Fern Constituents: *Adiantum cuneatum*. II. Six New Triterpenoids, Neohop-18-en-12α-ol, 13-Epineohop-18-en-12α-ol, Neohop-13(18)-en-19α-ol, Fern-7-en-25-ol, Fern-9(11)-en-25-ol, and Adian-5-en-25-ol

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Six new triterpenoids, neohop-18-en-12α-ol (1), 13-epineohop-18-en-12α-ol (2), neohop-13(18)-en-19α-ol (3), fern-7-en-25-ol (4), fern-9(11)-en-25-ol (5) and adian-5-en-25-ol (6) were isolated from the fresh leaves of *Adiantum cuneatum*, and their structures were elucidated by means of spectroscopic analysis.

Key words fern; *Adiantum cuneatum*; triterpenoid; neohopane group; fernane group; adianane group

In the preceding paper of this series,[1] we reported the structure elucidation of sixteen triterpenoids, including three new compounds, isolated from the fresh leaves of *Adiantum cuneatum* LANGSD. *et* FISCH. (*A. raddianum* PRESL, Adiantaceae). Further investigation of the same extract has resulted in the isolation of six more new triterpenoids; neohop-18-en-12α-ol (**1**), 13-epineohop-18-en-12α-ol (**2**), neohop-13(18)-en-19α-ol (**3**), fern-7-en-25-ol (**4**), fern-9(11)-en-25-ol (**5**), and adian-5-en-25-ol (**6**) (Chart 1). This paper deals with the isolation, and structure elucidation of the new compounds.

Results and Discussion

The more polar fraction of the hexane extract of the fresh leaves[1] was purified by various chromatographic techniques (see Experimental) to give the triterpenoid alcohols **1**—**6**, which are summarized in Table 1 along with their physical constants and yields.

The high-resolution mass spectra (HR-MS) of compounds **1** and **2** indicated their molecular formulae to be $C_{30}H_{50}O$ (M^+ at *m/z* 426.3858 and 426.3850 respectively). Their low-resolution MS (LR-MS) showed identical fragmentation pattern with differences in the intensities of the peaks, indicating that the compounds may be stereoisomeric in nature. The spectra showed, besides the peaks at *m/z* 408 (M^+-H_2O), 393 ($M^+-H_2O-CH_3$), 383 ($M^+-C_3H_7$) and 365 ($M^+-C_3H_7-H_2O$), fragment peaks at *m/z* 203 (c), 206 (b), 234 (d), 191 (a) and 190 (e) (Chart 2). Both the compounds showed hydroxyl group absorptions in their IR spectra. Their ^{1}H-NMR spectra displayed signals due to six tertiary and two secondary methyls, one trisubstituted vinylic methine proton and one equatorially oriented hydroxyl methine proton. However, the ^{1}H- (Table 2) and ^{13}C- (Table 3) NMR chemical shifts of the two compounds were dissimilar. Detailed analyses of the two dimensional (2D) NMR spectra were, therefore, undertaken. Critical analyses of their heteronuclear multiple bond correlation (HMBC) spectra clearly revealed the presence of the partial structures showed by heavy lines in **1** and **2** in Fig. 1, thereby indicating that

Table 1. Triterpenoids Isolated from *Adiantum cuneatum*

	mp (°C)	$[\alpha]_D^{23}$ (°)[a]	Yield (%)
Neohop-18-en-12α-ol (**1**)	208—209	+77.1	0.0015
13-Epineohop-18-en-12α-ol (**2**)	157—158	+33.3	0.0003
Neohop-13(18)-en-19α-ol (**3**)	250—251	−1.9	0.0010
Fern-7-en-25-ol (**4**)	197—199	−18.3	0.0124
Fern-9(11)-en-25-ol (**5**)	190—191	−16.6	0.0085
Adian-5-en-25-ol (**6**)	190.5—191.5	+42.8	0.0030

a) Yield from the dried materials after removal of water by azeotropic distillation.

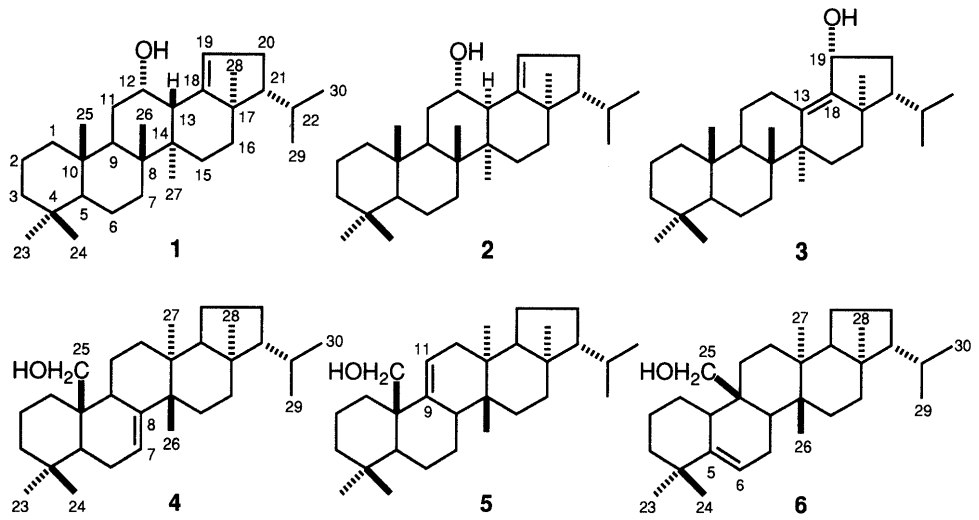

Chart 1

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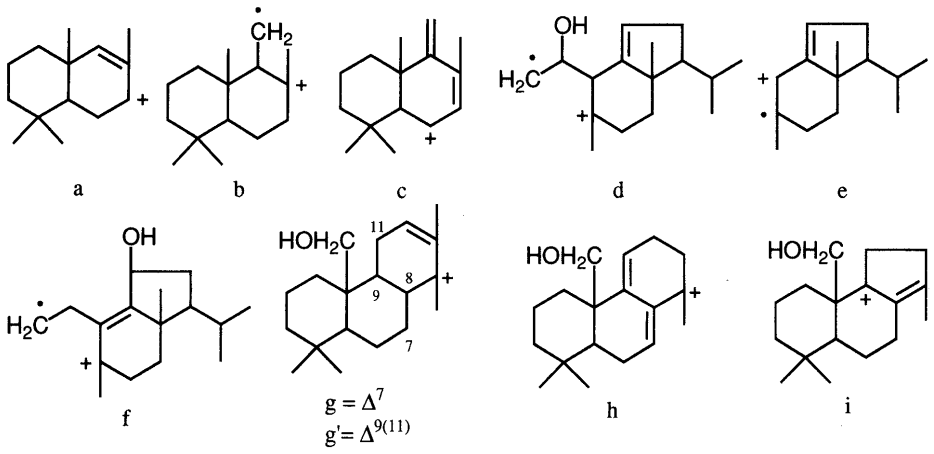

Chart 2

Table 2. ^{1}H-NMR Spectral Data[a] (δ, 500 MHz, $CDCl_3$)

	1	2	3	4	5	6
H-23	0.860	0.851	0.855	0.884	0.874	1.087
H-24	0.814	0.798	0.790	0.964	0.868	1.018
H-25	0.847	0.877	0.821	3.869 (s)	3.296	3.726
					(dd, 11.0, 1.5)	(d, 12.5)
					3.759	3.943
					(dd, 11.0, 11.0)	(d, 12.5)
H-26	0.906	1.068	0.887	1.125	0.744	1.134
H-27	1.259	1.219	1.125	0.908	0.848	0.942
H-28	1.067	0.932	1.084	0.739	0.768	0.785
H-29	0.896	0.925	1.010	0.902	0.893	0.891
	(d, 6.4)	(d, 6.4)	(d, 6.4)	(d, 6.4)	(d, 6.4)	(d, 6.7)
H-30	0.871	0.881	1.044	0.831	0.834	0.829
	(d, 6.4)	(d, 6.4)	(d, 6.4)	(d, 6.4)	(d, 6.4)	(d, 6.7)
>C=CH–	5.611 (H-19)	5.152 (H-19)	—	5.495 (H-7)	5.393 (H-11)	5.686 (H-6)
	(ddd, 3.0, 3.0, 2.1)	(ddd, 2.8, 2.8, 1.5)		(ddd, 3.7, 3.7, 3.1)	(ddd, 5.1, 2.4, 2.4)	(ddd, 5.8, 2.1, 2.1)

Multiplicity and coupling constants (J, Hz) are shown in parentheses. *a*) Assignments have been done on the basis of distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC and NOESY spectra.

Table 3. ^{13}C-NMR Spectral Data[a] (δ, 125 MHz, $CDCl_3$)

	1	2	3	4	5	6		1	2	3	4	5	6
C-1	40.04	40.49	40.54	33.84	34.94	26.75	C-16	35.38	39.41	38.62	36.16	36.07	35.51
C-2	18.64	18.62	18.75	19.27	19.61	19.91	C-17	46.36	47.12	42.37	42.85	42.95	42.78
C-3	42.03	41.99	40.02	42.31	42.12	40.62	C-18	155.69	153.39	138.94	54.12	51.77	51.70
C-4	33.31	33.36	33.32	33.18	33.59	36.05	C-19	121.39	116.52	72.84	20.00	20.12	21.83
C-5	56.76	56.05	56.75	51.11	44.89	147.14	C-20	35.40	36.15	38.62	28.21	28.17	28.31
C-6	18.33	18.05	18.83	24.69	18.94	119.96	C-21	64.20	59.59	63.77	59.52	59.64	60.04
C-7	33.45	36.40	34.42	117.88	17.34	24.33	C-22	28.81	29.44	25.13	30.67	30.96	30.78
C-8	41.83	42.50	41.35	146.70	40.34	43.40	C-23	21.61	21.58	21.57	33.14	32.72	29.83
C-9	45.16	46.20	52.13	47.71	144.15	39.13	C-24	33.42	33.31	33.34	21.07	22.19	29.50
C-10	37.18	37.35	37.76	39.56	44.62	50.68	C-25	15.90	17.02	16.72	62.82	61.09	66.30
C-11	27.83	26.50	21.42	17.44	121.66	28.61	C-26	15.69	21.03	18.57	24.43	15.73	15.64
C-12	66.73	70.20	27.00	32.61	36.66	29.58	C-27	26.40	30.79	26.59	21.26	16.07	15.10
C-13	43.80	48.44	132.94	36.09	36.79	39.55	C-28	16.77	18.07	19.67	14.11	14.01	16.09
C-14	39.41	41.69	42.42	42.12	37.94	38.83	C-29	22.65	22.91	22.36	22.10	22.11	21.95
C-15	31.12	31.18	28.81	30.43	29.03	29.09	C-30	22.78	22.59	23.07	22.98	22.97	22.92

a) Assignments were based on DEPT, H–H COSY, C–H COSY and HMBC spectra.

both compounds possess the neohop-18-en-12-ol structure. That **1** and **2** differ only with respect to the stereochemistry at C-13 was established by the nuclear Overhauser effect (NOE) interactions observed in their NOE spectroscopy (NOESY) spectra (Fig. 2). Thus, while H-13β (δ, 2.416) of **1** showed interactions with H-26 (β-Me), the spectrum of **2** showed cross peaks due to interaction of H-13α (δ, 2.136) with H-27 (α-Me) and H-28 (α-Me). The configuration of the hydroxyl group of **1** was decided based on the NOEs between H-12β (δ, 4.444 m) and H-13β which were confirmed by the NOE difference spectrum to be α-oriented. Compound **2** showed NOE

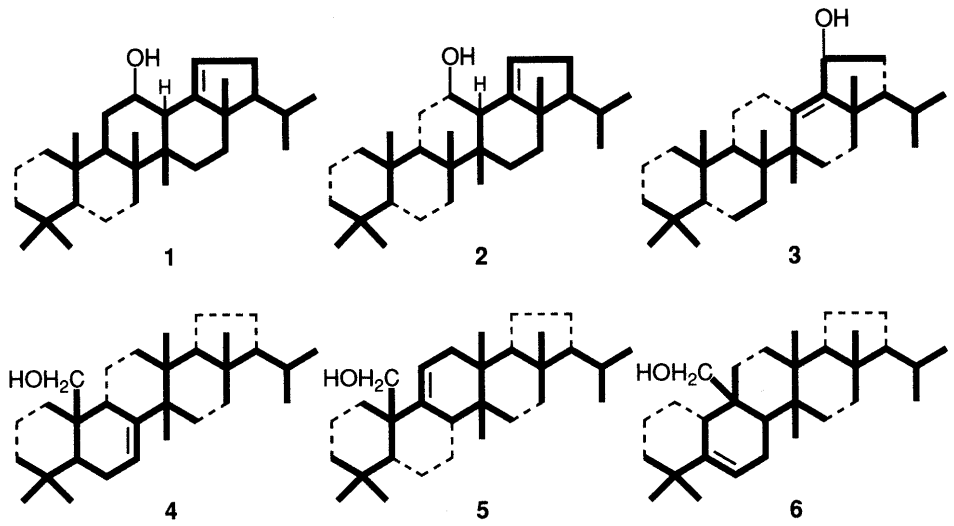

Fig. 1. Partial Structures of **1**, **2**, **3**, **4**, **5** and **6** Based on the HMBC Spectra

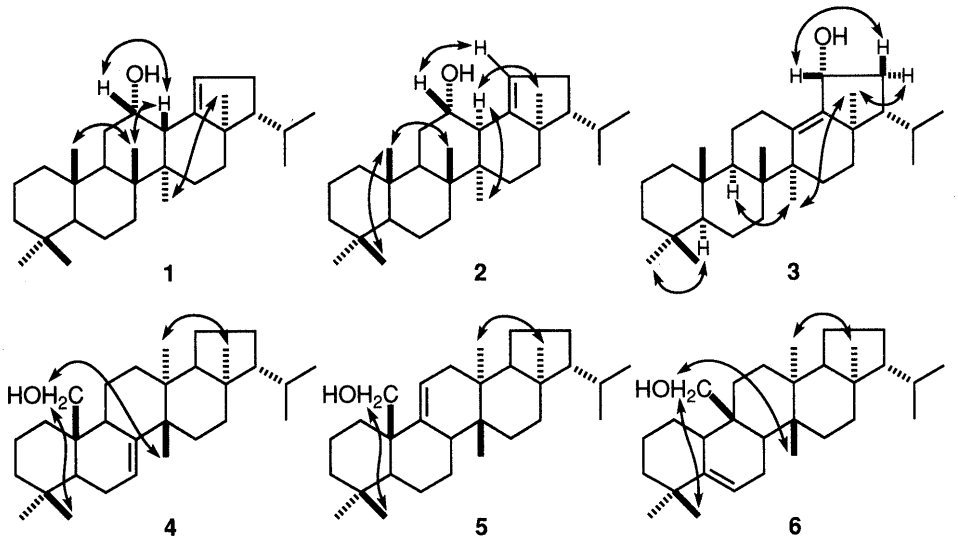

Fig. 2. NOEs Obtained from NOESY

between the H-19 olefinic proton and H-12β (δ, 4.535 m), reflecting conformational distortion. The *cis* juncture of the C/D rings compels H-12β and H-19 to lie close to each other. Thus, compounds **1** and **2** are neohop-18-en-12α-ol and 13-epineohop-18-en-12α-ol, respectively.

The IR spectrum of compound **3** showed a hydroxyl absorption band. The HR-MS of **3** showed M^+ at *m/z* 426.3901 (Calcd, 426.3861) suggesting the molecular formula to be $C_{30}H_{50}O$. Its LR-MS showed the base peak at *m/z* 191 (a), and an intense peak at *m/z* 234 (58, f), indicating that the hydroxy group is located in the D/E ring system. This fragment pattern suggested that **3** is a hopane or neohopane with a hydroxyl group in the right-hand part of the molecule.[1] The ^{1}H-NMR spectrum of **3** exhibited signals due to six tertiary methyl groups, two secondary methyl groups, and a hydroxymethine group. The chemical shifts of H-23, H-24, H-25, H-26 and H-27 were close to those of neohop-13(18)-ene (**7**), while the H-28, H-29 and H-30 signals appeared at comparatively lower fields.[2] The ^{13}C-NMR spectrum of **3** (Table 3) was similar to that of **7**,[2] except for the signals of C-18 to C-22. The hydroxyl group of **3** was determined to be located at C-19 based on the HMBC spectrum, which clearly showed the presence of the partial structure indicated by heavy lines in **3** (Fig. 1). Two protons (δ 2.855, 2.296) at lower field were identified as those at C-20 by examination of the ^{1}H–^{13}C correlation spectroscopy (COSY) spectrum. One (δ, 2.296) of them was elucidated to be α from the NOE between the methyl group of C-28(α) on NOESY, and the other (δ, 2.855) to be β. The configuration of the hydroxyl group was finally established by the NOE, which showed interactions between H-19β [d, 4.361 (dd, 5.8, 5.8)] and H-20β, and confirmed by the NOE difference spectrum to be α (Fig. 2). Thus, the structure of **3** was established as neohop-13(18)-en-19α-ol.

Compounds **4** and **5** showed M^+ at *m/z* 426.3862 and 426.3891 respectively in their HR-MS indicating their molecular formula to be $C_{30}H_{50}O$. Their LR-MS exhibited identical fragmentation patterns with slight differences in the intensities of the peaks. Their ^{1}H-NMR spectra (Table 2) displayed signals due to five tertiary methyl and two secondary methyl groups, one hydroxy methylene group and one trisubstituted olefinic proton. The typical splitting pattern[2] of the vinylic protons of **4** and **5** suggested that

the compounds have Δ^7 and $\Delta^{9(11)}$ pentacyclic triterpenoid skeletons, respectively. A careful comparison of the ^{13}C-NMR data (Table 3) of the compounds with those[2] of fern-7-ene (**8**) and fern-9(11)-ene (**9**) revealed that **4** and **5** are 25-hydroxy derivatives of **8** and **9** respectively, since in both cases, the signals due to C-1 were shielded by *ca.* 5 ppm and those for C-10 and C-25 were deshielded by *ca.* 4 and *ca.* 50 ppm, respectively. The assigned structures were fully supported by the correlations observed in the HMBC (Fig. 1) and NOESY (Fig. 2) spectra. It should be noted that in the mass spectra of **4** and **5**, the diagnostic peaks[3] for Δ^7 and $\Delta^{9(11)}$ pentacyclic triterpenoids at *m/z* 247, 259 and 273 were observed as very low intensity peaks (1—6% of the base peak). However, it seems that loss of a HCHO molecule from the above ions is very facile resulting in the formation of intense peaks at *m/z* 217 (12, i−HCHO), 229 (16, h−HCHO), 243 (12, g′−HCHO), respectively. On the basis of the above observations, **4** and **5** are considered to be fern-7-en-25-ol and fern-9(11)-en-25-ol, respectively.

Compound **6** showed the presence of a hydroxyl group in its IR spectrum. Its HR-MS showed M^+ at *m/z* 426.3829 suggesting the molecular formula to be $C_{30}H_{50}O$. Its LR-MS exhibited the base peak at *m/z* 395 ($M^+ - CH_2OH$), and peaks at *m/z* 289 and 137 diagnostic[3] of adian-5-ene (**10**), with a hydroxyl group in ring C, D or E of the molecule. The ^{1}H-NMR spectrum of **6** indicated the presence of five tertiary methyl groups, two secondary methyl groups, a hydroxyl methylene group, and an olefinic proton. The chemical shifts of methyl protons were close to those of **10** except for H-25 and H-26 (Table 2).[2] The ^{13}C-NMR spectrum of **6** was also close to that of **10**, except for the signals of C-9, C-11, and C-25 (Table 3),[2] strongly suggesting that the compound is 25-hydroxy-adian-5-ene. This structure was fully supported by the HMBC (Fig. 1) and NOESY (Fig. 2) spectra. Based on the above evidence, the adian-5-en-25-ol structure can be assigned to compound **6**.

Of these six new compounds, **2** seems to be most interesting because of its novel *cis*-fused C/D ring system.

Experimental

For details of general procedures and the plant material, see the preceding paper.[1]

Neohop-18-en-12α-ol (1), 13-Epineohop-18-en-12α-ol (2), Neohop-13(18)-en-19α-ol (3), Fern-7-en-25-ol (4), Fern-9(11)-en-25-ol (5), and Adian-5-en-25-ol (6) Fraction D (see the preceding paper[1]) was chromatographed on silica gel with hexane–benzene (1 : 1) and subjected to HPLC with CH_3CN–$CHCl_3$ (19 : 1) followed by recrystallization from methanol to give the following crystalline solids in pure form. **1**, 12 mg. IR ν_{max}^{KBr} cm^{-1}: 3470, 1076. MS *m/z* (rel. int.): 426 (18), 408 (21), 393 (6), 383 (10), 365 (16), 234 (4), 206 (19), 203 (25), 191 (100), 190 (44). **2**, 2 mg. IR ν_{max}^{KBr} cm^{-1}: 3350, 1090, 1030. MS *m/z* (rel. int.): 426 (15), 408 (13), 393 (3), 383 (5), 365 (4), 234 (8), 206 (12), 203 (12), 191 (83), 190 (100). **3**, 7 mg. IR ν_{max}^{KBr} cm^{-1}: 3525, 1048, 1040. MS *m/z* (rel. int.): 426 (28), 411 (8), 408 (10), 393 (5), 365 (12), 327 (3), 234 (58), 220 (12), 205 (36), 191 (100). **4**, 96 mg. IR ν_{max}^{KBr} cm^{-1}: 3530, 1044, 1020. MS *m/z* (rel. int.): 426 (25), 411 (23), 408 (100), 396 (73), 395 (33), 381 (83), 274 (4), 259 (6), 247 (3), 243 (27), 229 (68), 217 (24), 205 (41). **5**, 65 mg. IR ν_{max}^{KBr} cm^{-1}: 3530, 1029. MS *m/z* (rel. int.): 426 (1), 411 (2), 408 (8), 396 (62), 395 (100), 381 (11), 274 (2), 259 (3), 247 (1), 243 (12), 229 (16), 217 (12), 205 (28). **6**, 20 mg. IR ν_{max}^{KBr} cm^{-1}: 3370, 1023. MS *m/z* (rel. int.): 426 (5), 408 (4), 395 (100), 289 (3), 259 (10), 205 (35), 191 (44), 137 (29).

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