Fern Constituents: Sesterterpenoids Isolated from Fronds of *Aleuritopteris agetae*

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Five new sesterterpenoids were isolated from the fronds of *Aleuritopteris agetae*, together with the triterpenoids squalene, tirucalla-7,21-diene and hop-21-ene, and the flavonoids 3,5-dihydroxy-7,4′-dimethoxyflavone and 5-hydroxy-7,4′-dimethoxyflavone. The structures of five new compounds were determined to be (17*Z*)-cheilantha-13(24),17-diene-6α,19-diol (1), (17*Z*)-cheilantha-13(24),17-diene-1β,6α,19-triol (2), (17*Z*)-19-acetoxycheilantha-13(24),17-diene-1β,6α-diol (3), 17-oxo-18,19-bisnorcheilanth-13(24)-en-6α-ol (4) and 13,17-dioxo-18,19,24-trisnorcheilanth-6α-ol (5) on the basis of detailed spectral analyses.

Key words *Aleuritopteris agetae*; sesterterpenoid; (17*Z*)-cheilantha-13(24),17-diene-1β,6α,19-triol; 13,17-dioxo-18,19,24-trisnorcheilanth-6α-ol; triterpenoid; flavonoid

In the preceding paper[1] we have reported on the chemical constituents of the fronds of *Aleuritopteris mexicana* FÉE collected at Alishan in Taiwan. In the same area, one of the authors also found another species easily distinguishable from *A. mexicana* by TLC comparison of their extracts. This other species, named *A. agetae* SAIKI (Alishan-urajiro, nom. jap. nov.) by Saiki,[2] is morphologically very similar to *A. mexicana* FÉE and *A. pulverancea* FÉE, but it can be distinguished from them by its lemon yellow farina deposited on the undersurface of the fronds, and the characters of the scales and spores. This paper deals with the isolation and characterization of the sesterterpenoids, triterpenoids and flavonoids from the fronds of *A. agetae*, and the comparison of these compounds with those of *A. mexicana*.

Results and Discussion

The fresh fronds of the fern were extracted with *n*-hexane. The extract was subjected to silica gel and Sephadex LH 20 column chromatography followed by HPLC to give five new sesterterpenoids (**1**—**5**), together with three triterpenoid hydrocarbons, squalene,[3] tirucalla-7,21-diene,[4] and hop-21-ene,[5] and two flavonoids,[1] 3,5-dihydroxy-7,4′-dimethoxyflavone and 5-hydroxy-7,4′-dimethoxyflavone.

Compound **1** was shown to have the molecular formula $C_{25}H_{42}O_2$ by high-resolution mass spectrometry (HRMS), and its IR spectrum showed a hydroxyl absorption at 3350 cm^{-1} and an exocyclic methylene absorption at 1640 and 880 cm^{-1}. The ^{1}H-NMR spectrum (Table 1) showed signals due to four tertiary methyl groups, an olefinic methyl group (δ 1.743), a methine proton on carbon bearing a hydroxyl group (δ 3.968), methylene protons on carbon bearing a hydroxyl group (δ 4.039 and 4.074), exocyclic methylene protons (δ 4.564 and 4.870) and a proton of a trisubstituted double bond (δ 5.427). According to a distortionless enhancement by polarization transfer (DEPT) experiment, the numbers of various types of carbons are the same as those of cheilanthenetriol (**6**), except that **1** has tetrasubstituted and disubstituted olefin carbons, instead of an oxygenated quaternary carbon (C-13) and a methyl carbon (C-24) in **6**. This is in good agreement with the ^{1}H-NMR data described above. Furthermore, the ^{13}C-chemical shifts (Table 2) of C-1 to C-10 and C-20 to C-22 are similar to those of **6**. The heteronuclear multiple bond correlation (HMBC) (Table 3) and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra clearly demonstrated the connectivities of the skeletal carbons of **1** (Chart 1). In the nuclear Overhauser enhancement spectroscopy (NOESY) spectrum (Fig. 1), the

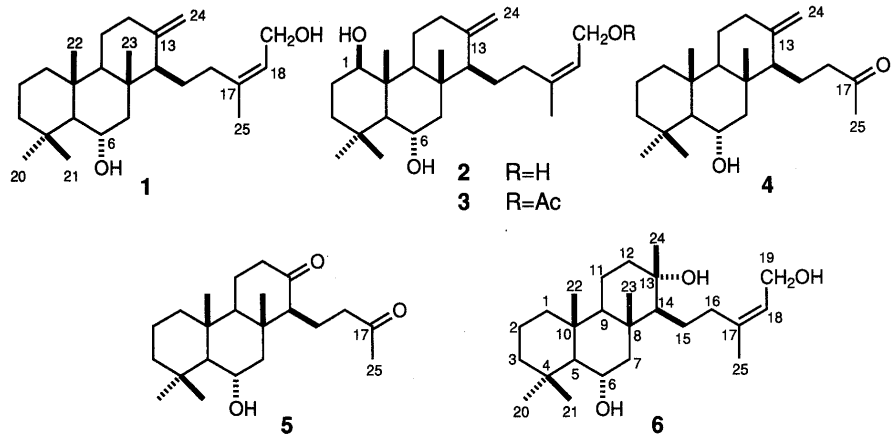

Chart 1

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Table 1. ^{1}H-NMR Spectral Data (500 MHz, $CDCl_3$, δ)

	1	2	3	4	5	6
H_3-20	1.168	1.170	1.173	1.164	1.185	1.167
H_3-21	1.014	1.086	1.088	1.015	1.033	1.014
H_3-22	0.848	0.934	0.935	0.852	0.906	0.846
H_3-23	0.740	0.746	0.746	0.756	0.809	0.859
Ha-24	4.564 (d, 1.2)	4.561 (d, 1.1)	4.553 (d, 1.2)	4.445 (d, 1.3)		
Hb-24	4.870 (d, 1.2)	4.876 (d, 1.1)	4.867 (d, 1.2)	4.826 (d, 1.3)		
H_3-24						1.126
H_3-25	1.743	1.740	1.760	2.107	2.097	1.780
H-1α		3.519 (dd, 5.2, 5.2)	3.521 (dd, 5.2, 5.2)			
H-6β	3.968 (ddd, 11.0, 11.0, 3.7)	4.037 (ddd, 11.0, 11.0, 4.3)	4.036 (ddd, 11.0, 11.0, 4.0)	3.967 (ddd, 11.0, 11.0, 4.0)	3.952 (ddd, 11.0, 11.0, 4.0)	3.962 (ddd, 10.7, 10.7, 3.6)
H-18	5.427 (dd, 6.7, 6.7)	5.429 (dd, 6.8, 6.8)	5.352 (dd, 7.3, 7.3)			5.464 (dd, 7.3, 7.3)
Ha-19	4.039 (dd, 11.7, 6.7)	4.04[a]	4.512 (d, 7.3)			4.055 (dd, 11.7, 7.3)
Hb-19	4.074 (dd, 11.7, 6.7)	4.07[a]	4.512 (d, 7.3)			4.129 (dd, 11.7, 7.3)
OAc			2.050			

Multiplicity and coupling constant (J in Hz) are shown in parentheses. *a*) Obscured by other signals.

Table 2. ^{13}C-NMR Spectral Data (125.65 MHz, $CDCl_3$, δ)

C	1	2	3	4	5	6
1	40.16	77.43	77.48	40.23	40.45	40.05
2	18.49	30.28	30.29	18.51	18.44	18.52
3	43.60	37.14	37.16	43.60	43.54	43.56
4	33.66	33.18	33.19	33.67	33.66	33.73
5	61.30	57.08	57.14	61.35	61.22	61.45
6	69.03	68.41	68.41	68.98	68.44	68.17
7	52.04	51.35	51.38	52.05	51.60	52.82
8	40.50	40.40	40.41	40.70	43.51	40.09
9	59.60	60.42	60.44	59.59	58.20	60.07
10	39.53	45.07	45.08	39.52	39.53	39.24
11	23.25	25.01	25.01	23.20	22.62	19.48
12	37.94	38.11	38.06	37.88	42.00	44.25
13	147.81	147.46	147.37	147.54	211.77	74.08
14	56.21	56.07	56.28	56.52	63.21	61.62
15	21.78	21.74	21.84	17.50	16.09	24.50
16	30.43	30.38	30.66	42.63	42.51	35.47
17	140.35	140.34	143.04	209.29	209.10	142.53
18	124.58	124.64	119.44	—	—	123.43
19	59.03	59.07	61.13	—	—	58.45
20	36.62	34.52	34.52	36.60	36.59	36.58
21	22.03	23.84	23.82	22.00	22.04	22.06
22	17.60	14.28	14.26	17.57	17.41	17.69
23	16.68	16.61	16.60	16.44	16.66	18.08
24	106.74	106.69	106.61	106.67	—	23.98
25	23.44	23.44	23.53	30.07	29.98	23.71
$OCO\underline{C}H_3$			21.14			
$O\underline{C}OCH_3$			171.13			

interactions of H-6 with other protons supported H-6 axial configuration, and that of H-18 with H_3-25 indicated the Z configuration of the Δ^{17} double bond. Consequently, the structure of compound **1** was concluded to be (17Z)-cheilantha-13(24),17-diene-6α,19-diol.

Compound **2**, $C_{25}H_{42}O_3$, showed a similar ^{1}H-NMR spectrum to that of **1**, except for an additional methine proton on carbon bearing a hydroxyl group (δ 3.519). The chemical shifts of H_3-23, H_3-24, H_3-25, H-18 and H_2-19, and of C-23 to C-25 and C-8, C-12 to C-19 in the ^{1}H- and ^{13}C-NMR spectra are almost the same as those of **1**. But the carbon chemical shifts in ring A are quite different from those in **1**. This indicated that **2** was a derivative of **1** having two secondary hydroxyl groups on the A and/or B rings. Two- and three-bond correlations from H_3-22, H_3-23, H-1 and H-6 in the HMBC spectrum suggested that the two hydroxyl groups were located at the C-1 and C-6 positions. Furthermore, the configurations of H-1 and H-6 were determined to be α and β, respectively, based on the NOESY finding that H-1 (α-side) interacted with H-5 (α), H-9 (α) and H_{eq}-11 (α), and H-6 (β-side) with H_3-21 (β), H_3-22 (β) and H_3-23 (β). All the above evidence led to (17Z)-cheilantha-13(24),17-diene-1β,6α,19-triol for the structure of compound **2.**

Compound **3**, $C_{27}H_{44}O_4$, showed absorptions due to hydroxyl groups, an acetoxyl group and an exocyclic methylene group in the IR spectrum. The ^{13}C-chemical shifts are almost the same as those of **2** except for C-17 to C-19 and two additional carbons (δ 21.1 and 171.1) due to an acetoxyl group. This indicated that **3** was a C-19 acetyl derivative of **2**. Since hydrolysis of **3** with 5% KOH solution gave **2**, this compound was concluded to be (17Z)-19-acetoxycheilanta-13(24), 17-diene-1β,6α-diol.

Compound **4**, $C_{23}H_{38}O_2$, showed absorptions due to a hydroxyl group, a carbonyl group and an exocyclic methylene group in the IR spectrum, and the ^{1}H-NMR spectrum showed signals due to four tertiary methyl groups, an acetyl group (δ 2.167), a methine proton on carbon bearing a hydroxyl group (δ 3.967) and exocyclic methylene protons (δ 4.445 and 4.826). The ^{13}C-NMR chemical shifts, except for those of C-15 to C-17 and C-25 are similar to those of **1**. The MS showed the fragment ion at m/z 257 generated by the elimination of a side chain and H_2O. These indicated that the side chain of **4** consisted of C_4H_7O with a carbonyl group. In addition, the C–H long-range correlations in the HMBC spectrum

showed the connectivities from H_2-16 with C-14, C-15 and C-17, and from H_2-12 with C-9, C-11, C-13, C-14 and C-24. The relative stereochemistry of the A, B and C ring moiety of **4** is the same as that of **1** based on the NOESY data. These results indicated that **4** was a 18,19-bisnor derivative of **1** with a carbonyl group at C-17, owing to an acetyl group (δ 209.39 and 30.07). Consequently, the structure of **4** was established as 17-oxo-18,19-bisnorcheilanth-13(24)-en-6α-ol.

Table 3. C–H Long-Range Correlations of **1**, **2**, **4** and **5** by HMBC in $CDCl_3$

	^{1}H signals	Correlated of carbons				
1	1.168 (H_3-20)	C-3	C-4	C-5	C-21	
	1.014 (H_3-21)	C-3	C-4	C-5	C-20	
	0.848 (H_3-22)	C-1	C-5	C-9	C-10	
	0.740 (H_3-23)	C-7	C-8	C-9	C-14	
	4.456 (Ha-24)	C-12	C-14			
	4.487 (Hb-24)	C-12	C-14			
	1.743 (H_3-25)	C-16	C-17	C-18		
	3.968 (H-6)	C-5	C-7	C-10		
	1.94 (H_{ax}-12)	C-9	C-11	C-13	C-14	C-24
	2.39 (H_{eq}-12)	C-9	C-11	C-13	C-14	C-24
	1.59 (H-14)	C-8	C-13	C-16	C-23	C-24
	5.427 (H-18)	C-16	C-19	C-25		
	4.039 (Ha-19)	C-17	C-18			
	4.074 (Hb-19)	C-17	C-18			
2	1.170 (H_3-20)	C-3	C-4	C-5	C-21	
	1.086 (H_3-21)	C-3	C-4	C-5	C-20	
	0.934 (H_3-22)	C-1	C-5	C-9	C-10	
	0.746 (H_3-23)	C-7	C-8	C-9	C-14	
	4.561 (Ha-24)	C-12	C-14			
	4.876 (Hb-24)	C-12	C-14			
	1.740 (H_3-25)	C-16	C-17	C-18		
	3.519 (H-1)	C-2	C-3	C-9	C-10	C-22
	2.401 (H_{eq}-12)	C-9	C-11	C-13	C-14	C-24
	5.429 (H-18)	C-16	C-19	C-25		
	4.04 (Ha-19)	C-17	C-18			
4	1.164 (H_3-20)	C-3	C-4	C-5	C-21	
	1.015 (H_3-21)	C-3	C-4	C-5	C-20	
	0.852 (H_3-22)	C-1	C-5	C-9	C-10	
	0.756 (H_3-23)	C-7	C-8	C-9	C-14	
	4.445 (Ha-24)	C-12	C-14			
	4.826 (Hb-24)	C-12	C-14			
	2.107 (H_3-25)	C-17				
	3.967 (H-6)	C-5	C-7	C-10		
	1.93 (H_{ax}-12)	C-9	C-11	C-13	C-14	C-24
	2.38 (H_{eq}-12)	C-9	C-11	C-13	C-14	C-24
	2.33 (Ha-16)	C-14	C-15	C-17		
	2.57 (Hb-16)	C-14	C-15	C-17		
5	1.185 (H_3-20)	C-3	C-4	C-5	C-21	
	1.033 (H_3-21)	C-3	C-4	C-5	C-20	
	0.906 (H_3-22)	C-1	C-5	C-9	C-10	
	0.809 (H_3-23)	C-7	C-8	C-9	C-14	
	2.097 (H_3-25)	C-17				
	3.952 (H-6)	C-5	C-7	C-10		
	2.26 (H_{ax}-12)	C-9	C-11	C-13	C-14	
	2.39 (H_{eq}-12)	C-9	C-11	C-13	C-14	
	2.21 (Ha-16)	C-14	C-15	C-17		
	2.59 (Hb-16)	C-14	C-15	C-17		

Compound **5**, $C_{22}H_{36}O_3$, showed signals due to four tertiary methyls, an acetyl group (δ 2.097) and a methine proton on carbon bearing a hydroxyl group (δ 3.956) in the ^{1}H-NMR spectrum. A comparison of the ^{13}C-NMR data of **5** with those of **4** revealed that **5** has another carbonyl carbon instead of a disubstituted and a tetrasubstituted carbon in **4**, suggesting **5** to be a 24-nor derivative of **4** having a carbonyl group at C-13. The cross peaks from H_2-12 in the HMBC spectrum indicated the connectivity of C-12 with C-9, C-11, C-13 and C-14. Since the NOE interactions in **5** were as shown in Fig. 1, the structure of **5** was established as 13,17-dioxo-18,19,24-trisnorcheilanth-6α-ol.

To our knowledge, this is the first report of the occurrence of a trisnorcheilanthane (C_{22} sesterterpenoid) such as **5** in nature, while the bisnorcheilanthane (C_{23}) **4** is the second example of its kind, following luteone from the dorid nudibranch, *Cadlina luteomarginata*.[6] Moreover, the isolation of cheilanthane-type sesterterpenoids with a hydroxyl group on the A ring such as **2** and **3** is characteristic of *A. agetae* (first examples).

We compared chemical constituents of *A. agetae* and *A. mexicana* which were collected simultaneously in the same region. The latter contains a large amount of sesterterpenoids (mainly cheilanthenetriol), while their quantity in the former is much smaller and further, the structures of these compounds isolated from the two species are different from each other, suggesting the differences of the biogenetic pathway following the cheilanthene cation.[2] The variety and quantity of triterpenoids were also greater in *A. mexicana* than in *A. agetae*, while the quantity of flavonoids is much less, which results in the lemon yellow color of the farina of the latter. Thus, from a chemotaxonomic point of view also, it is reasonable that *A. agetae* has been assigned as a new species.

Experimental

The instruments and experimental conditions are the same as described in the preceding paper.

Plant Materials The fronds of *Aleuritopteris agetae* were collected in August 1988 at Suh-Hao-Alishan, Taiwan. A voucher specimen has been deposited in the Herbarium of Shôwa College of Pharmaceutical Sciences, Tokyo.

Extraction and Separation The fresh fronds (192 g) were extracted with *n*-hexane to give the extract (2.9 g) and water (140 ml). The *n*-hexane extract was chromatographed on silica gel (Merck), which was eluted to afford tricalla-7,21-diene and hop-21-ene with *n*-hexane, squalene with *n*-hexane–benzene (4:1), two flavonoids, **3** and **4** with benzene–Et_2O

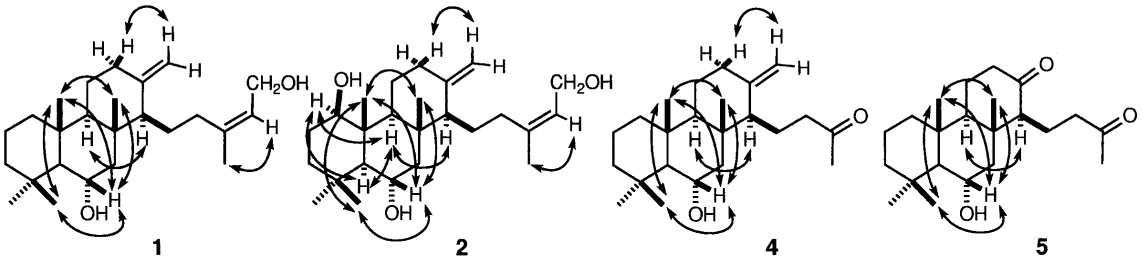

Fig. 1

(9:1), and **1**, **2** and **5** with benzene–Et_2O (1:1). Each compound was further purified by repeated chromatography (silica gel column chromatography (CC), Sephadex LH 20 CC and HPLC) and/or recrystallization.

Paraffins and Triterpenoid Hydrocarbons Each of the hydrocarbon fractions I (288 mg), II (0.9 mg) and III (7 mg) was subjected to GC and GC-MS: frac. I; Rt_R 1.13 [28% of total peaks, *m/z* 408 (M^+), $C_{29}H_{60}$], Rt_R 1.86 [32%, *m/z* 436 (M^+), $C_{31}H_{64}$], Rt_R 3.03 [15%, *m/z* 464 (M^+), $C_{33}H_{68}$]; frac. II; Rt_R 1.78 [58%, *m/z* 410 (M^+), 395, 297, 271, 257, 243, 231, 191, 189, tirucalla-7,21-diene], Rt_R 2.64 [15%, *m/z* 410 (M^+), 395, 367, 341, 231, 191, 189, hop-21-ene]; frac. III; Rt_R 0.90 [71%, *m/z* 410 (M^+), 341, 137, 69, squalene].

3,5-Dihydroxy-7,4′-dimethoxyflavone Yellow needles (512 mg) (from benzene), mp 182—184 °C. Identical (IR, MS and ^{1}H-NMR) with an authentic sample.

5-Hydroxy-7,4′-dimethoxyflavone Yellow needles (5 mg) (from benzene), mp 172—173 °C. Identical (IR, MS and ^{1}H-NMR) with an authentic sample.

(17*Z*)-Cheilantha-13(24),17-diene-6α,19-diol (1) Colorless needles (10 mg) (from MeOH–H_2O), mp 58.5—60 °C, $[\alpha]_D^{23}$ +19.5 °C (*c*=0.10, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3350, 1640, 990, 880. EI-MS (rel. int.) *m/z*: 374, (M^+, 3), 356 ($M^+ - H_2O$, 89), 341 (62), 338 (55), 323 (64), 271 (25), 257 (18), 243 (27), 203 (62), 190 (100), 189 (81). HRMS *m/z*: 374.3194 (M^+, Calcd for $C_{25}H_{42}O_2$: 374.3184).

(17*Z*)-Cheilantha-13(24),17-diene-1β,6α,19-triol (2) Colorless needles (8 mg) (from CH_3CN–EtOH), mp 147—149 °C, $[\alpha]_D^{24}$ +33.3° (*c*=0.05, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1040, 1010, 990, 890. EI-MS (rel. int.) *m/z*: 390 (M^+, 2), 375 (8), 372 (17), 357 (24), 354 (36), 339 (36), 321 (24), 231 (31), 219 (37), 213 (32), 205 (60), 201 (49), 189 (33), 187 (47), 123 (100). HRMS *m/z*: 390.3151 (M^+, Calcd for $C_{25}H_{42}O_3$: 390.3134).

(17*Z*)-19-Acetoxycheilantha-13(24),17-diene-1β,6α-diol (3) Colorless solid (2 mg), $[\alpha]_D^{23}$ +13.6° (*c*=0.10, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1650, 1240, 1030, 890. EI-MS (rel. int.) *m/z*: 432, (M^+, 2), 414 (23), 399 (29), 396 (43), 381 (30), 372 (21), 354 (98), 339 (57), 321 (54), 286 (50), 269 (35), 255 (38), 219 (72), 213 (60), 205 (100), 201 (76), 187 (89). HRMS *m/z*: 432.3172 (M^+, Calcd for $C_{27}H_{44}O_4$: 432.3237).

17-Oxo-18,19-bisnorcheilanth-13(24)-en-6α-ol (4) Colorless needles (6 mg) (from CH_3CN), mp 100—101 °C, $[\alpha]_D^{23}$ −13.2° (*c*=0.11, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1705, 1640, 1360, 1050, 890. EI-MS (rel. int.) *m/z*: 346 (M^+, 42), 328 (100), 313 (61), 295 (17), 270 (50), 257 (29), 243 (43), 221 (18), 203 (85), 191 (67), 190 (100), 189 (95). HRMS *m/z*: 346.2896 (M^+, Calcd for $C_{23}H_{38}O_2$: 346.2871).

13,17-Dioxo-18,19,24-trisnorcheilanth-6α-ol (5) Colorless solid (3 mg), $[\alpha]_D^{23}$ +54.3° (*c*=0.09, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 1705, 1360, 1160, 1050, 980: EI-MS (rel. int.) *m/z*: 348 (M^+, 19), 333 (17), 330 (9), 315 (100), 257 (9), 202 (19), 189 (11), 187 (16). HRMS *m/z*: 348.2674 (M^+, Calcd for $C_{22}H_{36}O_3$: 348.2664).

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