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FERN CONSTITUENTS: CYCLOPODMENYL ACETATE, A CYCLOARTANOID HAVING A NEW 33-CARBON SKELETON, ISOLATED FROM POLYPODIUM VULGARE

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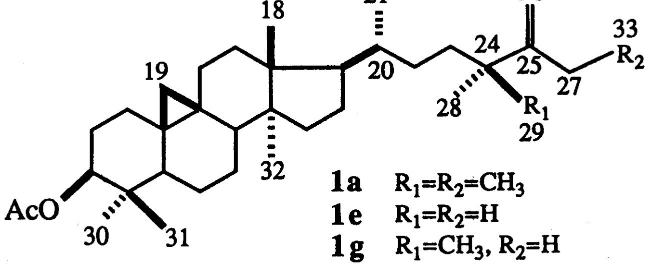
A cycloartane triterpenoid having a new 33-carbon skeleton, named cyclopodmenyl acetate, was isolated from the rhizomes of a fern, Polypodium vulgare Linné, and its structure was established to be 24,24,27-trimethyl-9,19-cyclolanost-25-en-3β-yl acetate (**1a**).

KEYWORDS fern constituent; triterpenoid; cycloartanoid; cyclopodmenyl acetate; 24,24,27-trimethyl-9,19-cyclolanost-25-en-3β-yl acetate; Polypodium vulgare

Common polypody, Polypodium vulgare Linné (Polypodiaceae, Oo-ezodenda 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 already been reported from this fern of European origin. In Japan, Oo-ezodenda is found in Oki Island (Shimane prefecture) and Hachinohe city (Aomori prefecture) as small colonies. This paper deals with the isolation and structure of a cycloartane derivative having a new 33-carbon skeleton, named cyclopodmenyl acetate (**1a**), together with various kinds of constituents described below.

n-Hexane extraction of the fresh rhizomes (105 g of the dried rhizomes estimated) collected at Hachinohe city gave 5.90 g of the extract, which was separated into the following fractions. 1) Triterpenoid hydrocarbons (0.58 g): fern-9(11)ene, neohop-13(18)-ene, fern-7-ene, hop-17(21)-ene, hop-22(29)-ene,[5] serrat-14-ene,[6] eupha-7,24-diene[7] and α-polypodatetraene.[8] 2) Fatty acid ester (1.94 g): β-Sitosteryl palmitate, and linolates of 31-norcycloartanol (**2b**), cycloartanol (**2c**), cycloartenol (**2d**), cyclolaudenol (**2e**) and cyclomargenol (**2f**).[9] 3) Acetates (0.40 g): acetates of **2b**, **2c**, **2d**, **2e**, **2f** and dryocrassol,[10] and **1a**. 4) Glyceride (1.65 g): glycerides of oleic and linoleic acids.

Compound **1a**, mp 116-118°C, $[\alpha]_D^{23}$ +52.0° (c=0.2, $CHCl_3$), was isolated from the acetate fraction by HPLC (C_{18} reverse phase, $CHCl_3/CH_3OH/H_2O$ 76/14/10). The content of **1a** was approximately 1% of the acetates fraction. The IR spectrum of **1a** (ν_{max}^{KBr} cm^{-1}: 3080, 1020; 1630, 890; 1730, 1245) indicated the presence of a cyclopropane ring, an endo methylene and an acetate groups in the molecule. The molecular formula $C_{35}H_{58}O_2$ for **1a** was obtained by its high-MS (M^+ 510.4421). Comparison of the MS fragmentation of **1a** with those of cyclolaudenyl acetate (**1e**) and cyclobalanyl acetate (**1g**) (Table I)[9] clearly indicated these three compounds have the same structure at cyclic parts of the molecule (common fragments: c, d, e, f, g and h) and their



1a $R_1=R_2=CH_3$
1e $R_1=R_2=H$
1g $R_1=CH_3$, $R_2=H$

Table I. EIMS Fragments (rel. int., 70 eV)

	M^+	M^+-Me	M^+-AcOH	M^+-Me -AcOH	a	b	c	d	e	f	g	h
1a	510(4)	495(45)	450(100)	435(47)	381(18)	328(33)	297(26)	255(6)	229(10)	215(10)	203(41)	175(50)
1e	482(15)	467(11)	422(100)	407(87)	353(32)	300(44)	297(58)	255(15)	229(27)	215(25)	203(70)	175(97)
1g	496(27)	481(15)	436(100)	421(91)	367(33)	314(38)	297(50)	255(14)	229(19)	215(24)	203(72)	175(89)

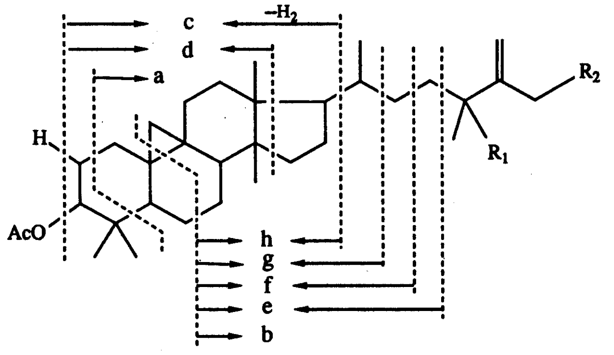


Table II. ^{1}H-Chemical shifts (δ, $CDCl_3$, 270 MHz)

	C-30	C-31	C-19	C-32	C-18	C-21	C-28/29	C-26	C-27	C-33	C-3α
1a	0.885	0.845	0.333d,0.570d (4.1)	0.885	0.943	0.841d (6.3)	1.019 1.019	4.762bd,4.785dt (1.2) (1.2,1.0)	1.990bq (7.4)	1.047t (7.4)	4.564dd (10.4,5.0)
1e	0.887	0.845	0.336d,0.571d (4.1)	0.887	0.952	0.845d (6.3)	0.997d (6.7)	4.665m ($w_{1/2h}$ 2.5)	1.639		4.562dd (10.5,5.2)
1g	0.887	0.845	0.335d,0.572d (4.1)	0.887	0.948	0.854d (6.3)	1.016 1.016	4.659bd,4.722dq (2.0) (2.0,1.0)	1.685d (1.0)		4.563dd (10.6,5.2)

Table III. ^{13}C-Chemical Shifts (δ, $CDCl_3$, 68 MHz)

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18
1a	31.9	26.9	80.7	39.5	47.3	21.0	28.1	47.9	20.2	25.9	26.1	35.6	45.3	48.9	32.9	26.6	52.2	17.9
1g	31.6	26.8	80.7	39.5	47.2	20.9	28.1	47.8	20.2	25.8	26.0	35.6	45.3	48.8	32.9	26.6	52.2	17.9

	C-19	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30	C-31	C-32	C-33	acetyl	
1a	29.8	36.7	18.5	30.9	37.7	39.2	157.8	106.5	23.4	27.8	27.5	25.5	15.2	19.3	13.1	170.9	21.3
1g	29.8	36.6	18.5	30.8	37.4	38.8	152.4	109.3	19.4	27.5	27.3	25.4	15.2	19.3	----	171.0	21.3

side chains are different, such as C_9 (**1e**), C_{10} (**1g**) and C_{11} (**1a**) (six fragments: M^+, M^+-CH_3, M^+CH_3COOH, M^+-CH_3-CH_3COOH, a and b).[10,11]

Comparison of the ^{1}H-NMR signals of **1a** with those of **1e** and **1g** (Table II) indicated these three compounds have the same structure at the cyclic parts and **1a** has an ethyl group (C-27,33) adjusting to the olefinic carbon (C-25), since the ethyl signals appeared at a lower field. This was proved by irradiating the signal of methylene (C-27) at δ1.990bq (J=7.4) resulting in the signal of methyl (C-33) at δ1.047t (J=7.4) being a singlet and also the proton signals at δ4.762bd and δ4.785dt (C-26) being sharper doublets. The remarkable differences in the values at C-25, C-27 and C-33 in the ^{13}C chemical shifts of **1a** and **1g** (Table III) also definitely indicated that **1a** has an extra methyl (C-33) at C-27. By the perfect coincidence of ^{1}H- and ^{13}C-chemical shifts of **1a** and **1g**, we concluded that **1a** has the same stereostructure as **1g** including the configuration at C-20.[12)] Thus, cyclopodmenyl acetate (**1a**) was established to be 27-methylcyclobalanyl acetate or 24,24,27-trimethyl-9,19-cyclolanost-25-en-3β-yl acetate.

Cyclopodmenyl acetate is the first example of a cycloartanoid having a 33-carbon skeleton. We know only one example of a triterpenoid having a 33-carbon skeleton, bosistoin (methyl pertyol),[13,14)] whose structures of the side chain and the cyclic part are different from those of **1a**. As far as Oo-ezodenda in Japan is concerned, this very restricted fern appeares to be the same species as the European Polypodium vulgare considering the many similarities in their chemical constituents.

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^{13}C- and ^{1}H-chemical shifts of the C-20 epimers, tirucalla-8,24-diene (**3a**) and eupha-8,24-diene (**3b**) are different enough to establish the stereochemistry of C-20. Signals of C-20: **3a** 36.4, **3b** 35.9; C-21: **3a** 18.8, 0.918d (J=5.6), **3b** 18.6, 0.860d (J=5.6).
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