Notes

Four New Triterpenes from the Heartwood of *Melaleuca leucadendron*

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Four new triterpenes, eupha-7,24-diene-3β,22β-diol (**1**), 20-taraxastene-3α,28-diol (**2**), 3α,27-dihydroxy-28,20β-taraxastanolide (**3**), and 3α-hydroxy-13(18)-oleanene-27,28-dioic acid (**4**) have been isolated from the heartwood of *Melaleuca leucadendron*. The structures and stereochemistry of **1**−**4** have been determined by spectroscopic analysis, with compounds **3** and **4** being investigated in the forms of their diacetate (**3a**) and dimethyl (**4a**) derivative, respectively.

Melaleuca leucadendron L. (Myrtaceae), a large tree cultivated in Taiwan,[1] is the source of the food additive cajeput oil.[2] Sesquiterpenoids, triterpenoid acids, neutral triterpenoids, and stilbenes and related aromatic compounds have been reported previously from this plant.[3−7] The major triterpenoids include compounds of the lupane, oleanane, and ursane types. In the present communication, we describe the isolation and identification of four compounds (**1**−**4**) from the heartwood of *M. leucadendron*, whose structures have been determined by spectroscopic analysis (Chart 1).

Compound **1** gave a molecular formula of $C_{30}H_{50}O_2$ by high-resolution mass spectrometry. EIMS fragment ions occurred at *m*/*z* 427 $[M - Me]^+$, 424 $[M - H_2O]^+$, and 409 $[M - Me - H_2O]^+$. The ions at *m*/*z* 372 and 69, which resulted from allylic cleavage of the C-22/C-23 bond, suggested the presence of a double bond at C-24 and also the presence of an isopropylidine group in the side chain.[8] The ^{1}H NMR data (Table 1) of **1**, which were compared with the data of the known compound **1a**,[9] displayed signals for five quaternary methyls at δ_H 0.72, 0.78, 0.83, 0.94, and 0.97, one secondary methyl at δ_H 0.86 (3H, d, J = 7 Hz), two vinylic methyls at δ_H 1.61 and 1.70 (each 3H, s), two doublet of doublets at δ_H 3.21 (J = 4, 11 Hz) and 3.63 (J = 8, 6 Hz) for two hydroxyl methine protons, and two olefinic protons at δ_H 5.11 (1H, t, J = 7 Hz) and 5.23 (1H, br d, J = 3 Hz). The position of the two hydroxyl groups was determined from the COSY-90 NMR spectrum, since the hydroxyl methine (δ_H 3.63) and allylic proton (δ_H 5.11) had the same cross-peaks at δ_H 1.97 and 2.26 which indicated that both these protons were coupled to the same set of two protons. In addition, the doublet of doublets proton (δ_H 3.21) and two methylene protons (δ_H 1.11, 1.62) had the same cross-peak at δ_H 1.54 and 1.58, which indicated clearly that these protons shared the same methene protons. From the HMBC spectrum of **1**, the signal at δ_H 3.63 was correlated with C-21 (δ_C 11.5) and δ_H 3.21 was correlated with C-28 (δ_C 27.6) and C-29 (δ_C 14.7), which allowed the placement of the two hydroxyl groups at C-22 and C-3, respectively.

The ^{13}C NMR data (Table 2) of **1** were similar to values published for compound **1a**.[9] Hence, **1** was considered to possess the same triterpenoid skeleton as **1a**. The ^{13}C NMR spectrum showed signals for six quaternary methyls at δ_C 11.5, 13.1, 14.7, 21.8, 27.4, and 27.6, two vinylic methyl carbons at δ_C 18.0 and 25.9, two hydroxyl methine carbons at δ_C 73.2 and 79.2, two trisubstituted olefinic carbons at δ_C 117.9 and 121.0, and two quaternary olefinic carbons at δ_C 134.3 and 145.7. An equatorial orientation (β-stereochemistry) of the C-3 hydroxyl was also supported by the large coupling constant (J = 11 Hz) between H-3 and H-2, and the coupling constant J_{20-22} (6 Hz) established the stereochemistry at C-22 as *S*.[10] From the above spectral data, compound **1** was determined structurally as eupha-7,24-diene-3β,22β-diol.

Compound **2** exhibited a molecular ion peak at *m*/*z* 442 in its mass spectrum and its molecular formula was determined as $C_{30}H_{50}O_2$ by HREIMS. The ^{13}C NMR data (Table 2) displayed seven methyls, ten methylenes, seven methines, and six quaternary carbons. A pentacyclic triterpenoid skeleton with one olefin accounted for the six degrees of unsaturation in **2**. The mass spectrum of **2** demonstrated the existence of characteristic ion fragments at *m*/*z* 424 $[M - H_2O]^+$, 411 $[M - CH_2OH]^+$, and 393 $[411 - H_2O]^+$. The fragment ions at *m*/*z* 207 and 235 were due to cleavage of the C-8/C-14 and C-11/C-12 bonds, respectively.[11] This fragmentation pattern indicated the location of a hydroxyl group in ring A at C-3 and a CH_2OH group in ring D or E.[11] The OH configuration of C-3 was determined as being α- from the coupling constant of H-3 (br s) and the ^{13}C NMR chemical shift of C-5 (δ_C 48.6).[12] The C-28 methine proton was correlated with the H-22 allyl protons (δ_H 1.51, 1.98) and the trisubstituted olefinic proton was correlated with the H-30 vinyl methyl proton (δ_H 1.62) by the HMBC technique. Therefore, compound **2** was assigned as 20-taraxastene-3α,28-diol.

Compounds **3** and **4** were found to be difficult to obtain in pure form. Therefore, the structural determinations of **3** and **4** were based mainly on their diacetate (**3a**) and dimethyl (**4a)** derivative, respectively.

Treatment of **3** with pyridine-acetic anhydride afforded the diacetate **3a** [$C_{34}H_{52}O_6$, M^+, *m*/*z* 556]. The IR spectrum of **3a** showed an absorption bond at 1736 cm^{-1} (carbonyl), while there was no evidence in the IR spectrum for the presence of a carbon−carbon double bond or a hydroxyl group. The ^{1}H NMR data of **3a** (Table 1) included signals

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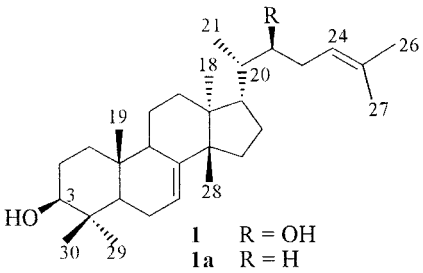
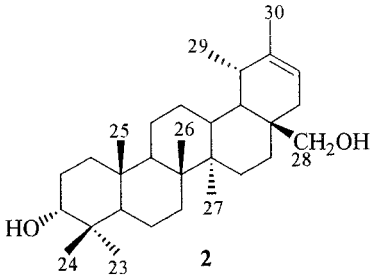
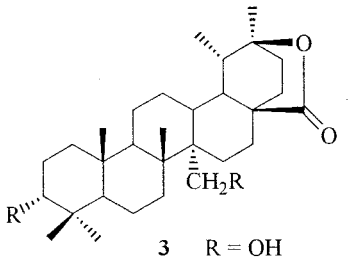
Chart 1



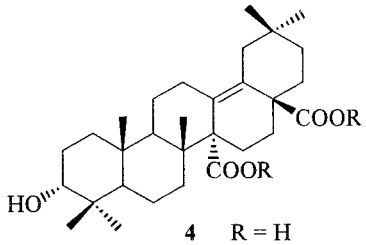
1 R = OH
1a R = H



2



3 R = OH
3a R = OAc



4 R = H
4a R = Me

Table 1. ^{1}H NMR Data of Compounds **1**, **2**, **3a**, and **4a** ($CDCl_3$, 300 MHz)

position	1	2	3a	4a
1	Ha 1.11 m	Ha 1.27 m	Ha 1.09 m	Ha 1.27 m
	Hb 1.62 m	Hb 1.42 m	Hb 1.42 m	Hb 1.42 m
2	Ha 1.54 m	Ha 1.53 m	Ha 1.58 m	1.49 m
	Hb 1.58 m	Hb 1.91 m	Hb 1.88 m	
3	3.21 dd (11, 4)	3.37 br s	4.60 br s	3.35 br s
5	1.28 m	1.23 m	1.13 m	1.24 m
6	Ha 1.92 m	Ha 1.07 m	Ha 1.28 m	Ha 1.23 m
	Hb 2.11 m	Hb 1.48 m	Hb 1.38 m	Hb 1.38 m
7	5.23 br d (3)	1.39 m	Ha 1.27 m	1.27 m
			Hb 1.52 m	
9	2.21 m	1.40 m	1.33 m	1.35 m
11	a	1.20 m	1.22 m	1.50 m
12	1.75 m	1.12 m	1.09 m	Ha 2.22 m
				Hb 2.81 m
13		1.56 m	1.29 m	
15	1.44 m	a	1.59 m	Ha 1.67 m
				Hb 1.87 m
16	Ha 1.24 m	Ha 1.10 m	Ha 1.52 m	Ha 1.28 m
	Hb 1.92 m	Hb 1.63 m	Hb 1.61 m	Hb 1.81 m
17	1.83 m			
18	0.72 s	1.21 m	1.21 m	
19	0.78 s	1.63 m	1.52 m	Ha 1.51 m
				Hb 2.49 m
20	1.38 m			
21	0.86 d (7)	5.27 br d (7)	1.87 m	1.21 m
22	3.63 dd (8, 6)	Ha 1.51 dd (16, 7)	0.87 m	Ha 1.51 m
		Hb 1.98 dd (16, 7)		Hb 2.15 m
23	Ha 1.97 m	0.91 s	0.81 s	0.91 s
	Hb 2.26 m			
24	5.11 t (7)	0.98 s	0.84 s	0.79 s
25		1.00 s	0.84 s	0.86 s
26	1.70 s	0.83 s	0.93 s	0.90 s
27	1.61 s	0.97 s	Ha 4.27 d (13)	
			Hb 4.40 d (13)	
28	0.94 s	Ha 3.45 d (11)		
		Hb 3.64 d (11)		
29	0.83 s	0.98 d (7)	0.97 d (7)	0.89 s
30	0.97 s	1.62 s	1.29 s	0.74 s
OMe				3.63 s, 3.63 s
OAc			2.08 s, 2.08 s	

[a] These protons were not observed in an HMQC experiment.

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, **3a**, and **4a** ($CDCl_3$, 75 MHz)[a]

carbon	1	2	3a	4a
1	37.2 t	33.3 t	34.1 t	33.2 t
2	27.7 t	25.3 t	21.9 t	25.2 t
3	79.2 d	76.2 d	78.0 d	76.0 d
4	38.9 s	37.5 s	37.5 s	37.4 s
5	50.6 d	48.6 d	50.5 d	48.4 d
6	23.9 t	18.2 t	18.0 t	18.4 t
7	117.9 d	34.0 t	34.8 t	35.5 t
8	145.7 s	41.3 s	41.5 s	42.3 s
9	48.9 d	50.1 d	51.6 d	52.3 d
10	34.9 s	37.2 s	36.7 s	37.7 s
11	18.1 t	21.3 t	22.9 t	20.4 t
12	33.8 t	26.7 t	25.0 t	26.6 t
13	43.4 s	38.2 d	43.6 d	133.0 s
14	51.1 s	42.2 s	41.8 s	59.1 s
15	34.0 t	27.5 t	20.9 t	23.9 t
16	27.6 t	30.2 t	32.1 t	33.4 t
17	49.2 d	38.6 s	43.9 s	48.4 s
18	13.1 q	49.0 d	48.3 d	131.1 s
19	21.8 q	36.3 d	42.2 d	41.4 t
20	40.4 d	141.0 s	84.2 s	33.6 s
21	11.5 q	117.8 d	26.9 t	37.0 t
22	73.2 d	35.0 t	27.9 t	35.5 t
23	34.3 t	28.2 q	27.7 q	28.2 q
24	121.0 d	22.9 q	21.8 q	22.3 q
25	134.3 s	16.1 q	16.5 q	16.0 q
26	25.9 q	16.0 q	16.0 q	18.2 q
27	18.0 q	14.9 q	62.7 t	177.0 s
28	27.6 q	60.1 t	177.0 s	176.5 s
29	14.7 q	22.1 q	18.7 q	32.1 q
30	27.4 q	20.9 q	23.9 q	24.0 q
OMe				51.3 q, 51.7 q
$OCOCH_3$			21.4 q, 21.5 q	
$OCOCH_3$			170.9 s, 171.6 s	

[a] Multiplicity (q = CH_3, t = CH_2, d = CH, s = C) determined by DEPT experiments.

due to a downfield methyl (δ_H 1.29), a secondary methyl (δ_H 0.97, J = 7 Hz), and four tertiary methyls of an ursane skeleton.[13] Also, it showed one acetate at δ_H 4.60 (br s), assignable to 3β-H, and an AB system (δ_H 4.27 and 4.40, J = 13 Hz), indicative of the presence of a hydroxymethylene group attached to an asymmetric center (C-14).[13] The mass spectrum included peaks at m/z 248 (100%) and 189, which suggested that an acetate group was situated on either the A or B ring. The methine proton (δ_H 1.52) correlated with a second methyl (δ_H 0.97) from the COSY-90 experiment and the downfield methyl (δ_C 23.9) and δ-lactone (δ_C 177.0) similarly correlated with the signal at δ_H 1.52 in the HMBC spectrum. Thus, the structure of **3a** was assigned as 3α,27-diacetoxy-28,20β-taraxastanolide.

Compound **4** was obtained as the dimethyl derivative **4a**. The mass spectrum in this derivative exhibited the $[M]^+$

ion at *m*/*z* 514 ($C_{32}H_{50}O_5$). The IR spectrum of **4a** showed the presence of carbonyl (1718 cm^{-1}) and hydroxyl (3582 cm^{-1}) functions. The structure of **4a** was determined from its ^1H and ^{13}C NMR data (Tables 1 and 2) and 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The ^1H NMR spectrum of **4a** showed the presence of six characteristic tertiary methyl and two *O*-methyl groups, which was reminiscent of an oleanane-type triterpene.[14] However, since protons at C-12 and C-18 were not observed, **4a** was assigned as an olean-13(18)-ene structure. The ^{13}C NMR spectrum revealed the presence of six methyl carbons which correlated in the HMQC spectrum with the six above-mentioned singlets, two olefinic signals (δ_C 131.1 and 133.0), a hydroxyl methine signal at δ_C 76.0, and two carboxylic groups at δ_C 176.5 and 177.0. The latter carbons were correlated in the HMBC spectrum with the C-15 and C-16 protons at δ_H 1.67, 1.87 and δ_H 1.28, 1.81, respectively. Thus, the structure of **4a** was determined as methyl 3α-hydroxy-13(18)-oleanene-27,28-dioate.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto (MP-500) micro-melting point apparatus. Optical rotation measurements were conducted on a JASCO DIP-1000 instrument; a quartz cuvette (length 10 cm) was used. IR spectra were recorded on a Nicolet Magna-550 spectrophotometer; UV spectra were recorded on a Hitachi U-3210 spectrophotometer. ^1H and ^{13}C NMR spectra were run on a Bruker AM-300 spectrometer, with 2D NMR spectra run on a Bruker DMX-500SB spectrometer, using $CDCl_3$ as solvent. The resonances of residual $CDCl_3$ at δ_H 7.24 and of $CDCl_3$ at δ_C 77.0 were used as internal references for the ^1H NMR and ^{13}C NMR spectra, respectively. Mass spectra were recorded (Finnigan TSQ-700 spectrometer) at an ionizing voltage of 70 eV. High-resolution mass spectra (HRMS) were recorded on a JEOL SX-102A spectrometer. 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 heartwood of *M. leucadendron* L. was collected in July 1994 on the campus of the National Taiwan University and was identified by Mr. Shing-Fan Huang, Department of Botany, National Taiwan University. A voucher specimen has been deposited at China Junior College of Medical Technology (C. C. M. T., accession # 8308).

Extraction and Isolation. The air-dried heartwood of *M. leucadendron* (10 kg) was crushed into small pieces, and extracted with Me_2CO (70 L × 3) at room temperature. The extract was concentrated and partitioned between $CHCl_3$ and water. The organic layer was concentrated to give an oily residue (125 g) that was chromatographed over silica gel 60 (Merck, 230−400 mesh) and eluted with hexane/EtOAc mixtures of increasing polarity to give 14 fractions. Fraction 10, eluted with hexane/EtOAc (10:3), was further separated or purified by repeated column chromatography and preparative HPLC (solvent system: hexane−EtOAc, 5:1; 7:3; 3:1, and 2.5:1), to give **1** (26 mg), **2** (2 mg), **3** (2 mg), and **4** (13 mg), respectively.

Eupha-7,24-diene-3*β*,22*β*-diol (1): amorphous solid; $[\alpha]^{25}_D$ −24° (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.12) nm; IR (neat) ν_{max} 3416 (OH), 2932, 2878, 1458, 1384, 1031, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 442 $[M]^+$ (8), 427 (6), 424 (7), 409 (6), 372 (73), 357 (100), 339 (25), 69 (24); HREIMS *m*/*z* 442.3806 (calcd for $C_{30}H_{50}O_2$, 442.3813).

20-Taraxastene-3α,28-diol (2): amorphous solid; $[\alpha]^{25}_D$ +48° (*c* 0.012, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.38) nm; IR (neat) ν_{max} 3442 (OH), 2935, 2866, 1453, 1379, 1027, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 442 $[M]^+$ (28), 424 (31), 411 (100), 393 (27), 385 (23), 367 (24), 339 (25); HREIMS *m*/*z* 442.3824 (calcd for $C_{30}H_{50}O_2$, 442.3813).

3α,27-Dihydroxy-28,20*β*-taraxastanolide (3): Treatment of impure **3** (2 mg) with Ac_2O (2 mL) and pyridine (2 mL) at 42 °C overnight followed by HPLC (hexanes−EtOAc 7:3) separation gave the corresponding diacetate **3a** (2 mg); amorphous solid; $[\alpha]^{25}_D$ −31° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.41), 296 (2.28) nm; IR (neat) ν_{max} 2938, 2872, 1736, 1453, 1378, 1246, 1034 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 556 $[M]^+$ (0.3), 496 (8), 436 (100), 248 (100), 202 (40), 189 (55); HREIMS *m*/*z* 556.3765 (calcd for $C_{34}H_{52}O_6$, 556.3766).

3α-Hydroxy-13(18)-oleanene-27,28-dioic acid (4): Treatment of impure **4** (11 mg) with diazomethane in methanol solution followed by HPLC (hexanes−EtOAc 3:1) separation gave its *O*-methylated product **4a** (12 mg); amorphous solid; $[\alpha]^{25}_D$ −42° (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.21), 306 (2.01) nm; IR (neat) ν_{max} 3582, 2948, 2870, 1718, 1458, 1387, 1264, 1215, 1156 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 514 $[M]^+$ (13), 496 (34), 482 (100), 464 (20), 437(26), 421 (23), 405 (25); HREIMS *m*/*z* 514.3661 (calcd for $C_{32}H_{50}O_5$ 514.3660).

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