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FERN CONSTITUENTS: TRITERPENOIDS ISOLATED FROM *POLYPODIUM VULGARE, P. FAURIEI* AND *P. VIRGINIANUM*

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Key Word Index—*Polypodium vulgare*; *P. fauriei*; *P. virginianum*; Polypodiaceae; rhizomes; hop-22(29)-ene; isohop-22(29)-ene; fernenes; serrat-14-ene; dammaradienes; eupha-7,21-diene; cycloartanoid; cyclopodmenyl acetate.

Abstract—The fresh rhizomes of *Polypodium vulgare*, *P. fauriei* and *P. virginianum* collected in Japan gave, in addition to 30 known compounds, three new triterpenoids; dammara-17,21-diene, cyclopodmenyl acetate and 21αH-hopan-22-ol. The structures were elucidated by their physical data and chemical correlations.

INTRODUCTION

Common polypody, *Polypodium vulgare* L. (Ooezo-denda in Japanese) is widely distributed in Europe, Asia and North America. Triterpenoid hydrocarbons [1], triterpenoid alcohols of the cycloartane group [2], ecdysones [3] and a sweet glycoside, osladin [4], have previously been reported from this fern of European origin. In Japan, Ooezo-denda is only found in Oki Island (Shimane prefecture) and at Hachinohe City (Aomori prefecture) as very small colonies.* This paper deals mainly with triterpenoid constituents which were isolated and identified from *Polypodium vulgare* and two other Japanese related ferns, *P. fauriei* Christ (Oshaguji-denda) and so-called *P. virginianum* L. (Ezo-denda in Japanese). Furthermore, the structures of a new dammarane-type hydrocarbon and a new cycloartane type triterpenoid having a C_{33} skeleton were established by their physical data and chemical correlation to known compounds.

RESULTS AND DISCUSSION

The fresh rhizomes of *P. vulgare* (Pv), *P. fauriei* (Pf) and *P. virginianum* (Pi) collected in Japan were extracted with *n*-hexane as described in the Experimental, and extracts of 5.90 g (Pv), 2.41 g (Pf) and 3.54 g (Pi) were obtained. The TLC patterns of the three extracts closely resembled each other, including remarkable spots of triterpenoid hydrocarbons, esters and triacyl glyceride. Each extract was chromatographed through silica gel to give the following fractions: hydrocarbons from *n*-hexane eluates, fatty acid esters from *n*-hexane–benzene (9:1) eluates, acetates from *n*-hexane–benzene (1:1) eluates and triacyl glyceride from benzene eluates. All triterpenoids isolated or identified from the various fractions of each material are listed in Table 1 with their RR_t and yields.

Several peaks were detected in the GC of pentacyclic triterpenoid hydrocarbon fraction from each material. The hydrocarbon mixtures of the extracts were repeatedly purified through $AgNO_3$–silica gel chromatography. Six compounds were identified as hop-22(29)-ene (**1**) [5], isohop-22(29)-ene (**2**) [6], hop-17(21)-ene (**3**) [5], neohop-13(18)-ene (**4**) [5], fern-7-ene (**5**) [5], fern-8-ene (**6**) [5] and fern-9(11)-ene (**7**) [5], which belong to triterpenoid hydrocarbons of the hopane and migrated hopane groups except for **2** being a member of isohopane group. These compounds, except for **2**, were characteristic triterpenoid hydrocarbons distributed widely in the ferns. In addition, serrat-14-ene (**8**) [7] was obtained from all of the three species and *Polypodium polypodioides* (L.) Watt. [8], but not from more than a hundred species of ferns, including *Polypodium someyae* Yatabe [9], *P. niponicum* Mett., *P. formosanum* Baker [5] and *P. amamianum* Tagawa [10]. All pentacyclic triterpenoid hydrocarbons were identified by comparison of their mp, IR and mass spectra with those of authentic samples.

Four compounds were obtained as oily substances from the fraction of the tetracyclic triterpenoid hydrocarbons. Three of them were identified as dammara-18(28),21-diene (**9**) [11], dammara-13(17),21-diene (**10**) [12] and eupha-7,21-diene (**11**) [12] by comparison of their ^{1}H NMR and mass spectra with those of authentic samples.

Compound **12**, mp 53–55°, RR_t 1.73, $[\alpha]_D^{23}$ +25° ($CHCl_3$; *c* 0.5), isolated from Pf, was a triterpenoid hydrocarbon of molecular formula $C_{30}H_{50}$ (its high resolution MS, $[M]^+$ at *m/z* 410.3906). The mass spectrum of **12** gave main fragment peaks of *m/z* 395, 341, 218, 191 and 69, which resembled those of **10** [11] (Scheme 1). However, unlike compound **10**, the fragment peaks of *m/z* 299 and 297 in the mass spectrum of **12** were not obvious. The ^{1}H NMR spectrum of **12** showed signals for eight methyl groups (δ0.804 × 2, 0.848 × 2, 0.974, 1.598, 1.666, 1.692) and one olefinic proton (δ5.120, *br t*, *J* = 7.0 Hz). The methyl proton signals of C-23, C-24 and C-25 were assigned by comparison with those of **9** and **10**. Thus, it is

*The dried rhizomes of *Polypodium vulgare* in Oki island has also been studied, and no other compounds than reported in this paper were obtained.

Table 1. Triterpenoids isolated or identified from the rhizomes of *Polypodium vulgare*, *P. fauriei* and *P. virginianum*

Compounds	RR_t	Yield (% × 10^3)		
		P. vulgare	*P. fauriei*	*P. virginianum*
Hop-22(29)-ene (**1**)	2.61	3.5	13.0	2.9
21αH-Hop-22(29)-ene (**2**)	1.99	2.5		
Hop-17(21)-ene (**3**)	1.67	3.0	0.6	1.8
Neohop-13(18)-ene (**4**)	1.91	3.8		
Fern-7-ene (**5**)	2.26	3.0	6.1	+
Fern-8-ene (**6**)	1.91		0.6	+
Fern-9(11)-ene (**7**)	2.00	80.0	121.0	70.0
Serrat-14-ene (**8**)	2.34	17.5	7.3	0.9
Dammara-18(28),21-diene (**9**)	1.53	+	+	1.2
Dammara-13(17),21-diene (**10**)	1.20	+	10.9	+
Eupha-7,21-diene (**11**)	1.62	1.8	4.5	+
Dammara-17,21-diene (**12**)	1.73	+	19.7	+
α-Polypodatetraene (**13**)	1.12	+	10.6	+
Onoceranoxide (**14**)	2.22	0.5	3.0	+
31-Norcycloartanol (**15**)	2.37	+	+	+
Cycloartanol (**16**)	2.80	+	+	+
Cyclolaudenol (**17**)	3.54	+	+	+
Cycloartenol (**18**)	3.10	+	+	+
Cyclomargenol (**19**)	4.36	+	+	+
31-Norcycloartanyl acetate (**20**)	3.10	13.0	1.4	1.2
Cycloartanyl acetate (**21**)	3.74	34.0	3.6	4.0
Cyclolaudenyl acetate (**22**)	4.49	36.0	1.7	7.6
Cycloartenyl acetate (**23**)	4.03	3.0	0.3	0.9
Cyclomargemyl acetate (**24**)	5.63	1.0	0.3	0.4
Dryocrassyl acetate (**25**)	6.41	3.0	0.1	0.3
Cyclopodomenyl acetate (**26**)	7.10	+		
Cycloartanone (**27**)	2.72	1.3	+	+
Cyclolaudenone (**28**)	3.30	+	+	+
Cycloartenone (**29**)	2.93	+	+	+
Cyclomargenone (**30**)	4.07	+	+	+
21αH-22-Hydroxyhopane (**31**)		+		10.0

Yield: +, Presence of the compound was confirmed, but its yield was unknown.

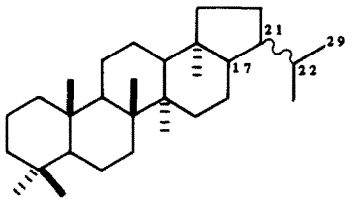
suggested that **12** has the same A, B and C ring system and terminal parts of the side chain as present in **10**. The presence of one olefinic proton signal and three olefinic methyl proton signals suggested an extra tetrasubstituted double bond and a methyl group in the side chain of **12**. The signals of two singlet methyl groups at δ1.598 and 1.666 were assigned as those of C-29 and C-30, respectively, having a double bond at C-17. This situation was only satisfied in the dammarane skeleton. The ^{13}C NMR signals of **12** were assigned by comparison with those of polypodatetraene (**13**) [13] and 18-hydroxydammar-21-ene (**32**) [11] (Table 2). The geometry of the side chain was confirmed by NOE difference spectrum measurement. The NOE enhancements which resulted from irradiation of methyl protons at C-26, C-28 and C-29 are shown in Scheme 1. In particular, the NOE enhancement between the H-12β and C-28 methyl protons gave clear evidence of the 17*E*-structure in **12**. Thus, **12** is (17*E*)-dammara-17,21-diene, and the structure was finally confirmed by comparison with the sample derived from **32** by dehydration with $POCl_3$.

α-Polypodatetraene (**13**) was detected as the most polar oily hydrocarbon, and was identified by comparison of its IR, ^{1}H NMR and mass spectra with those of an authentic sample from our laboratory [13].

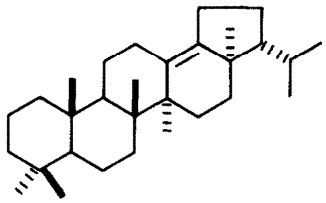
Compound **14** was obtained as a trace component from *n*-hexane–C_6H_6 (7:3) eluates of Pv. It was identified as onoceranoxide by comparison of the ^{1}H NMR and mass spectra with those of an authentic sample isolated from *Lemmaphyllum microphyllum* [7].

The *n*-hexane to *n*-hexane–benzene (7:3) eluates contained the principal components of each plant extract. The oily fractions were found to contain a trace of less polar compound (ester I) on TLC. This waxy ester I was separated by repeated chromatography. The ^{1}H NMR spectrum of ester I showed proton signals of five methyls (δ0.682, 0.846, 0.894, 0.958, 1.100), aliphatic methylene (δ1.258) and one olefinic proton (δ5.344). Alcohol and acid components of ester I were identified by hydrolysis with 5% KOH to give sitosterol accompanied by a trace of campesterol and palmitic acid. Thus, ester I is phytosteryl palmitate mainly constituted by the sitosterol ester.

A principal component of these ferns was an oily ester (ester II), which gave the ^{1}H NMR signals of a cyclopropane (δ0.140, 0.390; 0.328, 0.444), four methyl groups (δ0.838, 0.890, 0.958, 1.638), an aliphatic methylene



1 21βH $\Delta^{22(29)}$
2 21αH $\Delta^{22(29)}$
3 $\Delta^{17(21)}$



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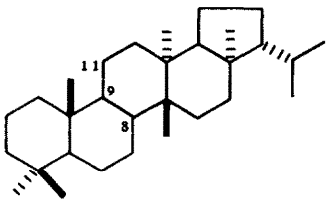
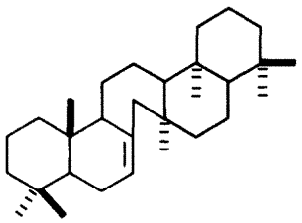


5 Δ^{7}
6 Δ^{8}
7 $\Delta^{9(11)}$



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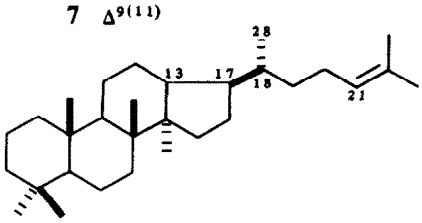
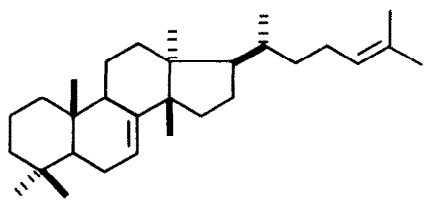


9 $\Delta^{18(28)21}$
10 $\Delta^{13(17)21}$



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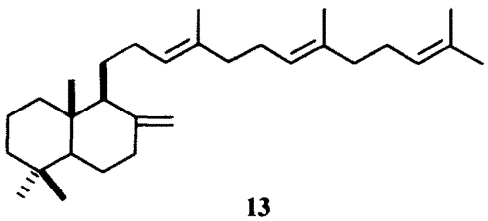

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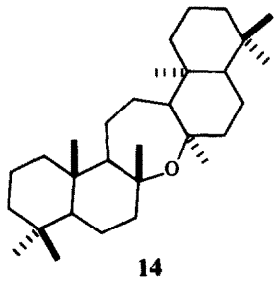

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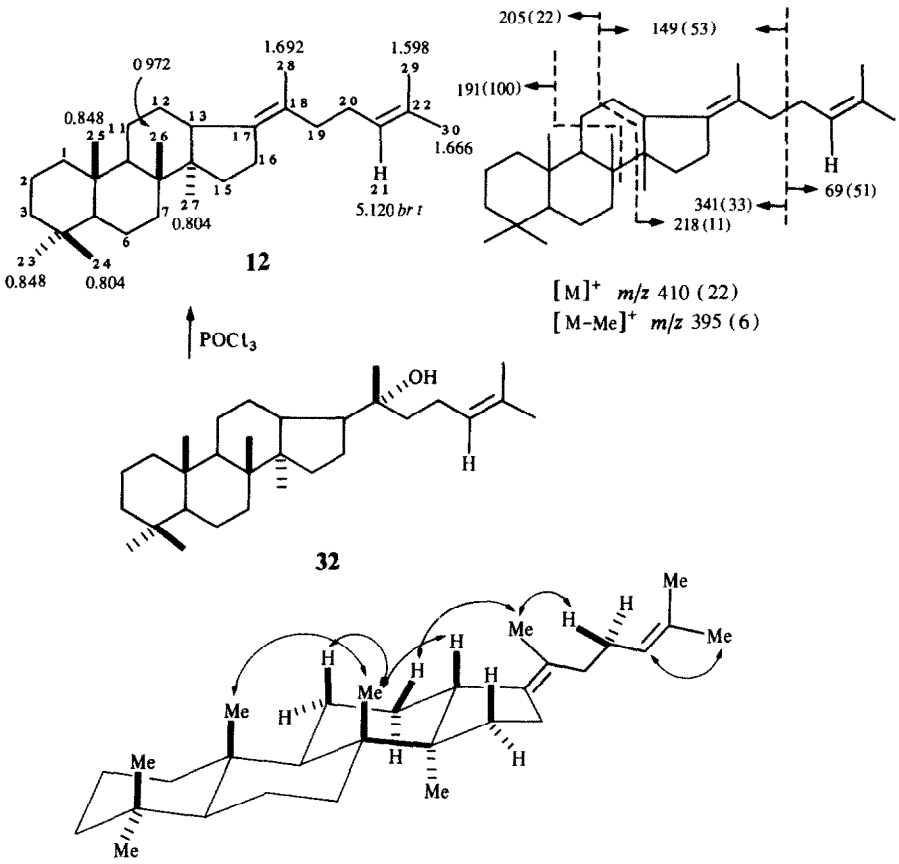
Table 2. ^{13}C NMR spectral data of dammara-17,21-diene (**12**) and related compounds (**13** and **32**)

C	12	13	32	C	12	13	32	C	12	13	32
1	40.7	39.1	40.6	11	21.5	23.8	21.3	21	124.7	124.4	124.7
2	18.8	19.4	18.9	12	27.2	29.7	25.3	22	131.0	131.1	131.3
3	42.2	42.3	42.1	13	47.1	125.1	42.1	23	33.4	33.6	33.4
4	33.4	33.6	33.3	14	49.7	134.8	50.0	24	21.5	21.7	21.5
5	57.1	55.6	56.9	15	30.3	39.8	31.0	25	15.7	14.5	15.6
6	18.6	24.5	18.6	16	28.8	26.8	27.5	26	16.4	106.1	16.4
7	35.6	38.4	35.2	17	125.8	124.3	49.6	27	16.5	16.0	16.1
8	40.1	148.4	40.6	18	136.6	134.8	75.7	28	17.6	16.0	23.6
9	50.7	56.2	50.7	19	37.4	39.8	41.8	29	17.6	17.1	17.6
10	37.2	39.6	37.4	20	26.1	26.8	22.3	30	25.7	25.7	25.7



Scheme 1.

(δ1.314), a terminal methylene (δ4.654) and an olefine (δ5.342) in its ^{1}H NMR spectrum. The molecular peaks in the mass spectrum of the ester II were found at *m/z* 702, 690, 688 and 676. The mixture was hydrolysed to give alcohols and an acid. The alcohol fraction contained three main peaks on GC, which were identified as 31-norcycloartanol (**15**) [14], cycloartanol (**16**) [15] and cyclolaudenol (**17**) [16] by GC-MS. Another two co-eluting compounds were identified as cycloartenol (**18**) [15] and cyclomargenol (**19**) [16] by GC-MS. The acid fraction was identified as linoleic acid by the ^{1}H NMR and mass spectra comparing with those of the respective methyl ester. The compositions of the alcohols in each extract are shown in Table 3.

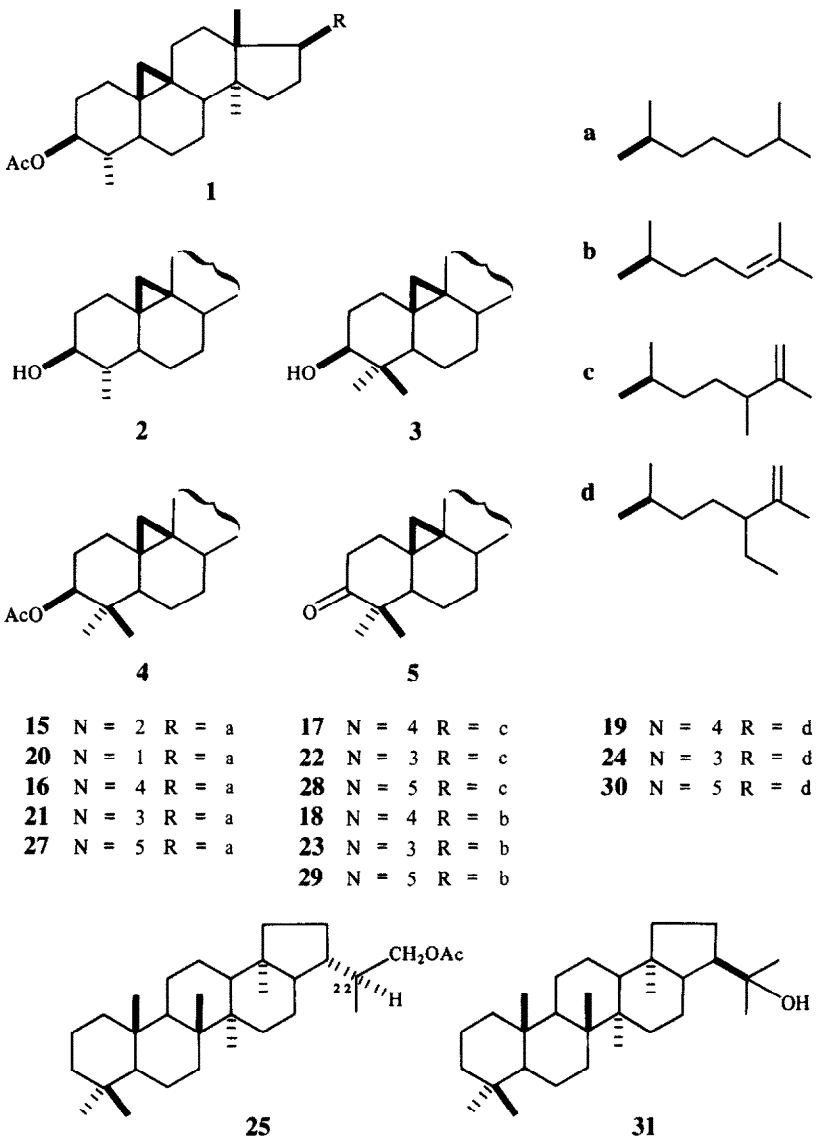
The GC patterns of the *n*-hexane–benzene (1 : 1) eluates of each extract resembled each other, composition ratios of the components and the main mass spectral fragments are listed in Tables 3 and 4. On the base of RR_t and the main fragment peaks, the compounds were identified as five cycloartanoid acetates: 31-norcycloartanyl acetate (**20**) [14], cycloartanyl acetate (**21**) [16], cyclolaudenyl acetate (**22**) [16], cycloartenyl acetate (**23**) [14], cyclomargenyl acetate (**24**) [16] and a pentacyclic triterpenoid acetate, dryocrassyl acetate (**25**) [17] of the hopane group, by comparison of its GC-MS with those of authentic samples.

A minor acetate (**26**), mp 116–118°, $[\alpha]_D^{23}$ +52° ($CHCl_3$; *c* 0.2), ν_{max}^{KBr} cm^{-1}: 3080, 1020; 1630, 890; 1730, 1245, named cyclopodmenyl acetate, was isolated from the acetate fraction of Pv by HPLC. This compound was suggested to have a cyclopropane ring, a terminal methylene and an acetate group in the molecule by its IR spectrum. The molecular formula was found to be $C_{35}H_{58}O_2$ by the high resolution mass spectrum ($[M]^+$ as *m/z* 510.4421). Comparison of its mass spectral fragmentation pattern with those of **22** and cyclobalanyl acetate (24,24-dimethyl-cycloart-25-en-3β-yl acetate) (**33**) [16] clearly indicated that these three compounds have the same structure in the cyclic part of the molecule and have a different side chain, namely C_9, C_{10} and C_{11} (Table 4). Comparison of the ^{1}H NMR signals with those of **22**, **24** and **26** also indicated that these three compounds have the same structure in the cyclic part. Compound **26** was found to have an ethyl group attached to the olefinic carbon, because the ethyl signals appeared at lower field. This was also confirmed by irradiation of the methylene signal at δ1.990 (*br q*, J = 7.4 Hz), which resulted in the signal of the C-33 methyl at δ1.047 (*t*, J = 7.4 Hz) being a singlet and also the proton signals at δ4.762 (*br d*) and δ4.785 (*dt*, C-26) being sharper doublets (Table 5). The remarkable differences in the values of C-25, C-27 and C-33 in the ^{13}C chemical shifts of **26** and **33** also definitely indicated that **26** has an extra methyl (C-33) at C-27 (Table 6). From the coincidence of ^{1}H and ^{13}C chemical shifts of **22** and **33**, it was concluded that **26** has the same stereostructure as **33**, including the configuration at C-20 [18]. Thus, cyclopodmenyl acetate is 24,24,27-trimethyl-9,19-cyclolanost-25-en-3β-yl acetate.

Table 3. The composition ratio (%) of cycloartanoid homologues in esters, alcohols and ketones

	Fatty acid ester			Acetate			Free alcohol and ketone						
	Pv	Pf	Pi	Pv	Pf	Pi	Pv	Pf	Pi		Pv	Pf	Pi
31-Norcycloartanol (**15**)	19	24	18	13	19	8	33	43	28				
Cycloartanol (**16**)	45	39	35	34	47	28	8	9	13	(**27**)	29	+	20
Cyclolaudenol (**17**)	27	23	36	36	23	52	4	2	6	(**28**)	51	+	57
Cycloartenol (**18**)	5	9	9	3	3	6	48	46	53	(**29**)	9	+	15
Cyclomargenol (**19**)	4	4	+	4	2	3	+			(**30**)	5	+	7

Pv: *Polypodium vulgare*; Pf: *P. fauriei*; Pi: *P. virginianum*.



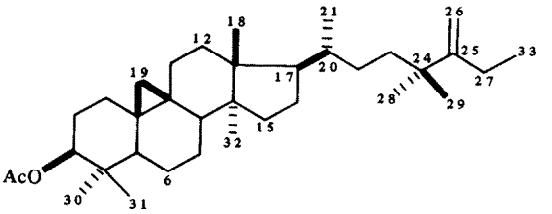
Cycloartanone (**27**) [14] from Pv was obtained by repeated purification with silica gel chromatography using *n*-hexane–benzene (1:1), and identified by comparison with the sample derived from cycloartanol [15] by mp, IR and mass spectra. Furthermore, it was supposed by GC-MS that cyclolaudenone (**28**) [16], cycloartenone (**29**) [15] and cyclomargenone (**30**) [16] coexist with **27**. The composition ratio and other physical data (MS) for three extracts are listed in Tables 3 and 4.

Triterpenoid alcohols in the three extracts were observed in small amount in the *n*-hexane–benzene (1:1) and benzene eluates, and the GC patterns of these

Table 4. Main mass spectral fragment ion peaks (*m/z*) of cycloartanoid homologues

Compounds	$[M]^+$	$[M-Me]^+$	$[M-H_2O]^+$ $[M-HOAc]^+$	a	b	c	d	e	f	g	h
31-Norcycloartanol (**15**)	414	399	396		288	283	241^2	229	215	203	175
31-Norcycloartanyl acetate (**20**)	456	441	396	341	288	283^1	241^1	229	215	203	175
Cycloartanyl acetate (**21**)	470	455	410	341	288	297^1	255^1	229	215	203	175
Cycloartanone (**27**)	426	411		341	288	313^3				203	175
Cycloartanol (**16**)	428	413	410	341	288	297^2	255^2	229	215	203	175
Cycloartenyl acetate (**23**)	468	453	408	339	286	297^1	255^1	229	215	203	175
Cycloartenone (**29**)	424	409		339	286	313^3		229	215	203	175
Cycloartenol (**18**)	426	411	408	339	286	297^2	255^2	229		203	175
Cyclolaudenyl acetate (**22**)	482	467	422	353	300	297^1	255^1	229	215	203	175
Cyclolaudenone (**28**)	438	423		353	300	313^3		229	215	203	175
Cyclolaudenol (**17**)	440	425	422	353	300	297^2	255^2	229	215	203	175
Cyclomargenyl acetate (**24**)	496	481	436	367	314	297^1	255^1	229	215	203	175
Cyclomargenone (**31**)	452	437			314	313^3		229	215	203	175
Cyclomargenol (**19**)	454	439	436	367	314	297^2	255^2	229	215	203	175
Cyclopodmenyl acetate (**26**)	510	495	450	381	328	297^1	255^1	229	215	203	175

c^1: $c-HOAc-H_2$; c^2: $c-H_2O-H_2$; d^1: $d-HOAc$; d^2: $d-H_2O$.



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fractions were very similar. The components were identified as 31-norcycloartanol (**15**) [14], cycloartanol (**16**) [15], cyclolaudenol (**18**) [16] and cyclomargenol (**19**) [16] by GC-MS. The composition ratios are also listed in Table 3.

As reported earlier, observation of the main mass spectral fragments and RR_t of cycloartanoid homologues is very useful for their structural identification. Characteristic fragments due to opening of the 9,19-cyclopropane ring and loss of the side chain and two protons from the molecule [16] were observed at *m/z* 297, 283; 286, 288, 300, 314 and 328 giving important data for structural elucidation (Table 4).

Compound **31**, mp 225–226°, $[\alpha]_D^{23}$ +21.3° ($CHCl_3$; *c* 0.4), IR ν_{max}^{KBr} cm^{-1}: 3480, 1135, $[M]^+$ at *m/z* 428.4015 ($C_{30}H_{52}O$), EIMS *m/z* 428 (10), 410 (16), 395 (24), 367 (4), 207 (20), 203 (22), 191 (100), 189 (87), was obtained from Pv. Compound **31** was identified as 22-hydroxyisohopane by comparison of its ^1H NMR and mass spectra with those of the authentic synthetic sample [19]. This is the first example of the occurrence of **31** from a natural source.

Another major oily substance, IR ν_{max}^{film} cm^{-1}: 1708, was isolated from benzene eluates of the extracts of fresh materials. The molecular peak was found at *m/z* 882 in the FD-MS, and this substance was established to be the glyceride of unsaturated fatty acids by ^1H and ^{13}C NMR data as follows. The ^1H NMR spectrum showed some characteristic proton signals of a methyl group (δ0.882, *t*), aliphatic methylene (δ1.270), methylenes adjacent to a double bond, a carbonyl and two double bonds (δ2.019, 2.350, 2.769, respectively). Further signals of olefinic protons (δ5.346, *m*), and protons of the glycerol part (δ4.189 and 4.240) were also recorded. The ^{13}C NMR spectrum showed characteristic carbon signals due to the carbons adjacent to oxygen (δ62.1, 69.0), olefinic carbon (δ127.9, 128.1, 130.0, 130.2) and carbonyl carbon (δ172.8, 173.2). Three signals of the glycerol part were confirmed for C-1 (δ62.1), C-2 (δ69.1) and C-3 (δ62.1) by comparison with those of commercial butylin. The fatty acid moieties were considered to be linoleic acid from the FD-MS. The chemical shifts of proton and carbon were assigned by comparison with those of linoleic and oleic acid standards. Distinction between linoleic acid and oleic acid, which are expected to coexist, was achieved by identification of two signals; δ25.7 at C-11 of linoleic acid and δ129.7 at C-9 of oleic acid. As a result, the triacyl glyceride of each extract was identified as trilinoleyl glyceride containing a trace of oleyl residue, but further details of the structure were not studied.

The chemotaxonomical features of the three Japanese *Polypodium* species reported in this paper can be concluded to be closely related: (i) very similar patterns of triterpenoid hydrocarbons were observed. (ii) In particu-

Table 5. ^{1}H NMR chemical shifts of cyclopodmenyl acetate (**26**) and related compounds

H	**26**	**22**	**33**	H	**26**	**22**	**33**
30	0.885	0.887	0.887	28/29	1.019	0.997 *d*	1.016
31	0.845	0.845	0.845			(6.7)	1.016
19	0.333 *d*	0.336 *d*	0.335 *d*	26	4.762 *d*	4.665 *m*	4.659 *br d*
	(4.1)	(4.1)	(4.1)		(1.2)	($W_{2/H}$=2.5)	(2.0)
	0.570 *d*	0.571 *d*	0.572 *d*		4.785 *dt*	—	4.722 *br q*
	(4.1)	(4.1)	(4.1)		(1.2, 1.0)		(1.0, 2.0)
32	0.885	0.887	0.887	27	1.990 *bq*	1.639	1.685 *d*
18	0.943	0.952	0.948		(7.4)		(1.0)
21	0.841 *d*	0.845 *d*	0.854 *d*	33	1.047 *t*	—	—
	(6.3)	(6.3)	(6.3)		(7.4)		
				3α	4.564 *dd*	4.562 *dd*	4.563 *dd*
					(10.4, 5.0)	(10.5, 5.2)	(10.6, 5.2)

J (in Hz) in parentheses.

Table 6. ^{13}C NMR spectral data of cyclopodmenyl acetate (**26**) and the related compound **33**

C	**26**	**33**	C	**26**	**33**	C	**26**	**33**
1	31.9	31.9	13	45.3	45.3	25	157.8	152.4
2	26.9	26.8	14	48.9	48.8	26	106.5	109.3
3	80.7	80.7	15	32.9	32.9	27	23.4	19.4
4	39.5	39.5	16	26.6	26.6	28	27.8	27.5
5	47.3	47.2	17	52.2	52.2	29	27.5	27.3
6	21.0	20.9	18	17.9	17.9	30	25.5	25.4
7	28.1	28.1	19	29.8	29.8	31	15.2	15.2
8	47.9	47.8	20	36.7	36.6	32	19.3	19.3
9	20.2	20.2	21	18.5	18.5	33	13.1	—
10	25.9	25.8	22	30.9	30.8	Acetyl	170.9	171.0
11	26.1	26.0	23	37.7	37.4		21.3	21.3
12	35.6	35.6	24	39.2	38.8			

Table 7. ^{1}H and ^{13}C NMR chemical shifts of trilinoleyl glyceride

H, C	^{1}H	^{13}C	H, C	H	^{13}C
α	4.142 *dd* (11.9, 5.8)	62.1	8	2.019 *m*	27.2
	4.298 *dd* (11.9, 4.2)		9	5.350 *m*	130.0
β	5.266 *m*	69.0	10	5.350 *m*	128.1
α′	4.142 *dd* (11.9, 5.8)	62.1	11	2.769	25.7
	4.298 *dd* (11.9, 4.2)		12	5.350 *m*	127.9
1	—	173.2‡	13	5.350 *m*	130.2
2	2.350 *t* (7.0)	34.0	14	2.019 *m*	27.2
3	*	24.9	15	†	29.2
4	*	29.2	16	*	31.9
5	*	29.1	17	*	22.6
6	*	29.1	18	0.889 *t* (6.6)	14.1
7	†	29.7			

*,†Proton signals were observed at 1.30–1.31 and 1.25–1.27, respectively.
‡^{13}C signal of C-1 attached to C-β was observed at 172.8.

lar serratene was found in the three species together with tetracyclic and pentacyclic triterpenoid hydrocarbons. (iii) The composition ratios of triterpenoid alcohol constituting the long chain fatty acid esters and acetates were almost the same. (iv) Trilinoleyl glyceride was obtained in a remarkable amount.

It is noteworthy that the triterpenoid constituents of *Polypodium vulgare* and its related species (this paper,

genuine *Polypodium sensu stricto*) and the other group such as *P. niponicum* and *P. formosanum* (*Polypodiodes niponica* Ching and *P. formosana* Ching) [5, 16] were quite different from the biogenetic point of view. The chemotaxonomical studies on these ferns will be published elsewhere.

EXPERIMENTAL

General. Mps: uncorr. $[\alpha]_D$s were observed in $CDCl_3$ soln (*c* 0.2–0.5) at 23°. IR spectra were recorded for KBr pellets. ^1H and ^{13}C NMR spectra were taken in 270 MHz (400 MHz for NOE) 68 MHz in $CDCl_3$ soln. TMS was used as an int. standard and chemical shifts are given as δ-values (ppm). MS were recorded by direct inlet at 70 eV unless otherwise stated, and the relative intensities of peaks are reported with reference to the most intense peak higher than *m/z* 100. TLC was carried out on silica gel (Merck 5721) with *n*-C_6H_{14}–EtOAc solvent system, the spray reagent was H_2SO_4. GC was performed on a 1 m glass column containing Chromosolve G HP with 1.4% SE-30 at 260°. Cholestane was used as an int. reference and its R_t was set at 3.0 min. HPLC was performed on a C-18 reverse phase column (detected by RI) with $CHCl_3$–MeOH–H_2O (38:7:5) as the eluent

Plant material. Polypodium vulgare (Pv) was collected at Hachinohe City, Aomori Prefecture, in Oct. 1984 (841003); *P. fauriei* (Pi) at 2 Gome of Mt. Fuji, Yamanashi prefecture, in Oct. 1982 (821004); *P. virginianum* (Pi) on the shore of Kussyaro lake, Hokkaido, in July 1984 (840701). The voucher specimens are deposited in the Herbarium of Showa College of Pharmaceutical Sciences, Tokyo. *Polypodium virginianum* of the type locality was found to be a tetraploid ($2n = 148$) and the so-called *P. virginianum* of Hokkaido was a diploid ($2n = 74$). Thus, the name *P. virginianum* for the material used may not be correct. Professor C. Haufler of Kansas University suggested to us the designation *P. sibiricum* Siplivinskij for the material, but we reserve this name until completing the chemical studies of *P. sibiricum* of Baical origin.

Extraction and separation. Cut material of the fresh rhizomes [400 g (Pv), 330 g (Pf) and 170 g (Pi)] was extracted with *n*-hexane to give extracts of 5.90 g (Pv), 2.41 g (Pf) and 3.54 g (Pi) being removed azeotropic H_2O, 295, 266 and 107 ml, respectively. The extracts were chromatographed on silica gel to give the following frs.

Fr.	Solvent	Pv	Pf	Pi	Main contents
1	*n*-C_6H_{14}	0.58 g	0.76 g	0.15 g	Hydrocarbons
2	*n*-C_6H_{14}–C_6H_6 (9:1)	1.94 g	0.53 g	1.13 g	Esters
3	*n*-C_6H_{14}–C_6H_6 (1:1)	0.39 g	0.06 g	0.11 g	Acetates
4	C_6H_6	1.65 g	0.62 g	1.78 g	Triacyl glyceride
5	Et_2O	0.71 g	0.35 g	0.24 g	Others

Triterpenoid hydrocarbons. Fr. 1 was chromatographed repeatedly on 20% $AgNO_3$–silica gel to give the following triterpenoid hydrocarbons in order of elution in the *n*-C_6H_{14} eluates.

Neohop-13(18)-*ene* (**4**). Plates (15 mg) (Me_2CO) from Pv. Mp 192–194°, 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).

Fern-8-*ene* (**6**). Plates (2 mg) (Me_2CO) from Pf. Mp 190–191°, IR ν_{max}^{KBr} cm^{-1}: 862, 857; EIMS *m/z* (rel. int.): 410 $[M]^+$ (31), 395 (95), 257 (17), 243 (100), 231 (17).

Fern-9(11)-*ene* (**7**). Plates (320 mg) (Me_2CO) from Pv. Mp 170–171°, IR ν_{max}^{KBr} cm^{-1}: 811, 799; EIMS *m/z* (rel. int.): 410 $[M]^+$ (32), 395 (86), 271 (4), 257 (20), 243 (100), 231 (16), 205 (8), 203 (7), 191 (9). 400 mg from Pf, 119 mg from Pi.

Fern-7-*ene* (**5**). Plates (20 mg) (Me_2CO) from Pf. Mp 211–212°, 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), 12 mg from Pv.

Serrat-14-*ene* (**8**). Plates (70 mg) (Me_2CO) from Pv. Mp 240–241°, IR ν_{max}^{KBr} cm^{-1}: 3030, 1655, 790; EIMS *m/z* (rel. int.): 410 $[M]^+$ (31), 395 (16), 218 (80), 204 (49), 203 (35), 191 (100), 24 mg from Pf and 5 mg from Pi.

Hop-17(21)-*ene* (**3**). Plates (12 mg) (Me_2CO) from Pv. Mp 183–185°, IR ν_{max}^{KBr} cm^{-1}: 851; EIMS *m/z* (rel. int.) 410 $[M]^+$ (45), 395 (31), 367 (100), 231 (76), 203 (13), 191 (76), 189 (51), 2 mg from Pf and 3 mg from Pi.

21α*H-Hop*-22(29)-*ene* (**2**). Needles (10 mg) (Me_2CO) from Pv. Mp 212–214°, IR ν_{max}^{KBr} cm^{-1}: 3049, 1643, 882; EIMS *m/z* (rel. int.): 410 $[M]^+$ (31), 395 (15), 191 (1), 189 (82).

Hop-22(29)-*ene* (**1**). Plates (43 mg) (Me_2CO) from Pf. Mp 210–211°, IR ν_{max}^{KBr} cm^{-1}: 3055, 1647, 1629, 887; EIMS *m/z* (rel. int.): 410 $[M]^+$ (19), 395 (10), 191 (100), 189 (94), 14 mg from Pv and 5 mg from Pi.

Eupha-7,21-*diene* (**11**). Oil (15 mg) from Pf, EIMS *m/z* (rel. int.): 410 $[M]^+$ (26), 395 (100), 297 (8), 257 (10), 243 (12), 203 (10), 191 (12), 189 (10), 69 (78). ^1H NMR ($CDCl_3$): δ0.845 (H-23), 0.882 (H-24), 0.745 (H-25), 0.987 (H-26), 0.818 (H-27), 0.850 (H-28), 1.603 (H-29), 1.684 (H-30), 5.239 (*ddd*, $J = 3.0$, 3.0, 3.7 Hz, H-7), 5.100 (*br t*, $J = 7.0$ Hz, H-21), 7 mg from Pv and a trace from Pi.

Dammara-13(17),21-*diene* (**10**). Oil (36 mg) from Pf. EIMS *m/z* (rel. int.): 410 $[M]^+$ (56), 395 (18), 299 (29), 297 (30), 218 (15), 205 (49), 191 (100), 69 (73); ^1H NMR ($CDCl_3$): δ0.848 (H-23), 0.801 (H-24), 0.848 (H-25), 0.848 (H-26), 1.071 (H-27), 0.919 (H-28), 1.590 (H-29), 1.686 (H-30), 5.100 (*br t*, $J = 7.0$ Hz, H-21).

Dammara-17,21-*diene* (**12**). Plates (65 mg) (Me_2CO) from Pf. A trace from Pi.

Dammara-18(28),21-*diene* (**9**). Oil (65 mg) from Pi. IR ν_{max}^{film} cm^{-1}: 3080, 1642, 886; EIMS *m/z* (rel. int.): 410 $[M]^+$ (23), 395 (3), 341 (3), 301 (4), 299 (7), 218 (18), 204 (30), 191 (100), 189 (34); ^1H NMR ($CDCl_3$): δ0.848 (H-23), 0.806 (H-24), 0.848 (H-25), 0.973 (H-26), 0.875 (H-27), 4.708, 4.728 (H-28), 1.612 (H-29), 1.686 (H-30), 5.130 (*br dd*, $J = 7.1$, 7.1 Hz, H-21).

Dehydration of compound **32**. A mixt. of pyridine (20 ml), compound **32** (320 mg) obtained from Dammar [9] and $POCl_3$ (4 ml) was left to stand for 3 hr at room temp., and then treated in the usual manner to give a solid (270 mg). The reaction products showed 4 main peaks [RR_t: 1.53 (38%), 1.60 (20%), 1.73 (19%), 2.46 (23%)] in its GC, one (RR_t 1.73) of which was repeatedly purified by $AgNO_3$–silica gel and HPLC [MeOH–$CHCl_3$, 4:1], and identified as **12** by ^1H NMR: δ0.848 (C-23), 0.806 (C-24), 0.848 (C-25), 0.975 (C-26), 0.806 (C-27), 1.692 (C-28), 1.598 (C-29), 1.660 (C-30).

α-*Polypodatetraene* (**13**). Oil (35 mg) from Pf. IR ν_{max}^{Nujol} cm^{-1}: 3080, 1643, 887; EIMS *m/z* (rel. int.): 410 $[M]^+$ (31), 395 (32), 341 (6), 273 (12), 205 (17), 204 (15), 191 (38), 137 (100), 69 (>100); ^1H NMR ($CDCl_3$): δ0.862 (H-23), 0.798 (H-24), 0.664 (H-25), 4.540, 4.882 (H-26), 1.568 (H-27), 1.606 (H-28), 1.606 (H-29), 1.684 (H-30), 5.10–5.12 (*br t*, $J = 6.3$ Hz, H-13, H-17, H-21), a trace from Pv and Pi.

Onoceranoxide (**14**). Plates (2 mg) (Me_2CO) from *n*-C_6H_{14}–C_6H_6 (7:3) of Pv, mp 226–227°, EIMS *m/z* (rel. int.): 413 $[M-Me]^+$ (2), 410 (8), 395 (6), 218 (15), 204 (40), 191 (100); ^1H NMR ($CDCl_3$): δ0.845 (C-23), 0.740 (C-24), 0.767 (H-25), 1.255 (H-26), 1.255 (H-27), 0.767 (H-28), 0.740 (H-29), 0.845 (H-30), 10 mg from Pf and a trace from Pi.

Isolation of ester I and ester II. Each ester fr. was chromatographed through silica gel to give ester I (35, 10 and 16 mg) from the *n*-C_6H_{14}–C_6H_6 (9:1) eluate and ester II (300, 400 and 443 mg) from *n*-C_6H_{14}–C_6H_6 (7:3) eluate of Pv, Pf and Pi respectively.

Alkaline treatments of ester I and II. A small amount of ester I and ester II from each sample was hydrolysed with 5% KOH by the usual method, and the resulting alcohols were purified through silica gel chromatography to give the compounds in the C_6H_6 eluate. Free acids from the H_2O layer were esterified with CH_2N_2 in the usual manner, and chromatographed through silica gel to give the Me-ester from *n*-C_6H_{14}–C_6H_6 (9:1) eluates. Sitosterol. RR_t: 2.83, EIMS *m/z*: 414 $[M]^+$. Contaminated peak: RR_t: 2.33; EIMS *m/z*: 400 $[M]^+$. Methyl palmitate, EIMS *m/z* (rel. int.): 270 $[M]^+$ (100), 241 (25), 239 (37), 227 (81), 213 (13), 199 (25), 185 (50). **15**. RR_t: 2.37; EIMS *m/z* (rel. int.): 414 $[M]^+$ (7), 399 (54), 396 (54), 381 (100), 288 (28), 283 (51). Compound **16** RR_t: 2.80; EIMS *m/z* (rel. int.): 428 $[M]^+$ (4), 413 (14), 410 (21), 395 (100), 341 (14), 367 (14), 288 (29). Compound **17** RR_t: 3.54; EIMS *m/z* (rel. int.): 440 $[M]^+$ (12), 425 (10), 422 (39), 407 (100), 379 (96), 353 (27), 300 (39), 297 (33). Compound **18** RR_t: 3.10; EIMS *m/z* (rel. int.): 426 $[M]^+$ (10), 411 (69), 408 (50), 393 (100), 297 (10). Compound **19** RR_t: 4.36; EIMS *m/z* (rel. int.): 454 $[M]^+$ (2), 439 (30), 436 (26), 421 (100), 367 (14), 314 (10), 297 (80). Methyl linoleate, EIMS *m/z* (rel. int.): 294 $[M]^+$ (100), 270 (9), 263 (34), 262 (40), 220 (19), 182 (11), 178 (27), 164 (54), 150 (55).

Acetates. *n*-Hexane–C_6H_6 (1:1) eluates of each material were purified repeatedly by silica gel chromatography to give acetate mixts, 350 mg (Pv), 55 mg (Pf) and 100 mg (Pi). Compound **20** RR_t: 3.10; EIMS *m/z* (rel. int.): 456 $[M]^+$ (9), 441 (9), 396 (100), 381 (76), 341 (15), 288 (21), 283 (31). Compound **21** RR_t: 3.74; EIMS *m/z* (rel. int.): 470 $[M]^+$ (17), 455 (13), 410 (100), 395 (45), 367 (27), 341 (28), 297 (39), 288 (65). Compound **22** RR_t: 4.49; EIMS *m/z* (rel. int.): 482 $[M]^+$ (21), 467 (11), 422 (100), 407 (49), 379 (29), 300 (47), 297 (27). Compound **23** RR_t: 4.03; EIMS *m/z* (rel. int.): 468 $[M]^+$ (47), 453 (56), 408 (100), 393 (100), 297 (71), 286 (16). Compound **24** RR_t: 5.63; EIMS *m/z* (rel. int.): 496 $[M]^+$ (17), 481 (11), 436 (100), 421 (50), 367 (19), 314 (39), 297 (29). Compound **25** RR_t: 6.41; EIMS *m/z* (rel. int.): 470 $[M]^+$ (16), 455 (12), 410 (2), 191 (100), 189 (68).

Isolation of cyclopodmenyl acetate (**26**). Acetate mixts of Pv were chromatographed through $AgNO_3$–silica gel to give a main fr. of **26** from the *n*-C_6H_{14}–C_6H_6 (7:3) eluate, and followed by HPLC.

Cycloartanone (**27**). Plates (5 mg) from Pv. Mp 105–106°, RR_t: 2.76; IR ν_{max}^{KBr} cm^{-1}: 1743; EIMS *m/z* (rel. int.): 426 $[M]^+$ (61), 411 (34), 313 (100), 295 (12), 288 (27).

Synthesis of compound **27**. Compound **16** (5 mg) was oxidized to give **27** (3 mg) with CrO_3–pyridine complex by a usual method. Compound **27** EIMS *m/z* (rel. int.): 426 $[M]^+$ (61), 411 (35), 313 (100), 295 (10), 288 (38).

Cycloartanoid ketones. *n*-C_6H_{14}–C_6H_6 (1:1) eluates of Pi were repeatedly purified on silica gel to give an oily material (5 mg) from the C_6H_6 eluates, 3 mg from Pv. Compound **27** RR_t: 2.72; EIMS *m/z* (rel. int.): 426 $[M]^+$ (51), 411 (34), 397 (7), 313 (100), 295 (12), 288 (27). Compound **28** RR_t: 3.30; 438 $[M]^+$ (100), 423 (45), 340 (41), 313 (91), 300 (50). Compound **29** RR_t: 2.93; EIMS *m/z* (rel. int.); 424 $[M]^+$ (100), 409 (57), 313 (43), 286 (33). Compound **30** RR_t: 4.07; EIMS *m/z* (rel. int.): 452 $[M]^+$ (67), 437 (39), 314 (61), 340 (22), 313 (100).

Cycloartanoid alcohols. C_6H_6 eluates of Pf were chromatographed on silica gel to give an oily alcohol mixt. (10 mg) from C_6H_6. Traces from PV and Pi. Compound **15** RR_t: 2.37; EIMS *m/z* (rel. int.): 414 $[M]^+$ (19), 399 (57), 396 (91), 381 (100), 341 (20), 288 (37), 283 (38). Compound **16** RR_t: 2.80; EIMS *m/z* (rel. int.): 428 $[M]^+$ (22), 413 (41), 410 (81), 395 (100), 367 (31), 341 (38), 297 (52), 288 (53). Compound **17** RR_t: 3.54; EIMS *m/z* (rel. int.): 440 $[M]^+$ (33), 425 (44), 407 (100), 379 (37), 353 (26), 300 (63), 297 (30). Compound **19** RR_t: 4.36; EIMS *m/z* (rel. int.): 454 $[M]^+$ (27), 439 (38), 436 (73), 421 (100), 393 (43), 367 (38), 314 (50), 297 (31).

21α-H-22-hydroxyhopane (**31**). The Et_2O eluates of Pi were repeatedly purified on silica gel to give **31**, 17 mg needles (Me_2CO) from the *n*-C_6H_{14}–C_6H_6 (9:1) eluate. Mp 232–233°, IR ν_{max}^{KBr} cm^{-1}: 3330; EIMS *m/z* (rel. int.): 428 $[M]^+$ (10), 410 (16), 395 (24), 367 (4), 207 (20), 203 (22), 191 (100), 189 (87). ^1H NMR ($CDCl_3$): δ0.847 (C-23), 0.793 (C-24), 0.819 (C-25), 0.997 (C-26), 0.947 (C-27), 0.629 (*d*, $J = 1.0$ Hz, C-28), 1.118, 1.188 (C-29, C-30), a trace from Pv.

Synthesis of compound **31**. To the Et_2O soln of isoadiantone (**35**) [19] (500 mg) was added Grignard reagent prepared from Mg (1.9 g) and CH_3I (4 ml) in Et_2O. The reaction mixt. was treated by the usual methods to give a product, which was purified on an Al_2O_3 column. Elution with *n*-C_6H_{14}–C_6H_6 (1:1) gave a solid (470 mg), which was recrystallized from *n*-C_6H_{14} to give **31** as needles, mp 230–233°.

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