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FERN CONSTITUENTS: PENTACYCLIC TRITERPENOIDS ISOLATED FROM *POLYPODIUM NIPONICUM* AND *P. FORMOSANUM*

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Key Word Index—*Polypodium niponicum*; *Polypodium formosanum*; Polypodiaceae; pentacyclic triterpenoids; hopenes; fernenes; oleanenes; taraxerenes; multiflorenes; friedelelene.

Abstract—From the dried rhizomes of *Polypodium niponicum* and *P. formosanum*, 16 hydrocarbons, three alcohols, five acetates, one epoxide and two ketones of pentacyclic triterpenoids belonging to the hopane, neohopane, fernane, trisnorhopane, oleanane, taraxerane, multiflorane, friedelane and taraxastane groups were isolated and characterized.

INTRODUCTION

Many kinds of pentacyclic triterpenoids belonging to the hopane and migrated hopane groups have been reported in ferns. However, no compounds have been observed belonging to the oleanane or migrated oleanane groups. This paper deals with the isolation and characterization of 15 compounds of the oleanane and migrated oleanane groups and ψ-taraxastene in addition to 12 compounds of the hopane and migrated hopane groups from the rhizomes of *Polypodium niponicum* Mett. (= *Marginaria niponica* Nakai, 'Aone-kazura' in Japanese, habitat: Japan and China, Polypodiaceae) and *P. formosanum* Baker (= *M. formosana* Nakai, 'Taiwan-aone-kazura', Japan and Formosa). These ferns are beautiful species having green rhizomes covered with whitish bloom. The rhizomes of the former plant are used as a native drug 'shui long gu' in China [1].

RESULTS AND DISCUSSION

The dried rhizomes of four kinds of material (*Polypodium niponicum* (NA, NB and NC) and *P. formosanum* (F)), were extracted with hexane or chloroform–methanol, and the extracts separated into fractions containing hydrocarbons, ethers, ketones, acetates and alcohols, respectively. All pentacyclic triterpenoids isolated and identified from the fractions are listed in Table 1 together with their physical constants and yields from the four kinds of material. In the case of the former fern, the compounds isolated were rather different depending upon the origin and the seasons of collection of the material.

Triterpenoids of the hopane and migrated hopane series

Compounds **1**, **2** and **3** were the hydrocarbons of the hopane group, i.e. hop-22(29)-ene (hopene-b [2], diploptene [3]), hop-21-ene (hopene-a [2]) and hop-17(21)-ene (hopene-I [2]), respectively. Compound **4** was found to be identical with 17β,21β-epoxyhopane [4] by its mass and ^{1}H NMR spectra. Compounds **5**, **6** and **7** were presumed to be dryocrassol, dryocrassyl acetate [5] and hydroxyhopane (diplopterol [6]), respectively, by TLC (**5**, **7**) and GC (**6**). Compound **8** was considered to be neohop-13(18)-ene (hopene-II [2]) by GC analysis and IR spectroscopy. Compounds **9** and **10** were hydrocarbons of the fernane group, i.e. fern-8-ene and fern-7-ene [7], respectively. These two compounds can be identified by GC and by their IR and ^{1}H NMR spectra, and they also have characteristic mass spectral features. Compound **11**, ferna-7,9(11)-diene [7], was shown to be a heteroannular diene by its UV spectrum. Compound **12** was presumed to be 17α*H*-trisnorhopan-21-one by its ^{1}H NMR and mass spectra. All the compounds described above were identified by comparison of mp, $[\alpha]_D$, GC, IR, ^{1}H NMR and mass spectra with the authentic samples from our laboratory. As far as ^{1}H NMR spectra (Table 2) of the compounds were concerned, the assignments of methyl groups were established by comparison with related compounds (i.e. a hydrocarbon and its 3β-*O*-acetate) including deuteriochloroform–deuteriobenzene solvent and lanthanide shifts. The details of ^{1}H NMR spectra of all compounds examined will be published elsewhere.

Triterpenoids of the oleanane and migrated oleanane series

Compounds **13**, $C_{30}H_{50}$, and **16**, $C_{32}H_{52}O_2$, corresponded to each other, because the IR spectra of both compounds were very similar except that the latter had acetoxyl absorptions. The ^{1}H NMR spectra of **13** and **16** were also similar, giving eight singlet methyl signals (two of those for C-24 and C-25 were different and suggested **16** to be the 3β-acetoxyl compound of **13**) and one olefinic proton signal characteristic of the 18-ene of the oleanane skeleton. The same base peak in the mass spectra of both compounds at *m/z* 204, also supports the above conclusion. Compound **16** was identified as olean-18-en-3β-yl acetate and **13** was olean-18-ene by comparison of the compounds with samples of germanicyl acetate obtained from *Salvia officinalis* [8] and the derived hydrocarbon, respectively. Compounds **14**, $C_{30}H_{50}$, and **17**, $C_{32}H_{52}O_2$, were shown to have the same carbon skeleton by their IR and ^{1}H NMR spectra. The ^{1}H NMR spectra of both compounds showed eight singlet methyl signals (two of those for C-24 and C-25 were different, suggesting **17** to be the

Table 1. Pentacyclic triterpenoids isolated from the rhizomes of *Polypodium niponicum* (NA, NB, NC) and *P. formosanum* (F)

	mp (°)	$[\alpha]_D$ (°)	RR_t	Yield (% × 10^3)*			
				NA	NB	NC	F
Hop-22(29)-ene (**1**)	211–212	+60.2	2.61	8.8	15.6	1.5	1.0
Hop-21-ene (**2**)	194–195	+29.8	2.67	1.3	+	0.7	2.9
Hop-17(21)-ene (**3**)	188–189	+50.0	1.67	+	+	0.2	+
17β,21β-Epoxyhopane (**4**)	263–265	+47.9	2.30	+	±	0.4	−
Dryocrassol (**5**)	247–249	+51.8	5.15	+	0.1	+	+
Dryocrassyl acetate (**6**)	197–199	+58.8	6.47	0.3	0.5	0.9	+
22-Hydroxyhopane (**7**)	253–255	+43.0		0.1	+	+	6.2
Neohop-13(18)-ene (**8**)	200–201	+2.9	1.90	0.8	+	1.3	−
Fern-8-ene (**9**)	190–191	+25.3	1.91	+	+	+	10.3
Fern-7-ene (**10**)	212–214	−27.8	2.26	130.6	76.0	0.9	41.2
Ferna-7,9(11)-diene (**11**)	202–203	−189.5	1.87	0.3	+	0.4	−
17α*H*-Trisnorhopan-21-one (**12**)	243–245	+148.5	1.87	0.3	±	±	−
Olean-18-ene (**13**)	174–175	+6.2	1.57	1.1	4.9	+	6.5
Olean-12-ene (**14**)	162–164	+96.2	1.57	4.6	13.5	10.9	18.5
Oleana-11,13(18)-diene (**15**)	226–227	−65.8	1.48	0.3	0.2	+	−
Germanicyl acetate (**16**)	277–279	+17.0	4.15	3.3	0.2	+	0.6
β-Amyrin acetate (**17**)	241–242	+81.0	3.64	0.6	1.0	13.0	+
Oleana-11,13(18)-dien-3β-yl acetate (**18**)	223–225	−53.1	3.43	0.3	+	±	−
Taraxer-14-ene (**19**)	251–252	+3.0	1.50	15.6	11.6	0.4	+
16-Oxo-taraxer-14-ene (**20**)	>290	−38.5	2.95	0.3	±	±	0.1
7α-Hydroxytaraxer-14-ene (**21**)	194; 252–254	−24.0	2.17	0.6	0.2	±	−
Multiflor-9(11)-ene (**22**)	163–166	−2.0	1.65	0.1	+	0.7	24.4
Multiflor-8-ene (**23**)	188–189	+58.0	1.63	1.2	1.7	1.8	0.4
Multiflor-7-ene (**24**)	146–147	−20.0	1.87	30.6	69.8	26.1	+
Multiflor-7-en-3β-yl acetate (**25**)	237–239	—	4.40	±	0.5	±	−
Friedel-3-ene (**26**)	272–273	−18.0	2.29	6.9	56.3	0.9	0.9
ψ-Taraxastene (**27**)	183–184	+54.2	2.01	0.3	2.1	+	−

*Yield: +, presence of the compound was confirmed, but its yield was unknown; ±, presence of the compound was detected by GC or other methods, but not confirmed; −, presence of the compound was not detected.

3β-acetoxyl compound of **14**) and one olefinic signal (*t*, *J* = 3.4 Hz) characteristic of olean-12-ene. The same base peak at *m/z* 218 in the mass spectrum also supported this conclusion. Compound **17** was identified as β-amyrin acetate and **14** was olean-12-ene by comparison of the compounds with samples obtained from *Firmiana simplex* [9] and the derived hydrocarbon, respectively.

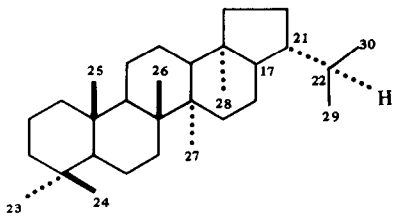
Compounds **15**, $C_{30}H_{48}$, and **18**, $C_{30}H_{50}O_2$, again corresponded to each other. The IR spectra of both compounds showed disubstituted double bond absorption and characteristic UV absorptions indicating the presence of a heteroannular diene system in the molecule. The ^{1}H NMR spectra of both compounds exhibited eight singlet methyl signals (two of those for C-24 and C-25 were different in chemical shift suggesting **18** to be the 3β-acetoxyl compound of **15**) and two olefinic proton signals of the ABX type. These observations suggested **15** to be oleana-11,13(18)-diene and **18** to be oleana-11,13(18)-dien-3β-yl acetate, and these were identified by comparison with the samples derived from **14** and **17**, respectively.

Compound **19**, $C_{30}H_{50}$, gave the absorption of a trisubstituted olefinic proton in its IR spectrum. The observation of the base peak at *m/z* 204 in the mass spectrum and the characteristic double doublets signal of the olefinic proton in the ^{1}H NMR spectrum suggested the compound to be a Δ^{14} pentacyclic triterpenoid. The identification of **19** as taraxer-14-ene was proved by comparison with the sample derived from taraxerol [10].

Compound **20**, $C_{30}H_{48}O$, was found to be a conjugated ketone because its UV absorption was observed at 247 nm (ε = 8500). The mass spectral fragmentation of **20** is shown in Scheme 1, and this, together with a very sharp signal of an olefinic proton in the ^{1}H NMR spectrum, suggested **20** to be a pentacyclic triterpenoid having a 14-en-16-one system. The identification of **20** as taraxer-14-en-16-one was proved by comparison with the sample obtained by chromate–*t*–butylate oxidation of **19**.

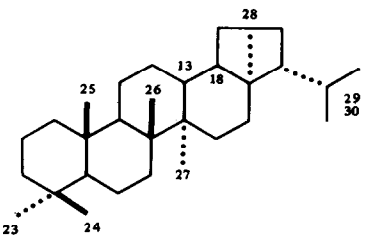
Compound **21**, $C_{30}H_{50}O$, was indicated to be an alcohol having a trisubstituted double bond by its IR spectrum. The mass spectral fragmentation of **21** is shown in Scheme 1. The characteristic double doublets signal of an olefinic proton and the eight singlet signals of methyl group protons in the ^{1}H NMR spectrum suggested **21** to be a derivative of taraxer-14-ene. As a proton signal adjacent to the hydroxyl group was observed as a triplet (*J* = 2.6 Hz), the axial hydroxyl group was assumed to be situated at a carbon next to a quarternary carbon, such as

Hopane



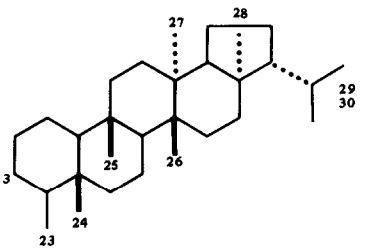
1 22(29)-ene
2 21-ene
3 17(21)-ene
4 17β, 21β-epoxyhopane
5 30-ol(22S)
6 30-yl acetate(22S)
7 22-hydroxy-

Neohopane



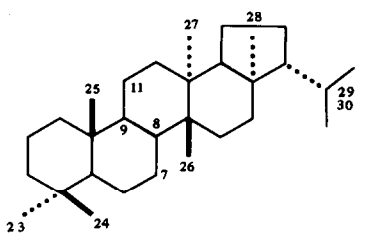
8 13(18)-ene

Filicane



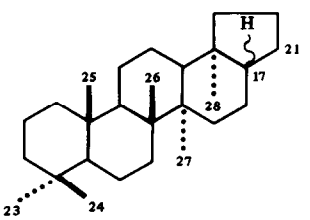
31 3-ene

Fernane



9 8-ene
10 7-ene, 9αH
11 7,9(11)-diene
29 9(11)-ene, 8αH

Trisnorhopane



12 17αH, 21-one

C-7. Only the two methyl signals of **21** (C-23 and C-26) were shifted compared with those of **19** and this also supported the 7α-position of the hydroxyl group in compound **21**. Oxidation of **21** with chromate–pyridine at 60°C gave a non-conjugated ketone (**28**), which was confirmed to be identical with 7-oxotaraxer-14-ene obtained from multiflor-7-ene (**24**) by chromate–acetic acid oxidation. Lithium aluminium hydride reduction of **28** gave two alcohols, the less polar one of which had the same TLC mobility as **21**. Thus, **21** was established to be 7α-hydroxytaraxer-14-ene.

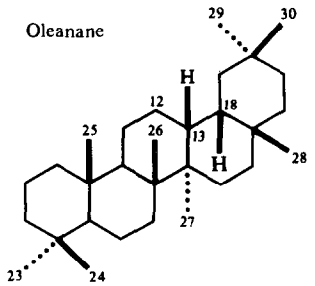
Compounds **22–24**, all $C_{30}H_{50}$, were hydrocarbons with the multiflorane skeleton. The ^1H NMR spectra of **22** and **24** both clearly indicated the presence of an olefinic proton and their patterns were very similar to those of fern-9(11)-ene (**29**) and **10**, respectively. All the compounds gave eight singlet signals of methyl groups and the chemical shifts of C-23–C-26 of **22–24** corresponded with those of **29**, **9** and **10**. Absorption of **24** on neutral alumina afforded **23**, and acid treatment of both **22** and **24** gave olean-12-ene(**14**) as the only product, thus confirming their carbon skeleton. Oxidation of **24** with selenium oxide gave a heteroannular diene (**30**) in a good yield to suggest the position of the double bond. Compound **24** was identified as multiflor-7-ene by direct comparison with the authentic sample [12], and compounds **22** and **23** were concluded to be multiflor-9(11)-ene and multiflor-8-ene, respectively. The mass spectra of **22–24** were rather different from those of **29**, **9** and **10** as shown in Table 3[11]. The fact could be explained as follows. (1) In the case of fernenes, **29** and **10** give almost the same fragmentation patterns and many of the same ions are seen in the spectrum of **9** so that the distinction of **29**, **9** and **10** is very difficult. (2) Because of instability of the molecular ion, **22** gives the corresponding ions to **23** and **14** and the fragment at *m/z* 218 is the base peak from the

Table 2. ^{1}H NMR chemical shifts for $CDCl_3$ solution (100 MHz)

Compound	Methyl or methylene signals								Other proton signals
	23	24	25	26	27	28	29	30	
1	0.845	0.794	0.818	0.963	0.948	0.727	4.777 *br s*	1.750	—
2	0.850	0.796	0.818	0.970	0.970	0.587	1.571	1.728	—
3	0.845	0.794	0.835	0.938	1.044	0.845	0.917 *d* (6.9)	0.978 *d* (6.9)	—
4	0.828	0.798	0.853	1.031	1.056	0.828	0.946 *d* (6.9)	1.060 *d* (6.9)	—
5	0.848	0.794	0.813	0.955	0.955	0.727	1.049 *d* (6.6)	3.387 *dd* (10.4, 5.7) 3.629 *dd* (10.4, 2.5)	—
6	0.845	0.791	0.813	0.953	0.953	0.725	1.013 *d* (5.6)	3.770 *dd* (10.7, 6.5) 4.072 *dd* (10.7, 2.2)	—
7	0.848	0.796	0.818	0.960	0.960	0.767	1.181	1.208	—
8	0.857	0.794	0.823	0.857	1.100	0.794	0.888 *d* (6.6)	0.935 *d* (6.6)	—
29	0.857	0.892	1.054	0.735	0.823	0.759	0.830 *d* (6.4)	0.889 *d* (6.4)	(11) 5.286 *ddd* (4.0, 3.0, 2.4)
9	0.875	0.828	0.946	0.946	0.769	0.769	0.826 *d* (6.2)	0.888 *d* (6.2)	
10	0.843	0.877	0.742	0.995	0.906	0.742	0.829 *d* (6.6)	0.897 *d* (6.6)	(7) 5.354 *ddd* (3.7, 3.2, 3.2)
11	0.857	0.911	0.911	0.911	0.705	0.759	0.830 *d* (6.9)	0.899 *d* (6.9)	(7) 5.404 *m* (11) 5.154 *m*
31	1.574 *d*	0.987	0.899	0.919	0.919	0.781	0.823 *d* (6.4)	0.887 *d* (6.4)	(3) 5.149 *m* (23) (1.5)
12	0.840	0.784	0.803	0.840	1.027	1.152			(17) 2.167
13	0.845	0.801	0.875	1.078	0.745	1.019	0.938	0.938	(19) 4.858 *d* (1.5)
14	0.872	0.821	0.931	0.968	1.144	0.833	0.872	0.872	(12) 5.186 *br t* (3.4)
15	0.865	0.808	0.892	0.752	0.955	1.054	0.713	0.955	(11) 6.368 *dd* (3.2, 10.4) (12) 5.551 *dd* (1.8, 10.4)
16	0.845	0.845	0.906	1.078	0.732	1.017	0.938	0.938	(19) 4.859 *d* (1.2)
17	0.872	0.872	0.970	0.970	1.132	0.830	0.872	0.872	(12) 5.181 *t* (3.4)
18	0.867	0.855	0.919	0.750	0.953	1.054	0.710	0.953	(11) 6.435 *dd* (2.9, 10.6) (12) 5.450 *dd* (1.8, 10.6)
19	0.848	0.828	0.914	1.088	0.948	0.828	0.914	0.914	(15) 5.525 *dd* (3.5, 7.9)
20	0.867	0.840	0.948	1.142	1.134	1.004	0.968	0.968	(15) 5.838
21	0.879	0.823	0.921	1.156	0.951	0.823	0.904	0.904	(15) 5.689 *dd* (3.4, 7.9) (7β) 4.005 *t* (2.6)
28	0.830	0.848	0.987	1.389	1.022	0.858	0.946	0.906	(15) 6.049 *dd* (3.5, 8.3)
22	0.848	0.897	1.054	0.784	0.897	1.054	0.975	0.975	(11) 5.299 *ddd* (2.5, 2.5, 2.5)
23	0.877	0.833	0.953	1.056	1.000	1.071	0.968	0.975	
24	0.853	0.887	0.740	1.073	1.098	1.058	0.970	0.970	(7) 5.470 *ddd* (4.0, 3.0, 3.0)
30	0.853	0.904	0.904	0.904	0.904	1.031	0.982	0.955	(7) 5.468 *m* (11) 5.206 *m*
25	0.857	0.936	0.764	1.076	1.082	1.058	0.973	0.973	(7) 5.469 *ddd* (4.0, 3.0, 3.0)
26	1.577 *d*	0.997	0.860	0.997	0.997	1.174	0.946	0.997	(3) 5.162 *m* (23) (1.5)
27	0.853	0.803	0.853	1.049	0.963	0.737	0.987 *d*	1.643	(21) 5.263 *br d* (6.6)

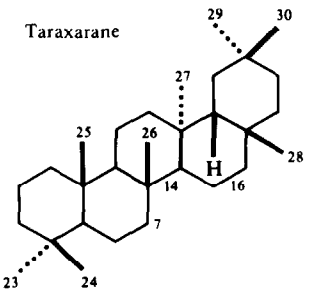
Signals, unless otherwise stated, were singlet. Assignments were confirmed by $CDCl_3$–C_6D_6 solvent shifts (for all compounds) and lanthanide shifts (if necessary).

Oleanane



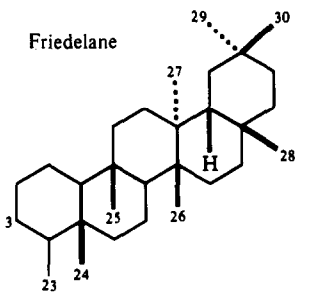
13 18-ene
14 12-ene
15 11,13(18)-diene
16 18-en-3β-yl acetate
17 12-en-3β-yl acetate
18 11,13(18)-dien-3β-yl acetate

Taraxarane



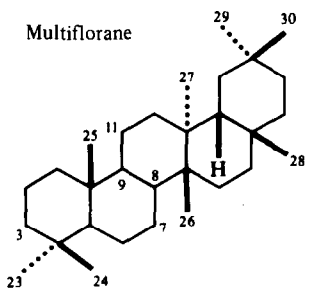
19 14-ene
20 16-oxo-14-ene
21 7α-hydroxy-14-ene
28 7-oxo-14-ene

Friedelane



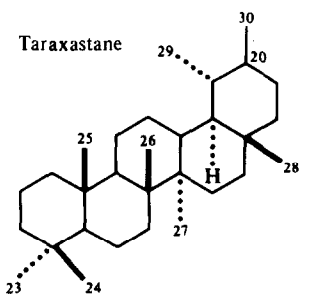
26 3-ene

Multiflorane



22 9(11)-ene
23 8-ene
24 7-ene
25 7-en-3β-yl acetate
30 7,9(11)-diene

Taraxastane



27 20-ene

latter, while **24** gives the corresponding ions seen in the spectra of **19** and **23**, and the base peak at *m/z* 204 is observed. Thus, the discrimination of multiflorenes from the other hydrocarbons, such as **14** and **19**, is confusing.

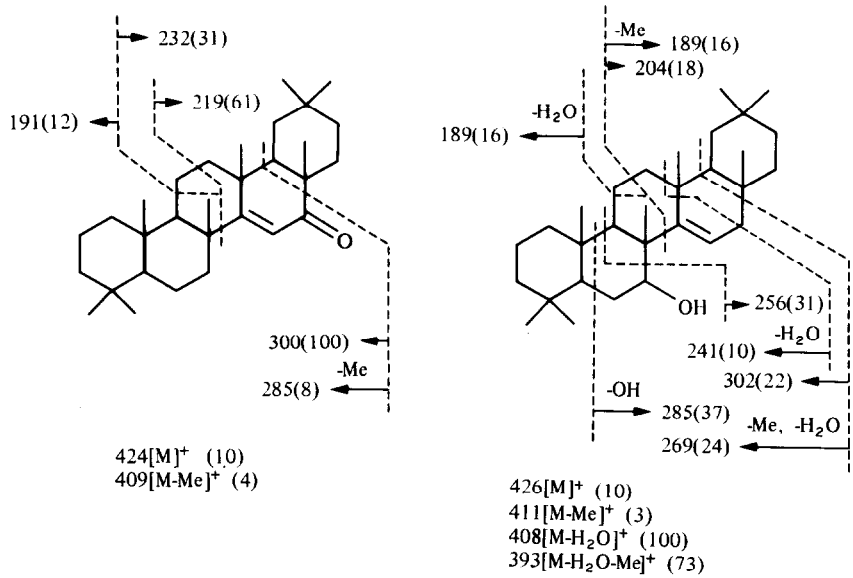
Compound **25**, $C_{32}H_{52}O$, was shown to be the corresponding 3β-yl acetate of **24** by comparison of its IR, ^{1}H NMR and mass spectra with those of **24**. The identity was established by comparison of **25** with the authentic sample of multiflorenyl acetate [12].

Compound **26**, $C_{30}H_{50}$, was indicated by its ^{1}H NMR spectrum to contain seven singlet methyls, one olefinic methyl and one olefinic proton. The similarity of the IR, ^{1}H NMR and mass spectra (base peak at *m/z* 218) with those of filic-3-ene (**31**), suggested the compound to be friedel-3-ene. The identity was established by comparison (GC, IR, ^{1}H NMR and mass spectra) of **26** with the sample derived from friedelin (cork) [13].

Triterpenoids of the taraxastane group

Compound **27**, $C_{30}H_{50}$, was shown by its ^{1}H NMR spectrum to be a pentacyclic triterpene hydrocarbon having six tertiary methyl groups, one secondary methyl group, one olefinic methyl group, and one olefinic proton. The similarity of the IR, ^{1}H NMR and mass spectra with those of ψ-taraxasterol suggested **27** to be ψ-taraxastene. The identity was established by comparison of **27** (GC, IR, ^{1}H NMR and mass spectra) with the sample derived from lup-20(29)-ene [14] by boron trifluoride–etherate treatment.

Among the compounds listed in Table 1, **13–15**, **20–24**, **26** and **27** are isolated for the first time from natural sources, and **16–19** and **25** are reported for the first time from fern plants. Although the most widely distributed pentacyclic triterpenoids among flowering plants are



Scheme 1. Fragmentation patterns of 16-oxo-taraxor-14-ene (**20**) and 7α-hydroxy taraxer-14-ene (**21**), 70 eV *m/z* (rel. int.).

Table 3. Mass spectra of fernane and multiflorane hydrocarbons

	m/z 410	395	286	271	257	243	231	218	206	205	204	203	191	189
Fern-9(11)-ene (**29**)	32	86	1	4	20	100	16	6	3	7	3	6	9	8
Fern-8-ene (**9**)	31	95	1	3	17	100	15	3	1	5	5	5	5	5
Fern-7-ene (**10**)	28	80	5	13	32	100	18	6	5	10	3	8	10	5
Multiflor-9(11)-ene (**22**)	18	20	1	5	12	50	40	100	40	38	17	28	75	17
Multiflor-8-ene (**23**)	16	20	1	4	9	100	72	20	36	53	12	5	35	8
Multiflor-7-ene (**24**)	9	14	3	7	9	47	46	19	26	55	100	8	24	12
Olean-12-ene (**14**)	5	3	1	1	5	1	1	100	1	5	9	33	16	11
Taraxer-14-ene (**19**)	14	12	61	54	20	4	14	40	8	32	100	12	18	26

oleanene derivatives having an oxygen function at C-3, the only example hitherto found among non-flowering plants is a migrated oleanene, taraxerene in a lichen [15]. Moreover, most of the pentacyclic triterpenoids found in fern plants are hopane and migrated hopane derivatives having no oxygen function at C-3. Under these situations discovery of various kinds of oleanane and migrated oleanane derivatives, including 3-acetoxyl derivatives from two species of *Polypodium* ferns, is very interesting from the chemotaxonomical point of view.

EXPERIMENTAL

General procedures. Mps were measured on a Kofler block and are corr. $[\alpha]_D$s were observed in $CHCl_3$ soln (*c* 0.2–0.5) at 22–24°. IR spectra were recorded for KBr pellets. ^1H NMR spectra were taken at 100 MHz in $CDCl_3$ soln. TMS was used as int. standard and chemical shifts are given in δ-values (ppm). Mass spectra were recorded for direct inlet at 70/eV unless otherwise stated and relative intensities of peaks are reported with reference to the most intense peak higher than *m/z* 120. TLC was carried out on Si gel (Merck 5721) with hexane–EtOAc solvent system, the spray reagent being H_2SO_4. GC were performed on a 1 m glass column containing Chromosorb G AW DMCS with 1.4% SE-30 at 260°. Cholestane was used as int. reference and its R_t was set at 3.5 min, and the RR_t of compounds are given in Table 1.

Plant materials. Polypodium niponicum: NA collected at Shizuoka City, Shizuoka Prefecture, on 20 Dec. 1970 (F701201); NB at Miyama, Gifu Prefecture, on 20 May 1971 (F710501); NC at Tomisawa, Yamanashi Prefecture, on 26 Aug. 1975 (F750801). *Polypodium formosanum*: F collected at Wulai, Taipei, on 6 Aug. 1971 (FF-690). The voucher specimens are deposited in the Herbarium of the Laboratory of Phytochemistry, Showa College of Pharmaceutical Sciences, Tokyo.

Extraction and separation. The cut and dried rhizomes of plant material were extracted with hexane (NA and NC) or $CHCl_3$–MeOH (NB and F) and the extracts were treated with MeOH to remove waxy substances. The resulting extracts were chromatographed on Si gel to separate the fractions shown in Table 4.

Triterpenoid hydrocarbons. Fraction 1 was chromatographed on $AgNO_3$–Si gel (1:4) to give three sub-fractions and the individual compounds were separated by repeated chromatography ($AgNO_3$–Si gel, Al_2O_3) and recrystallization. The compounds obtained are shown as follows in order of elution by chromatography ($AgNO_3$–Si gel) (see Tables 1 and 2 for data).

Multiflor-8-ene (**23**). 43 mg of colorless plates(Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 847. EIMS *m/z* (rel. int.): 410 $[M]^+$ (16),

Table 4. Chromatography of the extracts of *Polypodium* rhizomes

	NA	NB	NC	F
Dried material examined (kg)	3.6	9.6	2.3	3.4
Material extracted from the following fractions (g)				
Fraction 1 hexane,				
hexane-C_6H_6(8:2)	9.0	35.0	7.0	27.0
2 hexane–C_6H_6(8:2)–(7:3)	8.4	50.0	3.0	25.0
3 hexane–C_6H_6(7:3) C_6H_6	2.1	10.0	5.5	23.0
4 C_6H_6	1.6			2.6
5 C_6H_6	1.5			6.7
6 C_6H_6–Et_2O(9:1) Et_2O	6.0	35.0	5.0	5.3

395 (20), 257 (9), 243 (100), 231 (72), 218 (20), 206 (36), 205 (53), 191 (35). (Found: C, 87.89; H, 12.21. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 160 mg from NB-1, 41 mg from NC-1 and 15 mg from F.

Neohop-13(18)-*ene* (**8**). 30 mg of colorless plates (Me_2CO) from NC-1. IR ν_{max}^{KBr} cm^{-1}: 852, 845. EIMS *m/z* (rel. int.): 410 $[M]^+$ (20), 395 (4), 367 (3), 229 (23), 218 (58), 206 (27), 205 (61), 204 (36), 203 (24), 191 (100), 189 (27), 175 (25), (lit. [2] mp 194–196°, $[\alpha]_D$ +2°).

Fern-8-*ene* (**9**). 350 mg of colorless plates (Me_2CO) from F-1. IR ν_{max}^{KBr} cm^{-1}: 862, 857. EIMS *m/z* (rel. int.): 410 $[M]^+$ (31), 395 (95), 257 (17), 243 (100), 231 (17). (lit. [7] mp 211–213°, $[\alpha]_D$ −27°).

Hop-17(21)-*ene* (*(hopene-I)* (**3**). 5 mg of colorless plates (Me_2CO) from NC-1. IR ν_{max}^{KBr} cm^{-1}: 851. EIMS *m/z* (rel. int.): 410 $[M]^+$ (59), 395 (17), 367 (100), 231 (80), 203 (16), 191 (71), 189 (45), 175 (45), 161 (75), 136 (95), 135 (100). (lit. [2] mp 183.5–185°, $[\alpha]_D$ +49.5°).

Ferna-7,9(11)-*diene* (**11**). 12 mg of colorless needles (Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3030, 1633, 1614, 822, 817, 795. EIMS *m/z* (rel. int.): 408 $[M]^+$ (100), 393 (27), 365 (5), 269 (9), 257 (22), 255 (84), 243 (14), 241 (19), 229 (14), 215 (11), 203 (8), 199 (9), 187 (11), 175 (21). UV λ_{max}^{EtOH} nm (ε): 232 (12900), 204 (14900), 248 (9800). 10 mg from NC-1 and a trace from NB-1.

Multiflor-9(11)-*ene* (**22**). 15 mg of colorless plates (Me_2CO) from NC-1. IR ν_{max}^{KBr} cm^{-1}: 3030, 1650, 872, 861, 813. EIMS *m/z* (rel. int.): 410 $[M]^+$ (18), 395 (20), 257 (12), 243 (50), 231 (40), 218 (100), 206 (40), 205 (38), 203 (28), 191 (75),. (Found: C, 87.82; H, 12.42. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 5 mg from NA-1 and 830 mg from F-1.

Olean-12-*ene* (**14**). 165 mg of colorless plates(Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3030, 1655, 820, 811. EIMS *m/z* (rel. int.): 410 $[M]^+$ (6), 395 (3), 218 (100), 203 (33), 191 (16), 189 (11). (Found: C, 87.47; H, 12.41. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 1.30 g from NB-1 and 630 mg from F-1. (lit. [16] mp 162–163°, $[\alpha]_D$ +96°.

Taraxer-14-*ene* (**19**). 560 mg of colorless needles(Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3040, 1638, 816, 808. EIMS *m/z* (rel. int.): 410 $[M]^+$ (15), 395 (14), 286 (60), 271 (54), 257 (21), 231 (13), 218 (41), 204 (100), 191 (19), 189 (26). (Found: C, 87.45; H, 12.40. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 1.10 g from NB-1, 10 mg from NC-1 and a trace from F-1. (lit. [15] mp 237–238°, $[\alpha]_D$ +1°).

Multiflor-7-*ene* (**24**). 1.10 g of colorless plates (Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3020, 1659, 837, 821. EIMS *m/z* (rel. int.): 410 $[M]^+$ (9), 395 (14), 271 (7), 257 (9), 243 (47), 231 (46), 218 (19), 206 (26), 205 (55), 204 (100), 191 (24), 189 (12). (Found: C, 87.43; H, 12.40. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 6.70 g from NB-1 and 600 mg from NC-1. (lit. [12] mp 134–141°, $[\alpha]_D$ −20°).

Fern-7-*ene* (**10**). 4.70 g of colorless plates(Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3050, 1661, 828, 819. EIMS *m/z* (rel. int.): 410 $[M]^+$ (29), 395 (81), 271 (14), 257 (22), 243 (100), 231 (19), 205 (11), 203 (9), 191 (11), 189 (9). 7.30 g from NB-1, 20 mg from NC-1 and 1.40 g from F-1. (lit. [7] mp 211–213°, $[\alpha]_D$ −27°).

Olean-18-*ene* (**13**). 40 mg of colorless needles (Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3020, 1645, 846. EIMS *m/z* (rel. int.): 410 $[M]^+$ (20), 395 (19), 233 (11), 229 (6), 218 (24), 204 (100), 191 (44), 189 (70), 177 (70). (Found C, 87.81; H, 12.49. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 470 mg from NB-1 and 7.02 g from F-1. (lit. [17] mp 172°).

Ψ-*Taraxastene* (**27**). 13 mg of colorless needles(Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3020, 1677, 841, 779. EIMS *m/z* (rel. int.): 410 $[M]^+$ (33), 395 (12), 328 (4), 313 (2), 300 (2), 272 (10), 257 (8), 229 (5), 205 (6), 204 (10), 191 (100), 189 (26). (Found: C, 88.02; H, 12.40. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) (lit. [14] mp 182–184°, $[\alpha]_D$ +50°).

Hop-21-*ene* (**12**). 45 mg of colorless plates (Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 1699, 851. EIMS *m/z* (rel. int.): 410 $[M]^+$ (50), 395 (13), 367 (34), 341 (52), 231 (28), 218 (12), 205 (19), 203 (18), 191 (100), 189 (98), 161 (74), 149 (57), 135 (85). 15 mg from NC-1 and 100 mg from F-1. (lit. [2] mp 179–181°, $[\alpha]_D$ +25°).

Friedel-3-*ene* (**26**). 248 mg of colorless plates(Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3020, 1673, 849, 829, 793. EIMS *m/z* (rel. int.): 410 $[M]^+$ (43), 395 (30), 287 (12), 274 (20), 257 (24), 231 (20), 218 (100), 205 (59), 191 (41), 189 (39). (Found: C, 87.66; H, 12.27. $C_{30}H_{50}$ requires: C, 87.73; H, 12.27%.) 5.40 mg from NB-1 and 20 mg from NC-1. (lit. [13] mp 269–271°).

Oleana-11,13(18)-*diene* (**15**). 10 mg of colorless needles (Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3010, 1621, 830, 817, 810, 771. EIMS *m/z* (rel. int.): 408 $[M]^+$ (100), 393 (37), 271 (11), 270 (13), 269 (12), 255 (30), 243 (10), 229 (31), 215 (37), 205 (19), 204 (23), 203 (21), 191 (17), 189 (30). UV ν_{max}^{EtOH} nm (ε): 242 (26000), 250 (30000), 260 (21000). 23 mg from NB-1. (lit. [18] mp 223–224°, $[\alpha]_D$ −73°).

Hop-22(29)-*ene* (**1**). 317 mg of colorless needles (Me_2CO) from NA-1. IR ν_{max}^{KBr} cm^{-1}: 3055, 1647, 1629, 887. EIMS *m/z* (rel. int.): 410 $[M]^+$ (30), 395 (9), 367 (3), 299 (8), 218 (13), 205 (14), 204 (15), 203 (12), 191 (100), 189 (91). 1.50 g from NB-1, 35 mg from NC-1 and 33 mg from F-1. (lit. [2] mp 210–211°, $[\alpha]_D$ +61°).

17αH-*Trisnorhopane*-21-*one* (**12**). The most polar fraction of NA-1 was rechromatographed on $AgNO_3$–Si gel followed by recrystallization from Me_2CO to give 12 mg **12** (colorless needles). IR ν_{max}^{KBr} cm^{-1}: 1733. EIMS *m/z* (rel. int.): 384 $[M]^+$ (14), 369 (9), 207 (7), 191 (100), 177 (10).

17β,21β-*Epoxyhopane* (**4**). The first elute of NC-2 on Al_2O_3 (hexane–C_6H_6, 9:1) was recrystallized from Me_2CO to give 10 mg **4**. IR ν_{max}^{KBr} cm^{-1}: 1243, 998, 961, 896. EIMS *m/z* (rel. int.): 426 $[M]^+$ (100), 411 (11), 408 (7), 393 (8), 383 (6), 365 (8), 299 (13), 234 (12), 231 (11), 221 (21), 205 (40), 203 (23), 191 (97), 189 (16), 152 (100). (lit. [4] mp 268–270°, $[\alpha]_D$ +47°).

Germanicyl acetate (**16**). To fraction NA-2 was added a small amount of Me_2CO to give 120 mg **16** (colorless plates). IR ν_{max}^{KBr} cm^{-1}: 1733, 1251, 1025, 3020, 1650, 861, 847. EIMS *m/z* (rel. int.): 468 $[M]^+$ (15), 408 (13), 393 (3), 231 (12), 218 (16), 204 (100), 189 (81), 177 (73). (Found: C, 82.28; H, 11.44. $C_{32}H_{52}O_2$ requires: C, 81.99; H, 11.18.) 20 mg from NB-2, a trace from F-3.

7α-*Hydroxytaraxer*-14-*ene* (**21**). The mother liquor of recrystallization of **16** was chromatographed on $AgNO_3$–Si gel (hexane–C_6H_6, 1:1) followed by recrystallization from Me_2CO to give 20 mg **21** (colorless needles). IR ν_{max}^{KBr} cm^{-1}: 3500, 1070, 820. EIMS 300 eV *m/z* (rel. int.): 426 $[M]^+$ (10), 408 (100), 393 (73), 302 (22), 285 (37), 271 (14), 269 (24), 256 (31), 241 (10), 220 (10), 204 (18), 189 (16). A trace from NB-2.

β-Amyrin acetate (**17**), *dryocrassyl acetate* (**6**) *and multiflorenyl*

acetate (**25**). Fraction NA-2 after removing **16** and **21** was a mixture of some pentacyclic and tetracyclic triterpenyl acetates, and aliphatic compounds. Rechromatography on $AgNO_3$–Si gel (hexane–C_6H_6, 7:3–1:1) followed by recrystallization from Me_2CO gave the following compounds. Compound **17**, colorless plates, 19 mg. IR ν_{max}^{KBr} cm^{-1}: 1735, 1249, 1027; 3030, 1657, 822, 811. EIMS *m/z* (rel. int.): 468 $[M]^+$ (4), 453 (2), 218 (100), 203 (36), 189 (15). (Found: C, 81.76; H, 11.01. $C_{32}H_{52}O_2$ requires: C, 81.99; H, 11.18%.) 100 mg from NB-2 and 300 mg from NC-2. (lit. [19] mp 241°, $[\alpha]_D$ +85°. Compound **6**, colorless plates, 11 mg. IR ν_{max}^{KBr} cm^{-1}: 1730, 1226, 1030. EIMS *m/z* (rel. int.): 470 $[M]^+$ (8), 455 (5), 410 (4), 395 (4), 369 (11), 249 (50), 191 (100), 189 (90). 20 mg from NB-2 and a trace from NC-2. (lit. [5] mp 196–198°, $[\alpha]_D$ +58°). Compound **25**, colorless needles, 50 mg. IR ν_{max}^{KBr} cm^{-1}: 1733, 1252, 1025; 3060, 1658, 837, 821. EIMS *m/z* (rel. int.): 468 $[M]^+$ (12), 453 (11), 393 (8), 301 (25), 289 (13), 262 (100), 241 (24), 229 (38), 218 (22), 205 (64), 204 (27), 203 (29), 202 (44), 191 (18), 189 (17), 187 (25). (Found: C, 82.13; H, 11.18. $C_{32}H_{52}O_2$ requires: C, 81.99; H, 11.18%.) (lit. [12] mp 227–228°, $[\alpha]_D$ ±0°).

16-*Oxo-taraxer*-14-*ene* (**20**) *and oleana*-11,13(18)-*dien*-3β-*yl acetate* (**18**). After removing the crystalline acetate from NA-3, the mother liquor was chromatographed on Al_2O_3 (grade III, hexane–C_6D_6, 9:1) followed by recrystallization from Me_2CO to give 10 mg **20** (colorless needles). IR ν_{max}^{KBr} cm^{-1}: 1663; 1613, 849, 838, 827. EIMS *m/z* (rel. int.): 424 $[M]^+$ (16), 409 (4), 300 (100), 285 (7), 232 (22), 219 (45), 191 (12). (Found: C, 85.10; H, 11.29. $C_{30}H_{48}O$ requires: C, 84.84; H, 11.39%.) 5 mg from F-4. Elution of the column with hexane–C_6H_6 (8:2) followed by recrystallization from Me_2CO afforded 10 mg **18** (colorless needles). IR ν_{max}^{KBr} cm^{-1}: 1731, 1239, 1021; 3050, 1652, 831, 818, 812, 755. EIMS *m/z* (rel. int.): 466 $[M]^+$ (100), 451 (22), 406 (4), 391 (16), 323 (4), 271 (7), 270 (8), 255 (16), 243 (6), 241 (6), 229 (26), 215 (28), 205 (6), 204 (21), 203 (41), 202 (12), 191 (5), 189 (24). UV λ_{max}^{EtOH} nm (ε): 241 (22 000), 251 (26 000), 260 (19 600). A trace from NB-3. (lit. [20] mp 228–229°, $[\alpha]_D$ −62°).

22-*Hydroxyhopane* (**7**) *and dryocrassol* (**5**). Fraction NA-4 was chromatographed on Si gel to give **7** (hexane–C_6H_6, 7:3) and **5** (hexane–C_6H_6, 1:1). Compound **7**, 5 mg, colorless needles (Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3610, 3452, 1031. EIMS *m/z* (rel. int.): 428 $[M]^+$ (4), 413 (3), 410 (5), 395 (5), 369 (4), 207 (30), 205 (8), 203 (10), 191 (100), 189 (81), 177 (11), 163 (18). 13 mg from NC-3 and a trace from NB-3. (lit. [6] mp 254–256°, $[\alpha]_D$ +44.5°). Compound **5**, 5 mg, colorless needles (Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3350, 1026. EIMS *m/z* (rel. int.): 428 $[M]^+$ (4), 413 (5), 369 (8), 207 (100), 191 (64), 189 (10). A trace from NA-3 and a trace from NC-3. (lit. [5] mp 245–247°, $[\alpha]_D$ +68°). Tetracyclic triterpenoidal and aliphatic alcohols were also present in this fraction.

Sterol mixture. Fraction NA-6 was chromatographed on Al_2O_3 (grade III) to give a sterol mixture, 1.20 g, mp 129–134° (MeOH). RR_ts 2.38, 2.50 and 2.92.

Oxidation of taraxer-14-*ene* (**19**). Compound **19** (300 mg) was treated with CrO_3–*t*-butylate (1.2 ml) in C_6H_6 (20 ml) at 50° for 15 hr. The products were chromatographed on Florisil and the C_6H_6 elute was purified on Si gel TLC to give 16-oxotaraxer-14-ene, 10 mg, mp 290°, RR_t 2.95. IR ν_{max}^{KBr} cm^{-1}: 1663; 1613, 835.

7-Oxo-taraxer-14-ene (**28**). (a) Compound **21** (6 mg) was treated with CrO_3–pyridine for 24 hr at room temp. The product was separated by prep. TLC to give 5 mg **28** (Me_2CO), mp 242–243°, RR_t 2.19. (b) Compound **24** (700 mg) was treated with CrO_3 (160 mg in 20 ml HOAc) in CH_2Cl_2 (6.4 ml), HOAc (32 ml) and C_6H_6 (20 ml) for 24 hr at room temp. The products were chromatographed on Florisil and then $AgNO_3$–Si gel to give 8 mg **28**, mp 241–243° (Me_2CO), $[\alpha]_D^{23}$ −18.6°, RR_t 2.19. IR ν_{max}^{KBr} cm^{-1}: 1682; 823. EIMS 300 eV *m/z* (rel. int.): 424 $[M]^+$ (26), 409 (41), 391 (31), 300 (18), 285 (18), 267 (55), 243 (18), 220 (58), 205 (44), 189 (24), 123 (100).

Multiflora-7,9(11)-*diene* (**30**). Compound **24** (50 mg) was treated with SeO_2 (50 mg) in HOAc (10 ml) for 4 hr at 100°. The products were chromatographed on neutral Al_2O_3 to give 30 mg **30**, mp 122–123° (Me_2CO), RR_t 1.79. IR ν_{max}^{KBr} cm^{-1}: 3040, 1621, 816, 797. UV λ_{max}^{EtOH} nm (ε): 228 (12 300), 240 (14 500), 248 (9200). EIMS *m/z* (rel. int.): 408 $[M]^+$ (100), 393 (24), 284 (10), 255 (59), 241 (12), 229 (27), 205 (21).

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