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FERN CONSTITUENTS: CYCLOARTANE TRITERPENOIDS AND ALLIED COMPOUNDS FROM *POLYPODIUM FORMOSANUM* AND *P. NIPONICUM*

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Key Word Index—*Polypodium formosanum*; *P. niponicum*; Polypodiaceae; cycloartane triterpenoids; (24*R*)-cyclolaudenyl acetate; (24*R*)-cyclomargenyl acetate; 24-ethyl-9,19-cyclolanost-25-en-3β-yl acetate; (24*R*)-4α,24-dimethylcholesta-7,25-dien-3β-yl acetate; (24*R*)-4α-methyl-24-ethylcholesta-7,25-dien-3β-yl acetate.

Abstract—From the rhizomes of *Polypodium formosanum*, new triterpenoids of the cycloartane group, (24*R*)-cyclolaudenol and (24*R*)-cyclomargenol, were isolated as the corresponding acetates, alcohols and ketones, and their structures were established. Also, from the rhizomes of *P. niponicum* eight acetates of cycloartane derivatives and two acetates of new methyl sterols were isolated and characterized.

INTRODUCTION

In a previous paper [1] we reported the isolation and characterization of pentacyclic triterpenoids, 15 compounds of the oleanane and migrated oleanane groups, and 12 compounds of the hopane and migrated hopane groups from the rhizomes of *Polypodium niponicum* Mett. ('Aonekazura' in Japanese) and *P. formosanum* Baker ('Taiwan-aonekazura'). This paper deals with the identification of 12 kinds of cycloartane triterpenoids including six new compounds, and two new methyl sterols isolated from the same sources.

RESULTS AND DICUSSION

Cycloartane triterpenoids of the rhizomes of Polypodium formosanum [2]

Three fractions (see Experimental) obtained from the same material, from which we reported pentacyclic triterpenoids earlier [1], afforded a mixture (1:1) of two new compounds, (24*R*)-cyclolaudenyl acetate (**1a**) and (24*R*)-cyclomargenyl acetate (**1b**) [3]; another mixture (1:1) of the corresponding alcohols, **2a** and **2b**; and a third mixture (1:1) of the corresponding ketones, **3a** and **3b**, respectively. Separation of the mixtures was rather difficult and was achieved by repeated chromatography on silica gel impregnated with 20% silver nitrate, preparative GC, and/or HPLC on a reversed phase column.

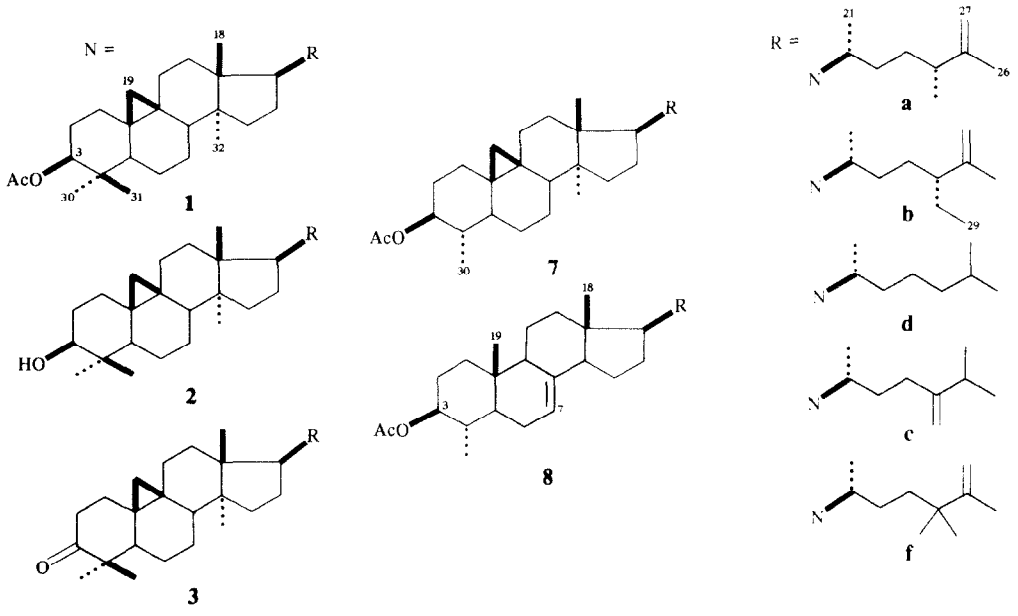
For compound **1a**, mp 127–128°, $[\alpha]_D + 53.5°$, elemental analysis and a molecular ion peak in the mass spectrum indicated the molecular formula $C_{33}H_{54}O_2$. The IR spectrum of **1a** showed the presence of a terminal methylene, an acetoxyl and a cyclopropane methylene group. The mass spectral fragments (Table 1) [4, 5] and the ^{1}H NMR spectrum (Table 2) [6, 7] of **1a** were almost superimposable with those of the known cyclolaudenyl acetate (**1c**), mp 121°, $[\alpha]_D + 55°$, of opium origin [8, 9], but a mixture of **1a** and **1c** melted at below 115° to provide evidence that the compounds were not identical.

Careful ozonolysis of **1a** followed by treatment with zinc powder and acetic acid, or osmium tetroxide and lead tetraacetate oxidation in a neutral condition afforded a methyl ketone (**4a**). The mass spectrum of **4a** showed appropriate shifts to mass numbers higher by two for the molecular ion and fragment ions containing the side chain, and the ^{1}H NMR spectrum (Table 2) indicated downfield shifts of the C-26 protons to δ2.128 (*s*) and C-28 protons to δ1.084 (*d*), and absence of C-27 methylene signals. These facts indicated the presence of the methyl ketone at the end of the side chain. Compound **4a** was further oxidized with oxygen in *t*-butanol–tetrahydrofuran with potassium *t*-butoxide [10], followed by acetylation to give another methyl ketone (**5a**). From the mass spectrum (further shifts to mass numbers lower by 28 for the molecular ion and ions containing the side chain) and the ^{1}H NMR spectrum [downfield shift of C-28 protons to δ2.140 (*s*)] and the absence of signals for the C-26 and C-27 hydrogens the structure of **5a** was confirmed.

On the other hand, **1c** was also oxidized in the same way as **1a** to afford the first methyl ketone (**4c**) and the second one (**5c**). As the identities of **5a** and **5c** were proved by mixed melting point determination, GLC and IR, ^{1}H NMR and mass spectra, **1a** was established as having the same structure as **1c** except for the absolute configuration at C-24.

Compound **1b**, $C_{34}H_{56}O_2$, exhibited an IR spectrum that closely resembled that of **1a**. The fact that the chemical shifts of the protons in **1b** attached to C-3, C-18, C-19, C-21, C-30, C-31 and C-32 were almost identical to those of **1a** and **1c** (Table 2), suggested that **1b** had the same structure of the cycloartane nucleus, including the configuration at C-20. The proton signals of **1b** were markedly different from those of **1a** and **1c** at a methyl (C-26), a terminal methylene (C-27) and a triplet methyl [C-29, instead of a doublet methyl (C-28) in **1a** or **1c**]. Similarly, **1b** provided a mass spectrum with shifts to mass numbers higher by 14 for the molecular ion and ions containing the side chain, and therefore the presence of an ethyl group at C-24 was suggested.

Oxidation of **1b** in the same way as **1a** gave a methyl ketone (**4b**). The mass spectrum (Table 1, shifts to mass



1a N = 1 R = a
1b N = 1 R = b
1d N = 1 R = d
1e N = 1 R = e
1f N = 1 R = f

2a N = 2 R = a
2b N = 2 R = b
3a N = 3 R = a
3b N = 3 R = b

7a N = 7 R = a
7d N = 7 R = d
7c N = 7 R = e
8a N = 8 R = a
8b N = 8 R = b

numbers higher by two for the molecular ion and fragment ions containing the side chain) and the ^{1}H NMR spectrum [Table 2, downfield shifts of the C-26 protons to δ2.108 (*s*) and the C-29 protons to δ0.868 (*t*)] of **4b** clearly demonstrated its methyl ketone structure. Compound **4b** was further oxidized to give an ethyl ketone (**5b**), and its structure was also confirmed by the mass spectrum (Table 1, further shifts to mass numbers lower by 28 for the molecular ion and ions with side chain) and the ^{1}H NMR spectrum (Table 2, a clear triplet at δ1.046 assigned to the C-29 protons).

Proof for the configuration at C-24 of **1a** and **1b** was accomplished by measuring the circular dichroism (CD) curves of **4a** and **4b** and comparing them with those of **4c**, **4a'** and **4c'**, which were obtained by alkaline treatment of **4a** and **4c**, respectively. As shown in Fig. 1, the curves of **4a** and **4b** were quite similar and found to be opposite to that of **4c**, centring at those of **4a'** and **4b'**. The CD curve of (3*R*)-(−)-3,7-dimethyloctan-2-one (**6**), derived from (+)-citronellol [11], was also used as a reference to confirm the absolute configuration. Hence **4a** and **4b** were clearly demonstrated to have (24*R*)- and **4c** (24*S*)-configurations, respectively; **1a** and **1b** were concluded to have (24*R*)- and **1c** (24*S*)-configurations [9].

The free alcohols, (24*R*)-cyclolaudenol (**2a**) and (24*R*)-cyclomargenol (**2b**), were separated as acetates, each component of which was proved to be identical to **1a** and **1b**. The mass spectrum (Table 1) and ^{1}H NMR spectrum (Table 2) of **2a** and **2b** were found to be consistent with the structure proposed.

The ketones, (24*R*)-cyclolaudenone (**3a**) and (24*R*)-cyclomargenone (**3b**), were also separated by preparative GC from the mixture. The identity of **3a** and **3b** with the compounds obtained from **2a** and **2b** by CrO_3–pyridine oxidation, respectively, were proved by mixed melting point determination and IR, mass and ^{1}H NMR spectra.

Compounds **1a** and **1b** were also found in other polypodiaceous ferns: *Drynaria foutunei* J. SM. and *Pseudodrynaria coronans* Ching [3]. Two 24-ethyl-cycloart-25-en-3β-ols, polysthicol [12] and tryphyllol [13], were also reported recently.

Cycloartane triterpenoids and methyl sterols of the rhizomes of Polypodium niponicum

Fresh material was subjected to investigation of the triterpenoids and steroids of these types. The components obtained were quite different from those of *P. formosanum* and the presence of the acetates of eight kinds of cycloartane derivatives was proved but no alcohols or ketones were isolated.

The cycloartane triterpenoid fraction was chromatographed on 20% silver nitrate impregnated silica gel to give seven fractions, as shown in Table 3 with their GC/MS data. Each fraction was separated by HPLC into individual compounds and their structures were established as described below.

The least polar fraction, ND-21, consisted of three compounds: **7d**, **1d** and a hopane derivative, dryocrassyl acetate [1]. Compound **7d** had the molecular formula $C_{31}H_{52}O_2$, which indicated the corresponding alcohol to be $C_{29}H_{50}O$. The mass (Table 1) and ^{1}H NMR (Table 2) spectra of **7d** clearly demonstrated that **7d** lacked a methyl group (C-31) in the nucleus part of the molecule, and had a C_8H_{17}-side chain. Thus, **7d** must be 31-norcycloartanyl

Table 1. GC and EIMS data of cycloartane triterpenoids and 4-methyl sterols

	RR_t	$[M]^+$	Fragments (rel. int.) 70 eV
(24*R*)-Cyclolaudenyl acetate (**1a**)	4.61	*482* (31)	467 (13), 422 (100), 407 (39), 379 (24), 357 (10), 353 (16), *300* (50), *297* (27), 175 (50)
(24*R*)-Cyclomargenyl acetate (**1b**)	5.6	*496* (31)	481 (12), 436 (100), 421 (37), 393 (18), 367 (15), 357 (10), *314* (42), *297* (23), 175 (42)
(24*S*)-Cyclolaudenyl acetate (**1c**)	4.60	*482* (30)	467 (13), 422 (100), 407 (56), 379 (24), 357 (19), 353 (19), *300* (46), *297* (23), 175 (42)
Cycloartanyl acetate (**1b**)	3.73	*470* (6)	455 (5), 410 (10), 395 (80), 367 (38), 341 (42), *297* (44), *288* (22), 255 (10), 203 (48), 191 (30)
24-Methylenecycloartanyl acetate (**1e**)	4.66	*482* (6)	467 (5), 422 (100), 407 (95), 355 (44), 353 (42), *300* (54), *297* (41), 281 (37), 203 (95), 189 (50)
24,24-Dimethylcycloart-25-enyl acetate (**1f**)	5.70	*496* (7)	481 (3), 436 (100), 421 (85), 393 (33), 369 (37), *314* (17), *297* (56), 255 (22), 203 (81)
(24*R*)-Cyclolaudenol (**2a**)	3.51	*440* (57)	425 (62), 422 (100), 407 (80), 379 (25), 353 (22), 315 (20), *300* (64), *297* (26), 175 (59)
(24*R*)-Cyclomargenol (**2b**)	4.40	*454* (53)	439 (48), 436 (90), 421 (100), 393 (27), 367 (18), 315 (21), *314* (40), *297* (18), 175 (39)
(24*R*)-Cyclolaudenone (**3a**)	3.64	*438* (100)	423 (19), 395 (7), 313 (43), *300* (28), 175 (19)
(24*R*)-Cyclomargenone (**3b**)	3.95	*452* (100)	437 (19), 409 (4), *314* (33), 313 (42), 175 (19)
(24*R*)-26-Nor-25-oxocyclolaudenyl acetate (**4a**)	6.01	*484* (10)	469 (4), 424 (100), 409 (80), 381 (27), 357 (14), 355 (35), *302* (35), *297* (53), 175 (51)
(24*R*)-26-Nor-25-oxocyclomargenyl acetate (**4b**)	7.89	*498* (12)	483 (9), 438 (100), 423 (62), 395 (21), 369 (23), 357 (20), *316* (56), *297* (45), 175 (100)
(24*S*)-26-Nor-25-oxocyclolaudenyl acetate (**4C**)	6.01	*484* (15)	469 (3), 424 (100), 409 (74), 381 (23), 357 (7), 355 (35), *302* (25), *297* (53), 175 (39)
26,27-Bisnor-24-oxocycloartanyl acetate (**5a**)	5.71	*456* (8)	441 (8), 396 (71), 381 (62), 357 (9), 353 (19), 327 (19), *274* (46), *297* (30), 175 (100)
27-Nor-24-oxocycloartanyl acetate (**5b**)	7.92	*470* (8)	455 (6), 410 (71), 395 (52), 367 (16), 357 (12), 341 (21), *288* (45), *297* (37), 175 (100)
31-Norcyclolaudenyl acetate (**7a**)	3.93	*468* (8)	453 (8), 408 (100), 393 (89), 356 (16), *300* (16), *283* (37), 201 (44), 189 (47)
31-Norcycloartanyl acetate (**7d**)	3.18	*456* (13)	441 (14), 396 (100), 381 (68), 341 (17), *288* (23), 283 (33), 206 (19), 189 (23)
Cycloeucalenyl acetate (**7e**)	3.93	*468* (3)	453 (3), 408 (100), 393 (94), 353 (19), *300* (10), *283* (36), 241 (19), 201 (28), 189 (53)
(24*R*)-4α,24-Dimethylcholesta-7,25-dien-3β-yl acetate (**8a**)	3.69	454 (36)	439 (20), 394 (8), 379 (10), *370* (13), *327* (100), 287 (13), 269 (39), 241 (21), 227 (41)
(24*R*)-4α-Methyl-24-ethylcholesta-7,25-dien-3β-yl acetate (**8b**)	4.08	*468* (31)	453 (20), 408 (6), 393 (9), *370* (25), *327* (100), 269 (29), 241 (17), 227 (30)
(24*R*)-4α,24-Dimethylcholesta-7,25-dien-3β-ol (**9a**)	2.78	*412* (48)	397 (25), 379 (5), 328 (10), 213 (7), *285* (100), 269 (25), 245 (13), 241 (11), 227 (19)
(24*R*)-4α-Methyl-24-ethylcholesta-7,25-dien-3β-ol (**9b**)	3.52	*426* (30)	411 (22), 408 (4), 393 (5), 328 (30), 313 (10), *285* (100), 269 (24), 245 (16), 241 (11), 227 (19)
(24*R*)-4α,24-Dimethylcholesta-7,25-dien-3-one (**10a**)	2.87	*410* (14)	395 (10), 326 (6), 312 (7), 311 (7), *283* (100), 269 (9), 258 (14), 243 (25)
(24*R*)-26-Nor-25-oxo-4α,24-dimethylcholest-7-en-3β-yl acetate (**11a**)	3.83	*456* (78)	441 (18), 396 (85), 381 (35), *269* (100), 343 (17), 227 (71)
(24*R*)-26-Nor-24-oxo-4α-methyl-24-ethylcholest-7-en-3β-yl acetate (**11b**)	5.03	*470* (75)	455 (20), 410 (100), 395 (37), 327 (27), *269* (75), 227 (50)
4α-24-Dimethylcholest-8 (14)-en-3β-yl actate (**12a**)	5.37	*456* (34)	441 (100), 396 (5), 327 (25), *269* (29), 243 (19), 227 (30)

Fragments in italic are some of the most characteristic features.

Table 2. ^{1}H NMR chemical shifts of cycloartane triterpenoids and 4-methyl sterols (δ, $CHCl_3$, 100 MHz)

	Methyl or methylene signals of										
Compound	C-30	C-31	C-19	C-32	C-18	C-21	C-28/29	C-26	C-27	3α-Protons	Acetyl methyl
1a	0.888	0.846	0.334 *d*, 0.504 *d* (4.2)	0.888	0.960	0.860 *d* (7.0)	0.992 *d* (7.0)	4.660, 4.670	1.640	4.54 *br dd* (10.8, 5.0)	2.044
1b	0.880	0.846	0.334 *d*, 0.574 *d* (4.2)	0.888	0.954	0.860 *d* (6.0)	0.806 *t* (7.8)	4.652 *d*, 4.720 *ddd* (2.4) (2.4, 1.4, 1.4)	1.574	4.54 *br dd* (10.6, 5.3)	2.052
1c	0.888	0.846	0.334 *d*, 0.574 *d* (4.2)	0.888	0.960	0.860 *d* (6.5)	0.992 *d* (7.0)	4.658, 4.670	1.640	4.54 *br dd* (10.7, 5.1)	2.044
1d	0.894	0.846	0.334 *d*, 0.580 *d* (4.2)	0.894	0.960	0.870 *d* (5.0)	—	0.872 *d* (6.0)	0.872 *d* (6.0)	4.54 *br dd* (10.0, 5.0)	2.046
1e	0.890	0.848	0.340 *d*, 0.578 *d* (4.1)	0.890	0.966	0.868 *d* (5.0)	4.668 *br s* 4.714 *br s*	1.028 *d* (6.8)	1.028 *d* (6.8)	4.58 *br dd* (10.9, 5.2)	2.050
1f	0.884	0.846	0.332 *d*, 0.572 *d* (4.4)	0.888	0.946	0.872 *d* (6.5)	1.014 1.014	4.662 *d*, 4.718 *ddd* (2.5) (2.5, 1.4, 1.4)	1.682	4.58 *br dd* (10.0, 5.0)	2.050
2a	0.960	0.806	0.324 *d*, 0.556 *d* (4.4)	0.882	0.960	0.848 *d* (7.6)	0.994 *d* (6.8)	4.660, 4.670	1.638	3.27 *br dd* (10.0, 5.0)	—
2b	0.964	0.806	0.322 *d*, 0.554 *d* (4.8)	0.882	0.964	0.848 *d* (7.6)	0.806 *t* (7.6)	4.656 *d*, 4.724 *ddd* (2.4) (2.4, 1.4, 1.4)	1.566	3.27 *br dd* (10.0, 5.0)	—
3a	1.098	1.046	0.572 *d*, 0.786 *d* (4.6)	0.898	0.790	0.870 *d* (6.1)	1.002 *d* (7.1)	4.662, 4.674	1.642	—	—
3b	1.098	1.044	0.576 *d*, 0.786 *d* (4.6)	0.894	0.988	0.866 *d* (7.0)	0.806 *t* (7.6)	4.666 *d*, 4.728 *ddd* (2.3) (2.3, 1.4, 1.4)	1.574	—	—
4a	0.888	0.848	0.338 *d*, 0.576 *d* (4.5)	0.888	0.954	0.874 *d* (7.0)	1.084 *d* (7.1)	—	2.128	4.55 *br dd* (9.0, 5.1)	2.054
4b	0.888	0.848	0.338 *d*, 0.576 *d* (4.5)	0.888	0.952	0.864 *d* (7.0)	0.868 *t* (6.4)	—	2.108	4.55 *br dd* (9.0, 5.1)	2.052
4c	0.888	0.846	0.336 *d*, 0.572 *d* (4.5)	0.888	0.952	0.888 *d* (7.0)	1.084 *d* (6.8)	—	2.126	4.55 *br dd* (10.0, 5.0)	2.052
5a	0.888	0.846	0.334 *d*, 0.578 *d* (4.2)	0.888	0.954	0.866 *d* (6.5)	2.140	—	—	4.55 *br dd* (10.0, 5.0)	2.050
5b	0.888	0.846	0.336 *d*, 0.574 *d* (4.2)	0.888	0.952	0.866 *d* (6.5)	1.046 *t* (7.3)	—	—	4.55 *br dd* (10.0, 5.0)	2.052

7a	0.842 *d* (6.1)	—	0.144 *d*, 0.402 *d* (4.2)	0.888	0.960	0.858 *d* (5.9)	0.996 *d* (7.0)	4.660, 4.670	1.640	4.55 *br dt* (10.0, 10.0, 5.0)	2.050
7d	0.842 *d* (5.8)	—	0.144 *d*, 0.404 *d* (4.2)	0.898	0.960	0.858 *d* (5.8)	—	0.866 *d* (6.4)	0.866 *d* (6.4)	4.52 *br dt* (10.0, 10.0, 5.0)	2.052
7e	0.842 *d* (5.6)	—	0.146 *d*, 0.404 *d* (4.1)	0.896	0.970	0.892 *d* (6.0)	4.668 *br s* 4.712 *br s*	1.026 *d* (6.8)	1.026 *d* (6.8)	4.52 *br dt* (10.0, 10.0, 5.0)	2.050
8a	0.914 *d* (6.1)	—	0.838	—	0.528	0.852 *d* (6.1)	0.994 *d* (7.1)	4.658, 4.668	1.638	4.40 *br dt* (10.8, 10.8, 4.0)	2.054
8b	0.912 *d* (6.3)	—	0.838	—	0.528	0.850 *d* (5.9)	0.804 *t* (7.6)	4.654 *d*, 4.724 *ddd* (2.4), (2.4, 1.4, 1.4)	1.576	4.40 *br dt* (10.5, 10.5, 4.1)	2.050
9a	0.980 *d* (6.8)	—	0.926	—	0.528	0.992 *d* (6.8)	0.992 *d* (6.8)	4.654, 4.668	1.638	3.12 *m*	—
9b	0.980 *d* (6.8)	—	0.916	—	0.524	0.980 *d* (6.8)	0.822 *t* (5.9)	4.650 *br d*, 4.718 *ddd* (2.4) (2.4, 1.4, 1.4)	1.562	3.12 *m*	—
10a	0.994 *d* (6.8)	—	1.076	—	0.556	0.926 *d* (6.8)	0.994 *d* (6.8)	4.658, 4.668	1.638	—	—
11a	0.992 *d* (6.8)	—	0.836	—	0.528	0.858 *d* (6.1)	1.082 *d* (6.8)	—	2.128	4.52 *br dt* (10.8, 10.8, 4.0)	2.054
11b	0.902 *d* (7.4)	—	0.836	—	0.524	0.850 *d* (6.8)	0.866 *d* (6.8)	—	2.108	4.52 *br dt* (10.5, 10.5, 4.1)	2.054
12a	0.854 *d* (6.3)	—	0.834	—	0.754	0.888 *d* (6.3)	0.854 *d* (6.3)	0.854 *d* (6.3)	0.854 *d* (6.3)	4.52 *br dt* (10.5, 10.5, 4.1)	2.052

Signals are singlets unless stated otherwise. Coupling constants are shown in parentheses. Olefinic proton signals (C-7) were also observed at 5.176 *m* (**8a**), 5.176 *m* (**8b**), 5.178 *m* (**9a**), 5.174 *m* (**10a**), 5.178 *m* (**11a**) and 5.170 *m* (**11b**).

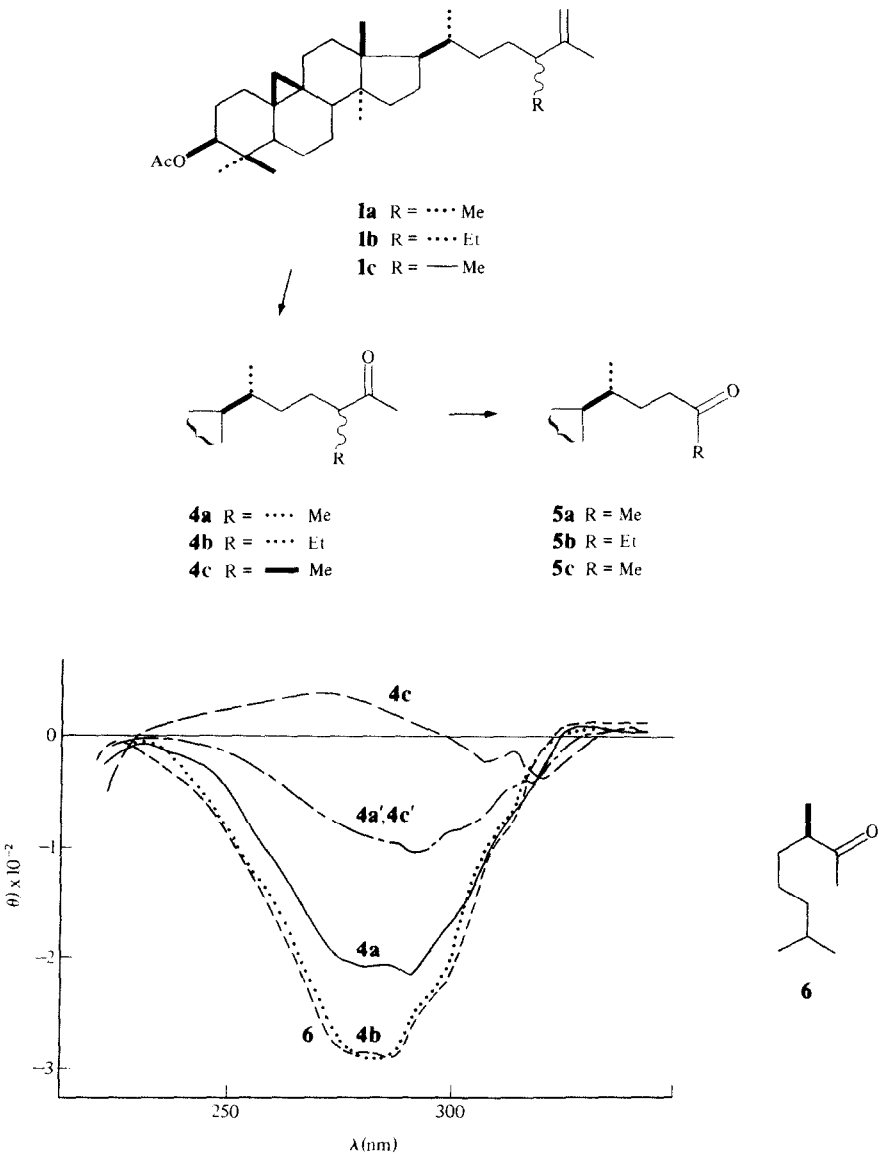


Fig. 1. CD curves of methyl and ethyl ketones.

acetate [14, 15]. Compound **1d** was identified as cycloartanyl acetate [14–16].

The second fraction, ND-22, was a mixture of at least seven components including β-amyrin acetate and dryocrassyl acetate. Overlap with the next fraction of the three compounds **8b**, **1a**″, and **1b**″ was proved by GC/MS. Compound **C** in Table 3 was very interesting because it seemed to be the acetate of a C_{33}-cycloartane derivative from its GC/MS, but further investigation of this compound was not successful.

Fraction ND-23 consisted of three components, one of which was a methyl sterol derivative (**8b**, described below). Two compounds, **1a**″ and **1b**″, were proved to be identical to **1a** and **1b** of *P. formosanum*, respectively. The absolute configuration at C-24 of **1a**″ was also established to be the same as that of **1a** (24*R*).

From the next fraction, ND-24, **7a**, **1a**″ and **1f** were isolated. Compound **7a** had the molecular formula $C_{32}H_{52}O_2$ and the identity of the structure of the side chain as that of **1a** as well as the 31-nor structure like **7d** was established by comparison of the ^{1}H NMR and mass spectra with those of **1a** and **7d**. Thus, **7a** was concluded to be 31-norcyclolaudenyl acetate, which was first reported from *Polypodium vulgare* [17]. The absolute configuration at C-24 was unknown. Meanwhile, **1f** had the molecular formula $C_{34}H_{56}O_2$ and the nucleus part of the compound was proved to be the same as **1a**–**1e** by comparison of its mass and ^{1}H NMR spectra. The presence of two methyl groups at C-24 and a double bond at C-25 was also established by the ^{1}H NMR spectrum. Thus, **1f** was proved to be 24,24-dimethylcycloart-25-enyl acetate (the corresponding acetate of cycloneolitsin [18, 19] or cyclobalanone [20]).

Fraction ND-25 consisted mainly of **1e**, which was identified with 24-methylenecycloartanyl acetate [21]. Both fractions ND-26 and ND-27 were mixtures of a

Table 3. Cycloartane triterpenoids and 4-methyl sterols of *Polypodium niponicum*

Fraction	Weight (mg)	RR_t	MS fragments	Compound
ND-21	130	3.18	456, 283, 288	**7d**
		3.73	470, 297, 288	**1d**
		6.42	470, 191, 189	**A**
ND-22	100	3.73	468, 218, 204	**B**
		4.08	458, 283, 288	—
			468, 327, 269	(**8b**)
		4.61	482, 297, 300	(**1a**)
		5.60	496, 297, 314	(**1b**)
		6.40	470, 191, 189	(**A**)
		6.40	510, 297, 328	**C**
ND-23	120	4.08	468, 327, 269	**8b**
		4.61	482, 297, 300	**1a**
		5.60	496, 297, 314	**1b**
ND-24	200	3.93	468, 283, 300	**7a**
		4.61	482, 297, 300	(**1e**)
		5.70	496, 297, 314	**1f**
ND-25	70	3.93	468, 283, 300	**7a**
		4.66	482, 297, 300	**1e**
ND-26	200	3.69	454, 370, 327	(**8a**)
		3.93	468, 283, 300	**7e**
ND-27	130	3.69	454, 370, 327	**8a**
		3.93	468, 283, 300	(**7e**)

Compounds in parentheses were not isolated. **A**: Dryocrassyl acetate [1]. **B**: β-Amyrin acetate [1]. **C**: Acetate of unknown C_{33}-cycloartane derivative.

methyl sterol (**8a**) and **7e** in different ratios. Compound **7e** was proved to be identical to cycloeucalenyl acetate by its ^{1}H NMR and mass spectra [6, 22].

As mentioned above, eight kinds of cycloartane derivatives were identified from this plant and the results were quite different from those obtained from *P. formosanum*. On the other hand, the alcohols corresponding to **1a**, **1d**, **1e**, **7a**, **7d** and **7e** were reported from the saponified extract of the rhizomes of *Polypodium vulgare* [14, 15, 17], although some ambiguity remained in the absolute configuration at C-24 of **1a** and **7a**. If we consider the unpublished data from our laboratory, the cycloartane triterpenoids seem to be widely distributed among polypodiaceous ferns. We also found that the combination of silver nitrate–silica gel chromatography and HPLC was very effective in separating these compounds from each other.

As far as ^{1}H NMR spectra of these compounds (Table 3) are concerned, the assignments were confirmed by $CHCl_3$–C_6D_6 solvent shift and lanthanide shift. The following common features were observed. (1) The signals of the C-19 methylene of the cyclopropane ring were observed at very high field in 31-nor compounds (**7a**, **7d** and **7e**), and at very low field in 3-one compounds (**3a** and **3b**) [17]. (2) A splitting pattern of endomethylene signals of four types was observed: (a) 25(26)-ene having a methyl group at C-24 (**1a**, **1c**, **2a**, **3a** and **7a**); (b) 25(26)-ene having an ethyl group at C-24 (**1b**, **2b** and **3b**); (c) 25(26)-ene having two methyl groups at C-24 (**1f**); and (d) 24(28)-ene (**1e** and **7e**). The splitting patterns were found to be a very effective means of distinguishing the compounds from each other.

Two new methyl sterols, **8a** and **8b**, were also obtained from ND-27 and ND-23, respectively. Compound **8a** had the molecular formula $C_{31}H_{52}O_2$; the molecular formula of the corresponding alcohol (**9a**), obtained by hydrolysis of **8a**, was $C_{29}H_{50}O$. CrO_3 oxidation of **9a** afforded the ketone **10a**. The mass spectra (Table 1) of **8a**, **9a** and **10a** demonstrated that these compounds had a C_{20} nucleus and a C_9 side chain. The ^{1}H NMR spectra (Table 2) of **8a**, **9a** and **10a** indicated that the structure of the side chain of these derivatives was the same as that of **1a** or **7a**. The absolute configuration of C-24 in **8a** was proved to be (24*R*) by the CD curve of **10a**. Liebermann tests and the ^{1}H NMR spectra of these derivatives indicated that the compounds had a steroid nucleus with 3β-hydroxyl and 4α-methyl groups, because a 3α-proton signal appeared as a broad triplet (J = 10.8, 10.8 and 4.0) such as those of the 31-norcycloartane derivatives (**7a**, **7d** and **7e**), and the doublet methyl signal at δ0.914 (**8a**) shifted to 0.980 (**9a**) and 0.994 (**10a**). The trisubstituted double bond was concluded to be at the $\Delta^{7(8)}$ position because the splitting pattern of the C-7 proton resembled those of Δ^7-triterpenoids like fern-7-ene and multiflor-7-ene [1], and the signal of the C-18 methyl group was observed at very high field (0.524–0.528 in **8a**, **9a** and **11a**). The fact that the reaction of **8a** under catalytic hydrogenation conditions afforded an isomer having a tetrasubstituted double bond at the $\Delta^{8(14)}$ position also gave evidence for the position of the double bond at $\Delta^{7(8)}$ in **8a**. Thus, the structure of **8a** was established to be (24*R*)-4α,24-dimethylcholesta-7,25-dien-3β-yl acetate.

The second compound, **8b**, had the molecular formula $C_{32}H_{52}O_2$. Compound **8b** gave the alcohol **9b**, $C_{30}H_{50}O$. The mass spectra of **8b** and **9b** suggested these compounds to be homologues of **8a** and **9a** having an extra methylene in the side chain, respectively. The fact that the ^{1}H NMR spectra of the side chain part of **8b** and **9b** were very similar to those of **1b** and **2b** indicated that these compounds also had 24-ethyl structures. Oxidation of **8b** gave the methyl ketone **11b**, the CD curve of which established the absolute configuration to be (24*R*). As the structure of the nucleus part of **8b** was demonstrated to be the same as that of **8a** by its ^{1}H NMR spectrum, **8b** was established to be (24*R*)-4α-methyl-24-ethylcholesta-7,25-dien-3β-yl acetate.

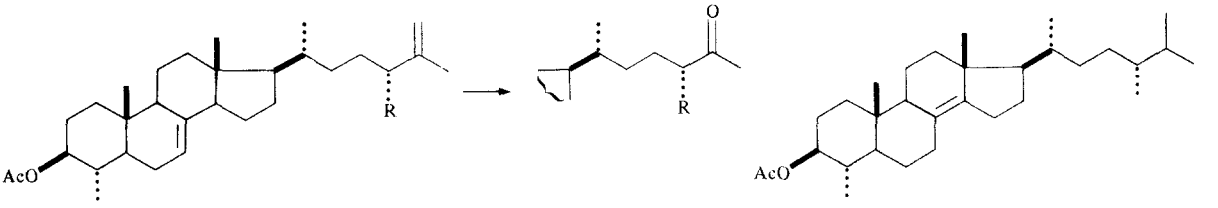
It is very interesting to note that all the 24-methyl compounds including campesterol as well as all the 24-ethyl compounds including sitosterol had the same (24*R*)-configuration, and the new biogenetic sequences cycloartenol → **1a** → **7a** → **8a** → campesterol and cycloartenol → **1b** → **7b** (not identified) → **8b** → sitosterol could be speculated to occur in these fern plants.

EXPERIMENTAL

General procedures were as described previously [1]. $[\alpha]_D$s were observed in $CHCl_3$ soln (*c* 0.2–0.5) at 22–24°. GC was performed on a 1 m glass column containing Chromosorb G HP with 1.4% SE-30 at 260°. Cholestane was used as internal reference.

Plant materials. Polypodium formosanum was collected at Wulai, Taipei on 26 August 1971 (FF-690) [1]. *Polypodium niponicum* was collected at Tomisawa, Yamanashi Prefecture on 24 May 1980 (F800504). Voucher specimens have been deposited at the Herbarium of the Laboratory of Phytochemistry, Shôwa College of Pharmaceutical Sciences, Tokyo.

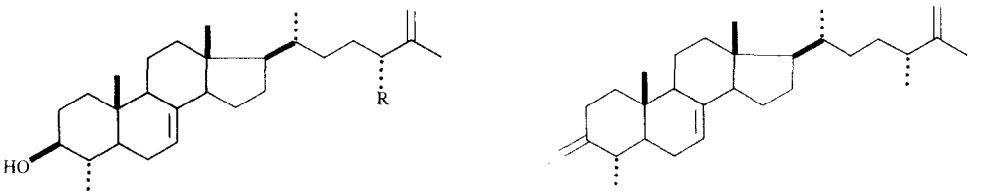
Components of the dried rhizomes of Polypodium formo-



8a R = Me
8b R = Et

11a R = Me
11b R = Et

12a



9a R = Me
9b R = Et

10a

sanum. The fractions obtained from the same material (3.4 kg) described earlier [1] were used.

(24R)-*Cyclolaudenyl acetate* (**1a**) *and* (24R)-*cyclomargenyl acetate* (**1b**). Fraction F-3 (23.0 g) was chromatographed on alumina and silica gel repeatedly to give a mixture (1:1) of **1a** and **1b** (9.2 g). The mixture was separated into two components by $AgNO_3$–silica gel prep. TLC followed by recrystallization from Me_2CO. Compound **1a**, more polar, colourless plates, mp 127–128°, $[\alpha]_D$ +53.5°, IR ν_{max}^{KBr} cm^{-1}: 1735, 1250, 1040; 3070, 1647, 888; 3050. (Found: C, 82.09; H, 11.27. $C_{33}H_{54}O_2$ requires: C, 82.00; H, 11.34%.) Compound **1b**, less polar, colourless plates, mp 144 145°, $[\alpha]_D$ +50.5°, IR ν_{max}^{KBr} cm^{-1}: 1735, 1250, 1040; 3070, 1647, 888; 3050. (Found: C, 82.20; H, 11.36. $C_{34}H_{56}O_2$ requires: C, 82.07; H, 11.47%.)

26-*Nor*-25-*oxocyclolaudenyl acetate* (**4a**) *and* , 26-*nor*-25-*oxocyclomargenyl acetate* (**4b**). (a) Compounds **1a** and **1b** (1.0 g of each) were separately oxidized with 3% O_3/O_2 in $CHCl_3$ soln at −60° and treated with Zn powder in HOAc. The products were chromatographed on silica gel and the eluates were recrystallized from MeOH to give **4a** (0.85 g) and **4b** (0.90 g), respectively. Compound **4a**, colourless plates, mp 142°, $[\alpha]_D$ +58.4°. IR ν_{max}^{KBr} cm^{-1}: 1730, 1240, 1022; 1710; 3050. CD $[\theta]_{305}$ −90.9°. Compound **4b**, colourless plates, mp 125–126°, $[\alpha]_D$ +51.5°. IR ν_{max}^{KBr} cm^{-1}: 1730, 1250, 1025; 1710; 3050. CD $[\theta]_{290}$ −214.6°. The CD curves of these specimens indicated them to be partially epimerized. (b) Compounds **1a** and **1b** (30 mg of each) were separately oxidized with 3% O_3/O_2 in a *n*-C_6H_{14} soln at −60° and treated as above. The oily specimens, **4a** and **4b**, obtained after chromatography gave the CD curves of highest intensity (Fig. 1). Addition of MeOH to the oils gave crystals having the same mp and IR and ^1H NMR spectra as those of **4a** and **4b** described above.

26,27-*Bisnor*-24-*oxocycloartanyl acetate* (**5a**). Compound **4a** (100 mg) in THF (10 ml) and *t*-BuOH (30 ml) with potassium (200 mg) was bubbled with O_2 at 5° for 10 min. After the mixture had been warmed at 70° for 20 min, the products were extracted with Et_2O and acetylated with Ac_2O–pyridine. Compound **5a** was obtained by prep. TLC (silica gel) and recrystallized from MeOH; 25 mg, colourless plates, mp 167°. IR ν_{max}^{KBr} cm^{-1}: 1730, 1250, 1025; 1710; 3050.

27-*Nor*-24-*oxocycloartanyl acetate* (**5b**). Compound **4b** (50 mg) was treated in the same way as **5a**. Compound **5b** obtained by chromatography on alumina and prep. GC was an oil. IR ν_{max}^{film} cm^{-1}: 1730, 1250, 1025; 1710; 3050.

(3R)-(−)-3,7-*Dimethyloctan-2-one* (**6**). Catalytic hydrogenation of (+)-citronellol (25 g, 75% purity), $[\alpha]_D$ +2.30° (neat), followed by acetylation with Ac_2O–pyridine gave (+)-dihydrocitronellyl acetate (12.7 g), $[\alpha]_D$ +1.16° (neat), which was heated drop by drop at 500° in N_2 atmosphere to give (3R)-(−)-3,7-dimethyloct-1-ene (3 g), $[\alpha]_D$ −4.49° (neat) [23]. ^1H NMR: δ0.87 (6H, *d*, *J* = 6 Hz), 0.90 (3H, *d*, *J* = 6 Hz), 5.71 (1H, *octet*, *J* = 17, 9.5, 7 Hz), 4.88 (1H, *q*, *J* = 3, 9.5 Hz), 4.93 (1H, *q*, *J* = 3.17 Hz). Oxidation of the compound with *m*-chloroperbenzoic acid in CH_2Cl_2 gave (3*R*)-(+)-3,7-dimethyloctan-1,2-oxide (1.7 g), $[\alpha]_D$ +0.62° (neat), $LiAlH_4$ treatment of which in Et_2O afforded (3*R*)-(+)-3,7-dimethyloctan-2-ol (1.1 g), $[\alpha]_D$ +6.8°. CrO_3–pyridine oxidation of the compound afforded **6** (650 mg), $[\alpha]_D$ −3.2° (neat), after chromatography (silica gel). ^1H NMR: δ0.88 (6H, *d*, *J* = 6 Hz), 0.97 (3H, *d*, *J* = 6 Hz), 2.11 (3H, *s*). Because of the impurity (geraniol) of the starting material, the optical purity of **6** was *ca* 80%.

(24R)-*Cyclolaudenol* (**2a**) *and* (24R)-*cyclomargenol* (**2b**). (a) Fraction FA-5 (6.7 g) [1] was chromatographed on alumina and separated by prep. TLC (silica gel) into a 1:1 mixture of **2a** and **2b** (270 mg). The mixture was separated into individual compounds by prep. GC using 1.4% SE-30 on Chromosorb G HP column, or by chromatography on 20% $AgNO_3$–silica gel column after acetylation as **1a** and **1b**. Compound **2a**, colourless needles (MeOH), mp 123–124°, $[\alpha]_D$ +36.5°. IR ν_{max}^{KBr} cm^{-1}: 3350, 1024; 3060, 1645, 880; 3040. EIMS: $[M]^+$ *m/z* 440.4025. Calc. for $C_{31}H_{52}O$: 440.4017. Compound **2b**, colourless needles (MeOH), mp 134–136°, $[\alpha]_D$ +34.3°. IR ν_{max}^{KBr} cm^{-1}: 3350, 1022; 3060, 1645, 880; 3040. EIMS: $[M]^+$ *m/z* 454.4173. Calc. for $C_{32}H_{54}O$: 454.4174. (b) Compounds of **1a** and **1b** (50 mg of each) were separately treated with $LiAlH_4$ in Et_2O and the products were recrystallized from MeOH. The specimens were identical to **2a** and **2b** mentioned above, respectively, in every respect (mp, $[\alpha]_D$, GLC, EIMS, IR and ^1H NMR).

(24R)-*Cyclolaudenone* (**3a**) *and* (24R)-*cyclomargenone* (**3b**). (a) Fraction FA-4 (2.6 g) [1] was separated by chromatography on alumina (grade III) and prep. TLC (silica gel) to give a mixture (1:1) of **3a** and **3b** (300 mg), oil, which was crystallized

from MeOH, mp 121°. The mixture was separated into pure compounds by prep. GC, followed by recrystallization from MeOH. Compound **3a**, colourless needles, mp 105°, $[\alpha]_D$ +14.2°. IR ν_{max}^{KBr} cm^{-1}: 1720; 3040, 1645, 880; 3050. EIMS: $[M]^+$ *m/z* 438.3858. Calc. for $C_{31}H_{50}O$: 438.3861. Compound **3b**, colourless needles, mp 122–124°, $[\alpha]_D$ +13.4°. IR ν_{max}^{KBr} cm^{-1}: 1720; 3040, 1645, 880; 3050. EIMS: $[M]^+$ *m/z* 452.4060. Calc. for $C_{32}H_{52}O$: 452.4017. (b) Compounds **2a** and **2b** (30 mg of each), prepared from natural acetates, were separately oxidized with CrO_3–pyridine. The products were chromatographed on Florisil and recrystallized from MeOH. The specimens were identical to **2a** and **2b** mentioned above in every respect (mp, $[\alpha]_D$, GLC, EIMS, IR and ^1H NMR).

Components of the fresh rhizomes of Polypodium niponicum. The fresh rhizomes (7.14 kg) were extracted 3 × with *n*-C_6H_{14} to give H_2O (4.2 kg) and an extract (56.1 g), the latter of which was chromatographed 2 × on silica gel. The *n*-C_6H_{14}–C_6H_6 (1:1) eluate (1.3 g) consisted mainly of acetates of the cycloartane triterpenoids, which were chromatographed on 20% $AgNO_3$–silica gel to give seven fractions, ND-21 to ND-27. These were checked by GC/MS as shown in Table 3.

31-*Norcycloartanyl acetate* (**7d**) *and cycloartanyl acetate* (**1d**). ND-21 was recrystallized from Me_2CO to remove dryocrassyl acetate (60 mg), and the filtrate (70 mg) was chromatographed on 20% $AgNO_3$–silica gel to give two fractions (ND-211 and ND-212). ND-211 (22 mg) was separated into **7d** (10 mg) and **1d** (10 mg) by HPLC [Radial Pak 8C1810, MeOH–$CHCl_3$–H_2O (74:16:10)]. Compound **7d**, colourless plates (Me_2CO), mp 91–92°, $[\alpha]_D$ +45.6° (lit. [14] mp 98–100°, $[\alpha]_D$ +57°). IR ν_{max}^{KBr} cm^{-1}: 1729, 1248. EIMS: $[M]^+$ *m/z* 456.3969. Calc. for $C_{31}H_{52}O_2$: 456.3966. Compound **1d**, colourless plates (Me_2CO), mp 130–132°, $[\alpha]_D$ +56.0°. (lit. [16] mp 130–132°, $[\alpha]_D$ +59°). IR ν_{max}^{KBr} cm^{-1}: 1730, 1252. EIMS $[M]^+$ *m/z* 470.4149. Calc. for $C_{32}H_{54}O_2$: 470.4123. ND-212 (45 mg) was separated into **7a** (20 mg) and **1a**″ (20 mg) by HPLC. These specimens were identified with those described below (mp, $[\alpha]_D$, GC, EIMS, ^1H NMR).

(24R)-*Cyclolaudenyl acetate* (**1a**″) *and cyclomargenyl acetate* (**1b**″). ND-23 was recrystallized to remove a steroidal component (30 mg, **8b**). The mother liquor was separated into **1a**″ (15 mg) and **1b**″ (a trace). Compound **1a**″, colourless plates (Me_2CO), mp 125–127°, which was identified with **1a** (GC, IR, EIMS, ^1H NMR). O_3-Oxidation of **1a**″ gave the methyl ketone **4a**″, $[\theta]_{290}$ −289°. Thus **1a**″ was proved to be identical to **1a**. Compound **1b**″ was also identified with **1b** (GC/MS, ^1H NMR).

31-*Norcyclolaudenyl acetate* (**7d**) *and* 24,24-*dimethyl-cycloart-25-enyl acetate* (**1f**). ND-24 was separated into three components, **7a** (100 mg), **1a**″ (50 mg) and **1f** (9 mg), by HPLC. Compound **7a**, colourless plates (Me_2CO), mp 105–107° (lit. [17] mp 108–110°), $[\alpha]_D$ +52.9°. IR ν_{max}^{KBr} cm^{-1}: 1735, 1242; 3060, 1645, 880; 3040. EIMS: $[M]^+$ *m/z* 468.3999. Calc. for $C_{32}H_{52}O_2$: 468.3966. Compound **1f**, colourless needles (Me_2CO), mp 176–179°, $[\alpha]_D$ +53.2° (lit. [19] mp 177–181°, $[\alpha]_D$ +58.0°). IR ν_{max}^{KBr} cm^{-1}: 1735, 1245; 3070, 1640, 890; 3035. EIMS: $[M]^+$ *m/z* 496.4298. Calc. for $C_{34}H_{56}O_2$: 496.4279.

24-*Methylenecycloartanyl acetate* (**1e**). ND-25 was separated into **1e** (65 mg) and **7a** (a trace) by HPLC. Compound **1e**, colourless needles (Me_2CO), mp 118–120°, $[\alpha]_D$ +57° (lit. [21] mp 116–117°, $[\alpha]_D$ +54.0°). IR ν_{max}^{KBr} cm^{-1}: 1738, 1245; 3070, 1640, 885; 3030. EIMS: $[M]^+$ *m/z* 482.4127. Calc. for $C_{33}H_{54}O_2$: 482.4123.

Cycloeucalenyl acetate (**7e**). ND-26 was chromatographed on 20% $AgNO_3$–silica gel to give two fractions of **7e** and **8a**, the former of which was purified by HPLC. Compound **7e**, colourless needles (Me_2CO), mp 108–109°, $[\alpha]_D$ +55.0° (lit. [22] mp 110°, $[\alpha]_D$ +63.0°). IR ν_{max}^{KBr} cm^{-1}: 1735, 1243; 3060, 1640, 890, 880; 3035. EIMS: $[M]^+$ *m/z* 468.3994. Calc. for $C_{32}H_{52}O_2$: 468.3966.

(24R)-4α,24-*Dimethylcholesta-7,25-dien-3β-yl acetate* (**8a**). Compound **8a** obtained from ND-27 was recrystallized from Me_2CO. Compound **8a** (45 mg), colourless plates, mp 167–168°, $[\alpha]_D$ +22.7°. IR ν_{max}^{KBr} cm^{-1}: 1740, 1240, 1038; 3040, 1640, 894; 820. (Found: C, 81.58; H, 11.37. $C_{31}H_{52}O_2$ requires: C, 81.88; H, 11.08%.) (b) Compound **8a** was also obtained from NA-2 (35 mg) and NC-3 (50 mg) [1].

(24R)-4α-*Methyl-24-ethylcholesta-7,25-dien-3β-yl acetate* (**8b**). (a) Fraction ND-23 was recrystallized from Me_2CO to give **8b** (30 mg), colourless plates, mp 167°, $[\alpha]_D$ +25.8°. IR ν_{max}^{KBr} cm^{-1}: 1740, 1250; 3040, 1640, 894, 820. (Found: C, 81.72; H, 11.32. $C_{32}H_{52}O_2$ requires: C, 81.99; H, 11.18%.) (b) Compound **8b** was also obtained from NA-2 (35 mg) and NC-3 (35 mg) [1].

(24R)-4α,24-*Dimethylcholesta-7,25-diene-3β-ol* (**9a**) *and* (24R)-4α-*methyl-24-ethylcholesta-7,25-dien-3β-ol* (**9b**). Compounds **8a** and **8b** (20 mg of each) were separately treated with $LiAlH_4$ in Et_2O and the products were passed through an Al_2O_3 column. Compound **9a**, colourless plates (MeOH), mp 175–177°, $[\alpha]_D$ +2.7°. IR ν_{max}^{KBr} cm^{-1}: 3350, 1020; 1640, 880. Compound **9b**, colourless plates (MeOH), mp 162–164°. IR ν_{max}^{KBr} cm^{-1}: 3350, 1020; 1640, 880.

(24R)-4α,24-*Dimethylcholesta-7,25-dien-3-one* (**10a**). Compound **9a** (10 mg) was oxidized with CrO_3–pyridine. Compound **10a**, colourless plates (MeOH), mp 149–151°. IR ν_{max}^{KBr} cm^{-1}: 1710; 1640, 880.

(24R)-26-*Nor-25-oxo-4α,24-dimethylcholest-7-en-3β-yl acetate* (**11a**) *and* (24R)-26-*nor-25-oxo-4α-methyl-24-ethylcholest-7-en-3β-yl acetate* (**11b**). Compounds **8a** and **8b** (10 mg of each) were separately treated with OsO_4 (10 mg) in a C_6H_6–pyridine soln for 5 hr at room temp., and with Na_2SO_3 and EtOH for 5 hr at 100°. The crystalline products were oxidized with neutral $Pb(OAc)_4$ in dry C_6H_6, and the products were chromatographed on silica gel. Compound **11a** (8 mg), colourless needles (Me_2CO), mp 145°. IR ν_{max}^{KBr} cm^{-1}: 1730, 1245; 1710, 820. CD $[\theta]_{297}$ −1756°, $[\theta]_{302}$ −1727°. Compound **11b** (7 mg), needles (Me_2CO), mp 140°. IR ν_{max}^{KBr} cm^{-1}: 1730, 1245; 1710; 825. CD $[\theta]_{300}$ −2115°.

4α,24-*Dimethylcholest-8-(14)-en-3β-yl acetate* (**12a**). Compound **8a** (30 mg) was treated with H_2–Pt_2O in an EtOAc soln for 1.5 hr. The product was recrystallized from EtOH. Compound **12a**, colourless plates, mp 103–105°. IR ν_{max}^{KBr} cm^{-1}: 1735, 1245.

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REFERENCES

1. Ageta, H. and Arai, Y. (1983) *Phytochemistry* **22**, 1801.
2. Ageta, H. and Arai, Y. (1982) *Chem. Letters* 881.
3. Tanaka, Y., Tôhara, K., Terasawa, K., Sawada, M. and Ageta, H. (1978) *Shôyakugaku Zasshi* **32**, 260.
4. Audier, H. E., Beugelmans, R. and Das, B. C. (1966) *Tetrahedron Letters* 4341.
5. Aplin, R. T. and Hornby, G. M. (1966) *J. Chem. Soc.* 1078.
6. Kocór, M. and St. Pyrek, J. (1973) *J. Org. Chem.* **38**, 3688.
7. Laonigro, G., Adinolfi, M., Barone, G., Lanzetta, R. and Mangoni, L. (1982) *Gazz. Chim. Ital.* **112**, 273.
8. Bentley, H. R., Henry, J. A., Irvine, D. S., Mukerji, D. and

Spring, F. S. (1955) *J. Chem. Soc.* 596.
9. Ourisson, G., Crabbé, P. and Rodig, O. R. (1964) *Tetracyclic Triterpenes*, p. 151. Hermann, Paris.
10. Bailey, E. J., Barton, D. H. R., Elks, J. and Templeton, J. F. (1962) *J. Chem. Soc.* 1578.
11. Klyne, W. and Buckingham, J. (1974) *Atlas of Stereochemistry*, p. 77. Chapman & Hall, London.
12. Laonigro, G., Siervo, F., Lanzetta, R., Adinolfi, M. and Mangoni, L. (1980) *Tetrahedron Letters* **21**, 3109.
13. Konno, C., Saito, T., Hikino, H. and Kabuto, C. (1981) *Planta Med.* **42**, 268.
14. Berti, G., Bottari, F., Marsili, A., Moreli, I., Polvani, M. and Mandelbaum, A. (1967) *Tetrahedron Letters* 125.
15. Alcaide, A., Devys, M., Pinte, F. and Barbier, M. (1970) *C. R. Acad. Sci. Ser. D* **271**, 862.
16. Bentley, H. R., Henry, J. A., Irvine, D. S. and Spring, F. S. (1953) *J. Chem. Soc.* 3673.
17. Berti, G., Bottari, F., Macchia, F., Marsili, A., Ourisson, G. and Piotrowska, H. (1964) *Bull. Soc. Chim. Fr.* 2359.
18. Ritchie, E., Senior, R. G. and Taylor, W. C. (1969) *Aust. J. Chem.* **22**, 2371.
19. Labriola, R. and Ourisson, G. (1971) *Tetrahedron* **27**, 1901.
20. Kamano, Y., Tachi, Y., Sawada, J. and Tanaka, I. (1976) *Yakugaku Zasshi* **96**, 1207.
21. Ohta, G. (1960) *Bull. Chem. Soc. Jpn.* **8**, 5.
22. Cox, J. S. G., King, F. E. and King, T. J. (1956) *J. Chem. Soc.* 1384.
23. Klein, E. and Rojahn, W. (1964) *Chem. Ber.* **97**, 2700.