A New Norlupene from the Leaves of *Melaleuca leucadendron*

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A new lupane-type nortriterpene and 13 known compounds were isolated from the leaves of *Melaleuca leucadendron* L. Based on chemical and spectral methods, the structure of the new compound was elucidated as 28-norlup-20(29)-ene-3β,17β-diol, while the known compounds were identified as (2*E*,6*E*)-farnesol, phytol, squalene, alloaromadendrene, ledene, palustrol, viridiflorol, ledol, betulinaldehyde, betulinic acid, 3β-acetyl-lup-20(29)-en-28-oic acid, 3-oxolup-20(29)-en-28-oic acid, and platanic acid.

Melaleuca leucadendron L. (Myrtaceae), known as paper-bark tree,[1] is widely distributed in Taiwan. Its bark and leaves are used in folk medicine as tranquiling, sedating, evil-dispelling, and pain-relieving agents.[2] Few reports of phytochemical work on *M. leucadendron* are available.[3,4] A systematic study of the chemical constituents of the leaves, leading to the isolation of a new lupane-type nortriterpene and 13 known compounds is reported herein.

The known compounds included three acyclic terpenoids, (2*E*,6*E*)-farnesol, phytol, and squalene, identified by direct comparison with authentic samples, and five related aromadendrane derivatives, alloaromadendrene,[5] ledene,[5] palustrol,[6] viridiflorol,[7] and ledol,[8] which were identified by comparing their MS and ^{1}H- and ^{13}C-NMR data with those reported in the literature. The other known compounds—betulinaldehyde,[9] betulinic acid,[10,11] 3β-acetyllup-20(29)-en-28-oic acid,[12] 3-oxolup-20(29)-en-28-oic acid,[13] and platanic acid[14]—belonged to the lupane group of triterpenes, the structure identification of which was performed mainly by the triterpenoid review report.[15]

A crystalline compound (**1**) was inferred to have the molecular formula $C_{29}H_{48}O_2$, from the ion $[M]^+$ at *m/z* 428.3662. The molecular formula indicated that **1** had six degrees of unsaturation. The MS showed the base peak at *m/z* 410, corresponding to a fragment from the loss of H_2O and two ions of major diagnostic importance: *m/z* 207 and 220. An IR absorption at 3453 cm^{-1} was attributable to a hydroxyl group. The ^{1}H-NMR spectrum showed five methyl groups, appearing as singlets, bonded to quaternary carbons. In addition, an isopropenyl group was evident from a lowfield methyl signal at δ 1.65, and two vinyl protons at δ 4.58 and 4.70. These data supported that **1** belongs to the lupane family. One of these was observed as a proton signal at δ 3.16 (dd, J = 10, 5 Hz), which was located at C-3 as evidenced from the HMBC spectrum. The large coupling constant (J = 10 Hz) between H-3 and H-2 pointed to their axial disposition, there indicating that the 3-hydroxyl group is equatorially oriented (β-face). The ^{13}C-NMR spectrum of **1** lacked a signal of carbonyl group and showed 29 carbon signals, to indicate **1** as a nortriterpenoid (Table 1). The signals at δ 79.0 (d) and 80.4 (s) indicated the presence of two hydroxyl groups. Acetylation of **1** with Ac_2O−pyridine afforded a monoacetyl product (**2**) ($C_{31}H_{50}O_3$), $[M]^+$ at *m/z* 470. The

Table 1. ^{1}H- and ^{13}C-NMR Data of **1**, **2** (δ, $CDCl_3$)

	compounds		
	1		**2**[a]
position	^{1}H (Hz)	^{13}C	^{13}C
1	0.88 (m)	38.7 t	38.46 t[b]
2	1.57 (m)	27.4 t	23.7 t
3	3.16 (dd, 10, 5 Hz)	79.0 t	81.0 t
4		38.8 t	37.8 t
5	0.66 (m)	55.3 d	55.4 d
6	1.40; 1.52 (m)	18.3 t	18.2 t
7	1.39 (m)	34.3 t	34.3 t
8		40.7 s	40.8 s
9	1.23 (m)	50.5 d	50.5 d
10		37.2 s	37.1 s
11	1.27; 1.41 (m)	20.9 t	21.0 t
12	1.07; 1.68 (m)	25.1 t	25.1 t
13	1.82 (m)	37.7 d	37.7 d
14		41.9 s	41.9 s
15	1.24; 1.39 (m)	29.4 t	29.4 t
16	1.09; 1.77 (m)	26.9 t	26.9 t
17		80.4 s	80.4 s
18	1.47 (m)	48.4 d	48.4 d
19	2.58 (ddd, J = 11, 5, 5 Hz)	48.1 d	48.1 d
20		150.0 s	150.0 s
21	1.47 (m); 1.52 (m)	38.4 t	38.43 t[b]
22	1.55 (m); 1.69 (m)	33.1 t	33.2 t
23	0.94 (s)	28.0 q	27.9 q
24	0.73 (s)	15.4 q	16.5 q
25	0.81 (s)	16.2 q	16.3 q
26	1.01 (s)	16.1 q	16.1 q
27	0.92 (s)	13.8 q	13.8 q
28			
29	4.58 (br s); 4.70 (br s)	109.8 t	109.8 t
30	1.65 (s)	19.3 q	19.3 q

[a] The ^{13}C signals of the acetoxyl groups in **2** appeared at 21.3 (q) and 171.0 (s). [b] The assignments can be interchanged.

^{13}C-NMR spectrum showed that A ring had significantly changed to indicate acetylation occurred only at a secondary hydroxy group (C-3), and the signal of the carbonyl group usually observed in betulinic acid was not present. A HMQC experiment was used to determine the ^{1}H- and ^{13}C-NMR vicinal correlations, and the HMBC technique confirmed that C-17 bears the hydroxy group. The stereochemistry of **1** was confirmed by NOESY spectroscopy. As shown in Figure 1, the methyl group at C-14 showed a correlation with the proton at C-18, indicating that the proton must be in the α position. The orientation of C-17 OH was difficult to elucidate. It was found that the chemical shift of C-13 showed no significant difference (about 38 ppm) between **1** and the analogous compounds **3**, **4**, and lupenyl acetate,[16] on which the functional group at C-17 is of

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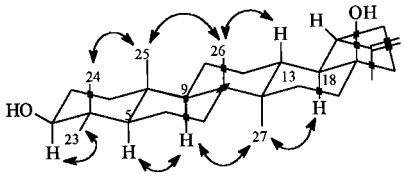
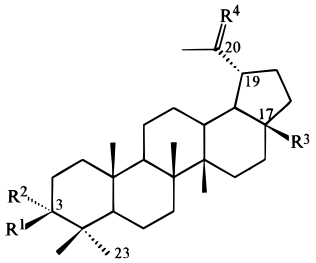


Figure 1. NOESY connections for compound **1**.

the β-configuration. In constrast, 17-epilupenyl acetate,[16] with α-oriented substitution at C-17, shows a very different chemical shift of C-13 (43 ppm). Thus, from these physical data and chemical evidence, the structure of the novel compound **1** was determined as 28-norlup-20(29)-ene-3β,17β-diol.



	R1	R2	R3	R4
(1)	OH	H	OH	CH2
(2)	OAc	H	OH	CH2
(3)	OH	H	CHO	CH2
(4)	OH	H	COOH	CH2

These lupane derivatives from *M. leucadendron* were obtained in pure form during the isolation process. The most plausible pathway for the formation of these compounds from betulinaldehyde **(3)** is by stepwise oxidation of C-3 or C-28 to a ketone or carboxylic acid, respectively, and by Baeyer−Villiger oxidation of C-28 to a formyl group and subsequent decarboxylation to the new nortriterpenol **1**.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto (MP-500) micro melting point apparatus. IR spectra were recorded on a Perkin−Elmer 983G infrared spectrophotometer. ^{1}H-NMR spectra were recorded at 300 or 400 MHz (Bruker AM-300 or AMX-400 spectrometer); ^{13}C-NMR spectra were recorded at 75 or 100 MHz. MS were recorded (Finnigan TSQ-46c spectrometer) at an ionizing voltage 70 eV. HRMS were recorded on a JEOL SX-102A spectrometer. Optical rotation measurements were conducted on Schmidt−Haensh polarimeter; a quartz cuvette (length 10 cm) was used. Merck silica gel 60F sheets were used for analytical TLC. HPLC was carried out on a Hichrosorb Si 60 (10-μm) column (25 cm × 1 cm).

Plant Material. The leaves of *Melaleuca leucadendron* L. were collected in July 1994, in Taipei. The plant material was identified by Shing-Fan Huang, a technician of the Department of Botany of the National Taiwan University, and a voucher specimen is deposited at the Department of Chemistry, National Taiwan University.

Extraction and Isolation. The fresh leaves (10 kg) were extracted exhaustively with Me_2CO (70 L × 3). The extract was concentrated *in vacuo*, and the filtrate was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble portion (230 g) was concentrated and chromatographed on a Si gel (1.5 kg) column by elution with EtOAc−hexane of increasing polarity. The appropriate portions were combined and further separated or purified by HPLC to give (2*E*,6*E*)-farnesol (10 mg), alloaromadendrene (34 mg), ledene (36 mg), phytol (15 mg), squalene (32 mg), palustrol (19 mg), viridiflorol (23 mg), ledol (17 mg), betulinaldehyde (9 mg), betulinic acid (97 mg), 3β-acetyl-lup-20(29)-en-28-oic acid (12 mg), 3-oxolup-20(29)-en-28-oic acid (15 mg), platanic acid (10 mg), and 28-norlup-20(29)-ene-3β,17β-diol (**1**) (12 mg).

28-Norlup-20(29)-ene-3β,17β-diol (1): colorless crystals (hexanes−EtOAc 50:10), mp 181.5−183.5 °C; $[\alpha]^{25}_D$ +31.8° (*c* 0.11, $CHCl_3$); IR (neat) ν_{max} cm^{-1} 3453 (OH), 2937, 1631, 1446, 1370, 1034, 877, 760; EIMS (70 eV); *m*/*z* (% rel int) 428 $[M]^+$ (21), 410 (100), 395 (6), 367 (5), 270 (19), 256 (19), 220 (37), 207 (46), 189 (57), 173 (48); HRMS $[M]^+$ for $C_{29}H_{48}O_2$, calcd 428.3654, found 428.3662; NMR data, see Table 1.

Acetylation of 1. Treatment of **1** (4 mg) with Ac_2O (2 mL) and pyridine (1 mL) at 45 °C overnight followed by HPLC (hexanes−EtOAc 70:30) gave the corresponding monoacetate **2**: HRMS $[M]^+$ for $C_{31}H_{50}O_3$, calcd 470.3762, found 470.3766; ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s), 0.82 (3H, s), 0.91 (3H, s), 1.00 (3H, s), 1.65 (3H, s), 2.01 (3H, s), 2.05 (3H, s), 2.57 (1H, dd, J = 11, 7 Hz, H-19), 4.45 (1H, dd, J = 9, 7 Hz, H-3), 4.58 (1H, br s, H-29), 4.70 (1H, br s, H-29), ^{13}C-NMR data, see Table 1.

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