TLC. Detection of components were made either by spraying with 1% $Ce(SO_4)_2$ or 10% H_2SO_4 soln, followed by heating or by use of a UV lamp.

Extraction. The systems of *M. diversifolia* used was from a collection made in December 1979 in Mt. Li-Long, Ping-tong Shen, Taiwan. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan. The ground air-dried stems of the plant (9.09 kg) were extracted with MeOH. Removal of solvent gave a syrup. Guided by the *in vivo* P-388 assay, the syrup (T/C = 164% at 25 mg/kg/day) was partitioned between MeOH–H_2O (3:1) and *n*-hexane. The active aq. MeOH layer was concd and extracted further with $CHCl_3$. Chromatography of the $CHCl_3$ extract (T/C = 178% at 12.5 mg/kg/day) on silica gel (8 × 140 cm) using $CHCl_3$–MeOH (19:1 and then 2:1, collected at 1 l. per fraction) as the eluting solvents yielded nine fractions. Fraction 1 showing three spots on TLC and was rechromatographed to give friedelin (**5**, 131 mg), canophyllal (**6**, 7 mg) and maytensifolin-A (**3**, 12 mg). Fraction 2 gave, upon evapn of solvents, crystalline residues. CC (C_6H_6–EtOAc) followed by recrystallization (C_6H_6–EtOAc) of these residues afforded maytensifolin-B (**4**, 129 mg). Purification of fraction 4 by HPLC (Silicar CC-7 special, 3 × 100 cm, C_6H_6–EtOAc 19:1) yielded the following compounds in order of elution: β-amyrin (**7**, 113 mg), canophyllol (**8**, 239 mg), pachysonol (**9**, 42 mg), 29-hydroxyfriedelan-3-one (**10**, 12 mg) and 30-hydroxyfriedelan-3-one (**11**, 77 mg). Fraction 8 showed antileukemic activity and was rechromatographed on silica gel with *n*-hexane–EtOAc and $CHCl_3$–MeOH. Further purification of the active fractions by HPLC ($CHCl_3$–MeOH) furnished maytenfoliol (**1**, 150 mg) and β-sitosterol-β-D-glucoside (110 mg). Maytansine (3.4 mg) was isolated from fraction 9 after purification by prep. TLC as described before [5].

The aq. MeOH layer after the $CHCl_3$ extraction as mentioned above still showed significant antileukemic activity (T/C = 150% at 100 mg/kg/day). CC of this aq. extract (15 g) on silica gel with $CHCl_3$–MeOH (9:1) followed by purification of its fraction 4 by HPLC ($CHCl_3$–MeOH, 19:1) twice led to the isolation of maytenfolic acid (**2**, 104 mg).

Maytenfoliol (**1**). Mp 290–291°; $[\alpha]_D$ −12.8° (*c* 1.13; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3480, 2920, 2860, 1700, 1449, 1383, 1042 and 1018; ^{13}C NMR ($CDCl_3$): δ6.82 (*q*), 14.70 (*q*), 18.19 (*q*), 18.31 (*t*), 18.65 (*q*), 19.13 (*q*), 22.29 (*t*), 28.32 (*t*), 28.60 (*q*), 29.17 (*t*), 29.96 (*t*), 30.20 (*t*), 31.11 (*t*), 31.51 (*t*), 33.30 (*s*), 35.15 (*s*), 35.48 (*t*), 37.54 (*s*), 38.39 (*s*), 38.88 (*d*), 39.59 (*s*), 41.36 (*t*), 41.51 (*t*), 42.12 (*s*), 52.49 (*d*), 58.32 (*d*), 59.65 (*d*) 69.02 (*t*), 73.36 (*t*) and 212.93 (*s*); MS *m/z* 427.3576 ($[M-CH_2OH]^+$; calcd. for $C_{29}H_{47}O_2$ 427.3576) (100), 409 (21), 396 (4), 287 (4) and 273 (18). *Acetate*: (**1a**): mp 138–139°; $[\alpha_D$ −29.4° (*c* 0.95; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2930, 2865, 1724, 1706, 1442, 1379, 1360, 1281 and 1025; ^{1}H NMR (250 MHz, $CDCl_3$): δ0.87 (3H, *d*, *J* = 6.0 Hz), 0.72, 0.86, 0.91, 1.02, 1.12, 2.06, 2.09 (each 3H, *s*), 2.18–2.45 (3H, *m*), 3.77, 3.83 (each 1H, ABq, *J* = 10.5 Hz), 3.99 and 4.18 (each 1H, ABq, *J* = 10.0 Hz); MS *m/z* 542.3971 ($[M]^+$; calc. for $C_{34}H_{54}O_5$ 542.3970).

Tosylation and reduction of maytenfoliol (**1**). Maytenfoliol (**1**, 90 mg) in dry pyridine was stirred with *p*-toluene sulphonic chloride (220 mg) at room temp. Usual work-up yielded a ditosylate (**1b**, 100 mg). To 80 mg of **1b** in dry THF, a suspension of $LiAlH_4$ (18 mg) in THF was added while stirring and the mixture refluxed for 6 hr. After excess $LiAlH_4$ was eliminated with MeOH followed by H_2O, the reaction product was extracted with Et_2O and purified by dry CC to give the 3β-hydroxy-28-monotosylate (**1c**, 20 mg). Compound **1b**: mp 110°; IR ν_{max}^{KBr} cm^{-1}: 2920, 1700, 1595, 1445, 1350, 1169, 806 and 662; ^{1}H NMR (250 MHz, $CDCl_3$): δ0.86 (3H, *d*, *J* = 6.0 Hz), 0.67, 0.70, 0.84, 0.92, 1.02, 2.45, 2.47 (each 3H, *s*), 3.46, 3.54 (each 1H, ABq, *J* = 9.0 Hz), 3.70, 3.94 (each 1H, ABq, *J* = 10.0 Hz), 7.34 (2H, *d*, *J* = 8.0 Hz), 7.36 (2H, *d*, *J* = 8.0 Hz), 7.78 (2H, *d*, *J* = 8.0 Hz) and 7.80 (2H, *d*, *J* = 8.0 Hz). Compound **1c**: mp 190–191° (decomp.); $[\alpha]_D$ −3.0° (*c* 0.80; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3540, 2915, 1440, 1324, 1162, 920, 835 and 678; ^{1}H NMR (250 MHz, $CDCl_3$): δ0.92 (3H, *d*, *J* = 6.0 Hz), 0.76, 0.78, 0.81, 0.91, 0.93, 1.02, 2.44 (each 3H, *s*), 3.71 (1H, *br s*), 3.88, 4.06 (each 1H, ABq, *J* = 9.5 Hz), 7.32 (2H, *d*, *J* = 7.5 Hz) and 7.78 (2H, *d*, *J* = 7.5 Hz).

Maytenfolic acid (**2**). Mp 281–282°; $[\alpha]_D$ +34.2° (*c* 1.20; C_5H_5N); IR ν_{max}^{KBr} cm^{-1}: 3600–3000, 3467, 1686, 1455, 1378, 1226 and 1014; ^{13}C NMR (pyridine-d_5): δ15.81 (*q*), 16.53 (*q*), 17.14 (*q*), 18.86 (*t*), 19.89 (*t*), 21.28 (*q*), 24.00 (*t*), 25.42 (*q*), 26.26 (*t*), 26.39 (*q*), 28.17 (*t*), 28.75 (*q*), 33.03 (*t*), 37.36 (*s*), 38.72 (*t*), 39.20 (*t*), 39.20 (*s*),

39.45 (*s*), 40.37 (*s*), 41.24 (*t*), 42.60 (*s*), 43.25 (*s*), 47.42 (*d*), 48.10 (*d*), 55.80 (*d*), 74.69 (*d*), 78.15 (*d*), 123.28 (*d*), 144.28 (*s*) and 180.67 (*s*); MS *m/z* 472.3554 ($[M]^+$; calc. for $C_{30}H_{48}O_4$ 472.3551) (8), 454 (8), 264 (91), 246 (100) (Scheme 1), 219 (49) and 207 (42).

Acetylation of maytenfolic acid (**2**). To maytenfolic acid (**2**, 8.4 mg) in pyridine (0.5 ml) was added Ac_2O (0.5 ml). The mixture was allowed to stand at room temp. overnight. Standard work-up followed by dry CC gave a monoacetate (**2a**) (4.2 mg): mp 312–315°; $[\alpha]_D$ +63.1° (*c* 0.10; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2930, 1762, 1720, 1445, 1365 and 1239; ^1H NMR ($CDCl_3$): δ0.86, 0.88, 0.93, 0.96, 1.07, 1.21, 1.57, 2.05 (each 3H, *s*), 4.14 (1H, *d*, *J* = 5.0 Hz), 4.48 (1H, *dd*, *J* = 6.0, 10.0 Hz), 5.29 (1H, *t*, *J* = 2.5 Hz); MS *m/z* 496.3551 ($[M]^+$; calc. for $C_{32}H_{48}O_4$ 496.3551).

Acid treatment of maytenfolic acid (**2**). Maytenfolic acid (15 mg) in 3 N H_2SO_4–EtOH (1:1) (2 ml) was refluxed for 2 hr. The mixture was diluted with H_2O and evap. to remove EtOH under red. pres. The aq. soln was extracted with $CHCl_3$, dried (Na_2SO_4), and evapd *in vacuo*. The crude product was purified by CC on silica gel to give **2b** (9 mg): mp 317–318°; $[\alpha]_D$ −33.4° (*c* 0.20; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3480, 2924, 1740, 1447 and 1166; ^1H NMR ($CDCl_3$): δ0.79, 0.87, 0.94, 0.94, 1.00, 1.08, 1.22 (each 3H, *s*), 3.22 (1H, *dd*, *J* = 6.0, 10.5 Hz), 4.15 (1H, *d*, *J* = 5.0 Hz), 5.30 (1H, *t*, *J* = 3.0 Hz); MS *m/z* 454. 3442 ($[M]^+$; calc. for $C_{30}H_{46}O_3$ 454.3446) (8), 436 (3), 246 (100), 207 (29) and 190 (35).

p-Bromobenzoylation of **2b**. Mp 220–223°; $[\alpha]_D$ +34.3° (*c* 2.50, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2940, 1780, 1770, 1710, 1586, 1479, 1452, 1394, 1270, 1170, 1000, 838 and 750; ^1H NMR ($CDCl_3$): δ0.86, 0.94, 0.95, 1.00, 1.03, 1.22 (each 3H, *s*), 4.13 (1H, *d*, *J* = 4.5 Hz), 4.70 (1H, *dd*, *J* = 5.5, 10.5 Hz), 5.28 (1H, *t*, *J* = 3.0 Hz), 7.66 and 7.97 (each 2H, *d*, *J* = 9.5 Hz).

Maytensifolin-A (**3**). Mp 234–236°: $[\alpha]_D$ −29.5° (*c* 0.46; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 1696, 1445, 1380, 883 and 880; ^{13}C NMR ($CDCl_3$): δ6.79 (*q*), 14.64 (*q*), 15.52 (*q*), 16.49 (*q*), 18.04 (*t*), 19.47 (*q*), 22.35 (*t*), 25.35 (*q*), 27.20 (*t*), 27.75 (*t*), 27.84 (*t*), 30.84 (*s*), 32.08 (*t*), 33.24 (*t*), 33.36 (*q*), 34.54 (*t*), 35.69 (*t*), 37.78 (*s*), 39.82 (*s*), 41.30 (*t*), 41.55 (*t*), 42.15 (*s*), 43.43 (*d*), 49.89 (*d*), 58.32 (*d*), 59.74 (*d*) 82.79 (*s*), 203.71 (*s*) and 212.87 (*s*); MS *m/z* 426.3497 ($[M-H_2O]^+$; calc. for $C_{29}H_{46}O_2$ 426.3497) (30), 411 (58), 408 (6), 395 (15), 393 (17), 357 (99), 301 (9), 273 (23), 177 (100) and 163 (22). *Acetate* (**3a**). ^1H NMR ($CDCl_3$): δ0.87 (3H, *d*, *J* = 7.5 Hz), 0.70, 0.93 × 2, 0.98, 1.19, 2.04 (each 3H, *s*) and 3.39 (1H, *d*, *J* = 11.0 Hz); MS *m/z* 486.3728 ($[M]^+$; calc. for $C_{30}H_{50}O_4$ 486.3709).

Maytensifolin-B (**4**). Mp 280–282°; $[\alpha]_D$ −21.8° (*c* 0.45; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 2925, 1709, 1684, 1449 and 1386; ^{13}C NMR ($CDCl_3$): δ6.79 (*q*), 14.67 (*q*), 16.22 (*q*), 17.34 (*q*), 18.62 (*t*), 20.29 (*q*), 22.23 (*t*), 27.38 (*q*), 27.59 (*s*), 29.11 (*t*), 30.78 (*t*), 31.11 (*q*), 31.66 (*t*), 35.21 (*q*), 35.42 (*t*), 35.48 (*t*), 37.66 (*s*), 39.15 (*s*), 40.45 (*s*), 41.03 (*t*), 41.42 (*t*), 42.09 (*s*), 44.00 (*d*), 45.31 (*s*), 50.22 (*t*), 52.37 (*d*), 58.17 (*d*), 59.35 (*d*), 212.48 (*s*) and 218.84 (*s*); MS *m/z* 440.3657 ($[M]^+$; calc. for $C_{30}H_{48}O_2$ 440.3654) (100), 425 (43), 355 (30), 273 (18), 219 (43) and 124 (32).

Oxidation of pachysonol (**9**). To a soln of pachysonol (10 mg), in Me_2CO (3 ml) was added one drop of Jones' reagent. After the mixture was kept at room temp. for 3 hr, it was diluted with H_2O and the product extracted with Et_2O. The Et_2O extract was washed, dried and evapd to give a ketone (8 mg). The spectrum of the ketone was identical to that of the naturally occurring maytensifolin-B (**4**).

Friedelin (**5**). Mp 258–260°; $[\alpha]_D$ −12.9° (*c* 0.87; $CHCl_3$); MS *m/z* 440.3657 ($[M]^+$; calc. for $C_{30}H_{50}O$ 426.3860). The spectrum of **5** was identical to that of an authentic sample of friedelin.

Canophyllal (**6**). Mp 263–265°; $[\alpha]_D$ −12.8° (*c* 0.54; $CHCl_3$); MS *m/z* 440.3657 ($[M]^+$; calc. for $C_{30}H_{48}O_2$ 440.3654). The spectrum of **6** was identical to that of an authentic sample of canophyllal and of one formed by Jones' oxidation of canophyllol.

β-Amyrin (**7**). Mp 195–196°; $[\alpha]_D$ +143° (*c* 0.04; $CHCl_3$); MS *m/z* 426.3863 ($[M]^+$; calc. for $C_{30}H_{50}O$ 426.3860). This compound and its acetate (mp 239–241°) were identical to authentic samples.

Canophyllol (**8**). Mp 282–283°; $[\alpha]_D$ −28.2° (*c* 0.46; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3490, 2926, 1700, 1450 and 1386; ^{13}C NMR ($CDCl_3$): δ6.79 (*q*), 14.67 (*q*), 18.08 (*q*), 18.25 (*t*), 19.07 (*q*), 19.16 (*q*), 22.25 (*t*), 28.14 (*s*), 29.14 (*t*), 30.11 (*t*), 31.32 (*t*), 31.44 (*t*), 32.84 (*q*), 33.41 (*t*), 34.26 (*q*), 34.54 (*t*), 35.20 (*s*), 35.48 (*t*), 37.51 (*s*), 38.21 (*s*), 39.42 (*s*), 39.51 (*d*), 41.30 (*t*), 41.51 (*t*), 42.09 (*s*), 52.55 (*d*), 58.28 (*d*), 59.56 (*d*), 68.11 (*t*) and 212.93 (*s*); MS *m/z* 442 ($[M]^+$, weak), 411 (100) and 273 (62). *Acetate*. Mp 163–165°, $[\alpha]_D$ −23.1° (*c* 0.80; $CHCl_3$); MS *m/z* 484.3919 ($[M]^+$; calc. for $C_{32}H_{52}O_3$ 484.3916). The identity of **8** was established by direct comparison with an authentic sample.

Pachysonol (**9**). Mp 278–280°; $[\alpha]_D$ −4.7° (*c* 3.2; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3380, 2925, 1700, 1445, 1380 and 999; ^{13}C NMR ($CDCl_3$): δ6.82 (*q*), 14.70 (*q*), 18.19 (*q*), 18.56 (*t*), 20.07 (*q*), 21.47 (*q*), 22.29 (*t*), 24.86 (*q*), 28.02 (*s*), 30.75 (*q*), 30.81 (*t*), 32.05 (*t*), 32.05 (*s*), 35.48 (*q*), 35.78 (*t*), 35.78 (*t*), 36.03 (*t*), 37.63 (*s*), 39.33 (*s*), 40.09 (*s*), 41.36 (*t*), 41.55 (*t*), 42.27 (*s*), 44.43 (*t*), 44.79 (*d*), 53.49 (*d*), 58.32 (*d*), 59.65 (*d*), 75.57 (*d*) and 212.841 (*s*); MS *m/z* 442.3815 ($[M]^+$; calc. for $C_{30}H_{50}O_2$ 442.3810) (6), 424 (36, 357 (11) and 273 (100). Compound **9** was identical to an authentic sample.

29-Hydroxyfriedelan-3-one (**10**). Mp 268–270°; $[\alpha]_D$ −12.1° (*c* 0.1; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3528, 2925, 1706, 1445, 1380 and 1034; ^{13}C NMR ($CDCl_3$): δ6.79 (*q*), 14.70 (*q*), 18.04 (*q*), 18.31 (*t*), 18.56 (*q*), 19.95 (*q*), 22.32 (*t*), 28.26 (*t*), 28.96 (*q*), 29.44 (*t*), 29.66 (*s*), 30.05 (*s*), 30.57 (*t*), 32.20 (*t*), 32.20 (*q*), 33.45 (*s*), 35.66 (*t*), 37.54 (*t*), 38.18 (*s*), 39.91 (*t*), 39.91 (*s*), 41.42 (*t*), 41.55 (*t*), 42.15 (*s*), 42.88 (*d*), 53.10 (*d*), 58.32 (*d*), 59.65 (*d*), 72.09 (*t*) and 212.90 (*s*); MS *m/z* 442.3815 ($[M]^+$; calc. for $C_{30}H_{50}O_2$ 442.3810) (90), 411 (33), 357 (17), 302 (65), 273 (75), 221 (54) and 141 (100). *Acetate*. Mp 188–190° (MeOH) MS *m/z* 484.3919 ($[M]^+$; calc. for $C_{32}H_{52}O_3$ 484.3916).

30-Hydroxyfriedelan-3-one (**11**). Mp 270–271°; $[\alpha]_D$ −17.9° (*c* 1.6; $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3490, 2919, 1699, 1446, 1381 and 1040; ^{13}C NMR ($CDCl_3$): δ6.82 (*q*), 14.70 (*q*), 17.90 (*q*), 18.29 (*t*), 18.44 (*q*), 20.77 (*q*), 22.32 (*t*), 25.87 (*q*), 27.87 (*t*), 29.82 (*t*), 29.82 (*s*), 30.63 (*t*), 32.14 (*q*), 32.81 (*t*), 33.17 (*s*), 35.72 (*s*), 35.96 (*t*), 37.51 (*s*), 38.31 (*s*), 39.56 (*t*), 40.03 (*s*), 41.37 (*t*), 41.55 (*t*), 41.97 (*d*), 42.18 (*s*), 53.47 (*d*), 58.31 (*d*), 59.58 (*d*), 74.79 (*t*) and 212.2 (*t*); MS *m/z* 442.3810 ($[M]^+$; calc. for $C_{30}H_{50}O_2$ 442.3810 (100), 411 (22), 357 (10), 302 (35), 273 (71), 221 (29) and 141 (75). This compound (**11**) was oxidized with Jones' reagent to give a monoaldehyde (mp 252–254°) and a monoacid [mp 275–276°; $[\alpha]_D$ −31.2° (*c* 0.78; $CHCl_3$)]; MS *m/z* 456.3608 ($[M]^+$; calc. for $C_{30}H_{48}O_3$ 456.3603). The latter acid was identical to an authentic sample of polpunonic acid.

β-Sitosterol-β-D-glucoside. Mp 280–282°; $[\alpha]_D$ −40.0° (*c* 2.0; C_5H_5N); MS *m/z* 576.4399 ($[M]^+$; calc. for $C_{36}H_{60}O_6$ 576.4390 and 396.3733 ($[M-\text{glucose}]^+$; calc. for $C_{29}H_{48}$ 396.3756). *Tetraacetate*. Mp 163–164°. The mps of both compounds were undepressed when mixed with authentic samples.

Maytansine. Mp 170–171°; $[\alpha]_D$ −58.8° (*c* 0.17; $CHCl_3$). The identity of this compound was confirmed by mp, TLC, $[\alpha]_D$ and spectroscopic comparison with an authentic sample [5].

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