Spiromarienonols A and B: Two New 7(8 → 9)*abeo*-Lanostane-Type Triterpene Lactones from the Stem Bark of *Abies mariesii*

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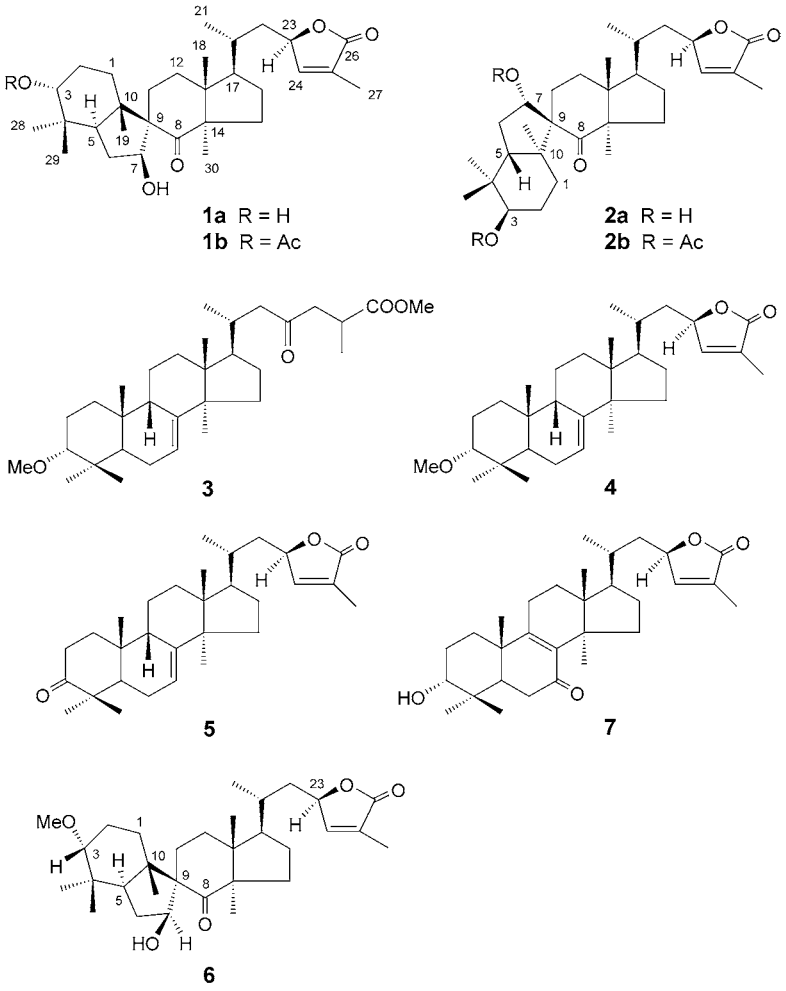
Two new skeletal triterpene lactones, spiromarienonols A (**1a**) and B (**2a**), were isolated from the stem bark of *Abies mariesii* Masters (Pinaceae). Based on spectral data and biogenetic considerations, their unique three-dimensional structures were determined to be (3*R*,7*S*,9*R*,23*R*)- (**1a**) and (3*R*,7*S*,9*S*,23*R*)-3,7-dihydroxy-8-oxo-7(8 → 9)*abeo*-lanost-24-eno-26,23-lactone (**2a**). Moreover, the potent activity of abiesenonic acid methyl ester (**3**) and abieslactone (**4**) against a disease-oriented panel of 39 human cancer cell lines were investigated.

Introduction. – *Abies mariesii* Masters (Pinaceae) is a tall evergreen tree growing in the mountains from central to northern Japan. Previously, we reported the isolation of abieslactone [1], three new gammacerane-type triterpenoids [2], and five new $\Delta^{8(9)}$-lanostane-type triterpene lactones [3][4] from the stem bark of this plant. In the present study, two unusually 'migrated' lanostane-type triterpene lactones, spiromarienonol A (**1a**) and spiromarienonol B (**2a**) were isolated from the stem bark. The structural elucidation and proposed biogenetic pathways of these compounds are reported here.

We previously found that abiesenonic acid methyl ester (**3**) is a chemical derivative of abieslactone (**4**), a main triterpenoid isolated from the stem bark of *Abies mariesii* [1] and *A. veitchii* [5], which shows significant anti-tumor activities in *in vivo* two-stage mouse-skin carcinogenesis assay with 7,12-dimethylbenz[*a*]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) [6]. Recently, we reported that compounds **3** and **4** inhibit rat hepatocarcinogenesis, as determined by a liver medium-term bioassay for carcinogenesis (*Ito*'s test) upon assessing immunohistochemically the number and area per cm^2 of preneoplastic lesions of glutathione *S*-transferase-placental-form-positive (GSTP-positive) foci [7]. Here, the potent activity of compounds **3** and **4** against a disease-oriented panel of 39 human cancer cell lines (HCC panel) [8] are also reported.

Results and Discussion. – Spiromarienonols A (**1a**) and B (**2a**) were found to have the same molecular formula, $C_{30}H_{46}O_5$, by HR-EI-MS. Their IR spectra indicated OH groups [**1a**: 3480 cm^{-1}; **2a**: 3443 cm^{-1}], an α,β-unsaturated γ-lactone [**1a**, **2a**: 1745 cm^{-1}], and a C=O group [**1a**: 1679 cm^{-1}; **2a**: 1685 cm^{-1}]. The ^{1}H- and ^{13}C-NMR spectra of **1a** and **2a** (*Table 1*) indicated five Me groups at C_q-atoms [**1a**: δ_H 0.70, 0.91, 0.97, 1.19, 1.44; **2a**: 0.73, 0.91, 0.93, 1.00, 1.18], one Me group at a secondary C-atom [**1a**:



δ_H 1.03 ($d, J = 6.5$); **2a**: 1.01 ($d, J = 6.5$)], eight CH_2, three CH, five C_q, and two secondary OH functions [**1a**: δ_H 3.46 (t), 4.25 (br. s); δ_C 75.0 (d), 80.2 (d); **2a**: δ_H 3.39 (t), 4.36 (dd); δ_C 74.9 (d), 78.0 (d)], a C=O group [**1a**: δ_C 215.8 (s); **2a**: 221.1 (s)], and an α,β-unsaturated γ-lactone [**1a**: δ_H 1.92 (t, Me(27)), 4.96 (ddd, H–C(23)), 6.99 (*quint.*, H–C(24)); δ_C 10.7 (q, C(27)), 78.7 (d, C(23)), 129.7 (s, C(25)), 149.4 (d, C(24)), 174.3 (s, C(26)); **2a**: δ_H 1.92 (t, Me(27)), 4.98 (ddd, H–C(23)), 7.00 (*quint.*, H–C(24)); δ_C 10.6 (q, C(27)), 78.7 (d, C(23)), 129.6 (s, C(25)), 149.5 (d, C(24)), 174.3 (s, C(26))], signals that were similar to those of abieslactone (**4**) and related triterpenes [1][3][9][10].

The (23*R*)-configuration of the lactone side chain in **1a** and **2a** was deduced from circular-dichroism (CD) measurements (*Fig. 1*), which gave rise to a negative *Cotton* effect similar to that of **3** and (23*R*)-3-oxo-9β-lanosta-7,24-dien-26,23-olide (**5**) [5][9].

Table 1. *^{1}H- and ^{13}C-NMR Spectral Data of Compounds* **1**, **2**, *and* **2a** (in $CDCl_3$)[a])

	1a		**2a**		**2b**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
H_α–C(1)	1.56 (*m*)	30.0 (*t*)	1.22 (*m*)	29.1 (*t*)	1.26 (*m*)	29.6 (*t*)
H_β–C(1)	2.10 (*m*)		1.44 (*m*)		1.40 (*m*)	
H_α–C(2)	1.67 (*m*)	26.2 (*t*)	1.89 (*m*)	26.2 (*t*)	1.88 (*m*)	24.2 (*t*)
H_β–C(2)	2.06 (*m*)		1.64 (*m*)		1.68 (*m*)	
H_β–C(3)	3.46 (*t*, *J* = 2.8)	75.0 (*d*)	3.39 (*t*, *J* = 3.0)	74.9 (*d*)	4.66 (*t*, *J* = 2.5)	76.3 (*d*)
C(4)		37.4 (*s*)		37.2 (*s*)		36.5 (*s*)
H–C(5)	1.84 (*m*)	43.5 (*d*)	2.80 (*dd*, *J* = 14.0, 6.0)	42.0 (*d*)	2.87 (*dd*, *J* = 14.5, 6.5)	43.4 (*d*)
H_α–C(6)	2.23 (*ddd*, *J* = 13.5, 7.5, 7.5)	34.4 (*t*)	1.43 (*ddd*, *J* = 14.0, 11.8, 7.0)	34.4 (*t*)	1.33 (*ddd*, *J* = 14.5, 12.0, 6.0)	32.2 (*t*)
H_β–C(6)	1.52 (*m*)		2.21 (*ddd*, *J* = 11.8, 6.0, 8.0)		2.41 (*ddd*, *J* = 12.0, 8.0, 6.5)	
H_β–C(7)	4.25 (br. *s*)	80.2 (*d*)	4.36 (*dd*, *J* = 8.0, 7.0)	78.0 (*d*)	5.19 (*dd*, *J* = 8.0, 6.0)	79.0 (*d*)
C(8)		215.8 (*s*)		221.1 (*s*)		218.9 (*s*)
C(9)		64.4 (*s*)		63.8 (*s*)		62.3 (*s*)
C(10)		48.9 (*s*)		50.4 (*s*)		50.2 (*s*)
H_α–C(11)	1.89 (*m*)	26.2 (*t*)	2.11 (*m*)	22.6 (*t*)	2.13 (*m*)	23.7 (*t*)
H_β–C(11)	1.42 (*m*)		2.11 (*m*)		2.13 (*m*)	
H_α–C(12)	2.04 (*m*)	30.8 (*t*)	1.98 (*m*)	33.2 (*t*)	2.00 (*m*)	33.3 (*t*)
H_β–C(12)	1.78 (*m*)		1.70 (*m*)		1.56 (*m*)	
C(13)		47.4 (*s*)		46.6 (*s*)		46.5 (*s*)
C(14)		61.2 (*s*)		61.1 (*s*)		61.0 (*s*)
H_α–C(15)	1.88 (*m*)	29.6 (*t*)	1.88 (*m*)	29.7 (*t*)	1.88 (*m*)	29.8 (*t*)
H_β–C(15)	1.26 (*m*)		1.18 (*m*)		1.18 (*m*)	
H_α–C(16)	1.30 (*m*)	27.0 (*t*)	1.26 (*m*)	27.1 (*t*)	1.29 (*m*)	27.0 (*t*)
H_β–C(16)	1.87 (*m*)		1.86 (*m*)		1.86 (*m*)	
H–C(17)	1.63 (*m*)	50.8 (*d*)	1.66 (*m*)	52.7 (*d*)	1.66 (*m*)	52.6 (*d*)
CH_3(18)	0.70 (*s*)	17.2 (*q*)	0.73 (*s*)	20.2 (*q*)	0.59 (*s*)	20.4 (*q*)
CH_3(19)	1.44 (*s*)	18.2 (*q*)	0.93 (*s*)	16.4 (*q*)	0.95 (*s*)	16.2 (*q*)
H–C(20)	1.82 (*m*)	32.9 (*d*)	1.79 (*m*)	32.8 (*d*)	1.78 (*m*)	32.8 (*d*)
CH_3(21)	1.03 (*d*, *J* = 6.0)	18.7 (*q*)	1.01 (*d*, *J* = 6.5)	18.1 (*q*)	0.99 (*d*, *J* = 6.5)	18.4 (*q*)
CH_2(22)	1.38 (*ddd*, *J* = 13.5, 10.5, 3.0), 1.51 (*m*)	40.3 (*t*)	1.39 (*ddd*, *J* = 14.1, 10.5, 2.0), 1.51 (*ddd*, *J* = 14.1, 11.0, 2.5)	40.1 (*t*)	1.42 (*m*), 1.52 (*m*)	40.0 (*t*)
H–C(23)	4.96 (*ddd*, *J* = 10.5, 2.0, 1.5)	78.7 (*d*)	4.98 (*ddd*, *J* = 11.0, 2.0, 1.5)	78.7 (*d*)	4.96 (*ddd*, *J* = 10.0, 2.0, 1.5)	78.7 (*d*)
H–C(24)	6.99 (*quint.*, *J* = 1.5)	149.4 (*d*)	7.00 (*quint.*, *J* = 1.5)	149.5 (*d*)	6.98 (*quint.*, *J* = 1.5)	149.3 (*d*)
C(25)		129.7 (*s*)		129.6 (*s*)		129.7 (*s*)
C(26)		174.3 (*s*)		174.3 (*s*)		174.2 (*s*)
CH_3(27)	1.92 (*t*, *J* = 1.5)	10.7 (*q*)	1.92 (*t*, *J* = 1.5)	10.6 (*q*)	1.91 (*t*, *J* = 1.5)	10.6 (*q*)
CH_3(28)	0.91 (*s*)	28.3 (*q*)	1.00 (*s*)	28.6 (*q*)	0.89 (*s*)	28.2 (*q*)
CH_3(29)	0.97 (*s*)	22.3 (*q*)	0.91 (*s*)	22.5 (*q*)	0.94 (*s*)	22.1 (*q*)
CH_3(30)	1.19 (*s*)	19.7 (*q*)	1.18 (*s*)	19.3 (*q*)	1.18 (*s*)	19.4 (*q*)
AcO					2.08 (*s*)	21.2 (*q*)
					2.08 (*s*)	21.3 (*q*)
						169.7 (*s*)
						170.9 (*s*)

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY experiments.

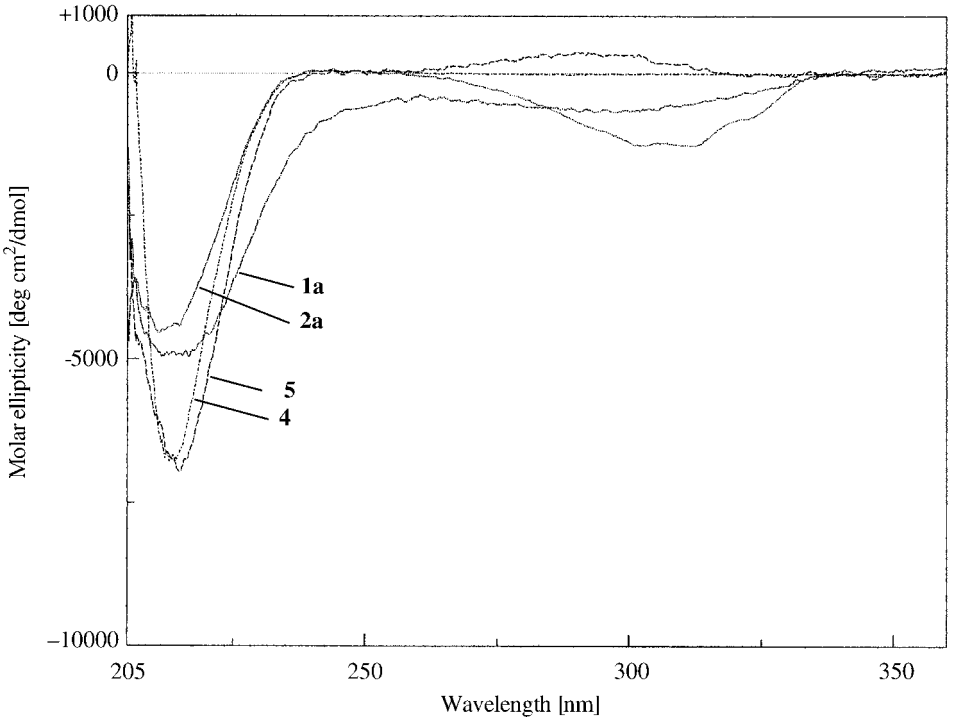


Fig. 1. *CD Spectra of selected triterpene lactones*

The DEPT and HMQC NMR spectra of **1a** and **2a** showed the same C-atom framework, except for an additional quaternary sp^3-hybridized C-atom instead of a CH_2 group relative to classical lanostane-type triterpenes [1][9][10]. In the mass spectra, **1a** and **2a** showed the same predominant fragment-ion peaks (*Fig. 2*) at *m/z* 468.3244 ($[M-H_2O]^+$, $C_{30}H_{44}O_4^+$; calc. 468.3237), 453 ($[M-H_2O-Me]^+$), 450

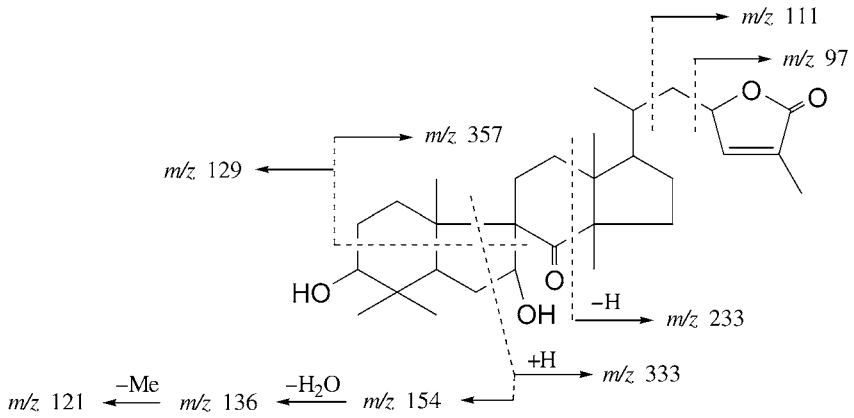


Fig. 2. *Observed EI-MS fragmentation pattern of* **1a** *and* **2a**

$[M-2\,H_2O]^+$), 397, 357, 355, 333 (100, $[C_{20}H_{29}O_4]^+$), 273, 233 ($[C_{15}H_{21}O_2]^+$), 154 ($[C_{10}H_{18}O]^+$), 136 ($[C_{10}H_{16}]^+$), 129, 121 ($[C_9H_{13}]^+$), 111 ($[C_6H_7O_2]^+$), and 97 ($[C_5H_5O_2]^+$). In particular, the appearance of a typical base peak at *m/z* 333 suggested that both **1a** and **2a** had an unusual type of a 'migrated' lanostane skeleton, involving a spiro-ring system, resembling spiroveitchionolide (**6**), which was first isolated from *A. veitchii* [11].

The HMBC NMR spectra of **1a** and **2a** indicated the same long-range correlations (*Fig. 3*) between Me(19) and C(1) (*t*), C(5) (*d*), C(9) (*s*), and C(10) (*s*) respectively; between Me(30) and C(8) (*s*), C(13) (*s*), C(14) (*s*), and C(15) (*t*) respectively; between H–C(7) and C(5) (*d*), C(6) (*t*), C(8) (*s*), C(9) (*s*), C(10) (*s*), and C(11) (*t*) respectively. In addition, the same correlations were revealed between H–C(3) and C(1) (*t*), C(2) (*t*), C(4) (*s*), C(5) (*d*), C(28) (*q*), and C(29) (*q*) respectively; between H–C(24) and C(22) (*t*), C(23) (*d*), C(25) (*s*), C(26) (*s*), and C(27) (*q*) respectively. Therefore, compounds **1a** and **2a** had the same unique overall structure of the 7(8→9)*abeo*-lanostane framework (6/5/6/5 ring system).

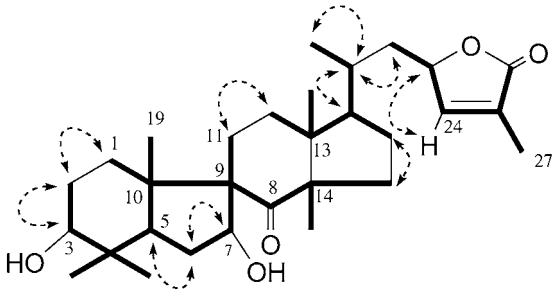
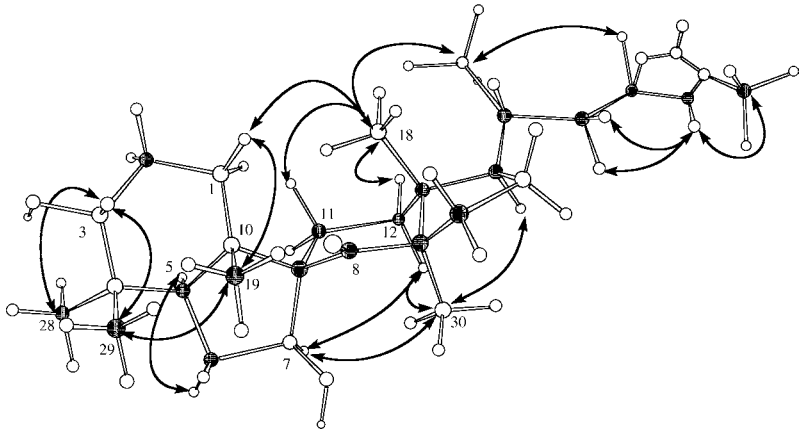


Fig. 3. *$^1H,^1H$-COSY* (- - →) ***and HMBC*** (——) ***Correlations of*** **1a** ***and*** **2a**

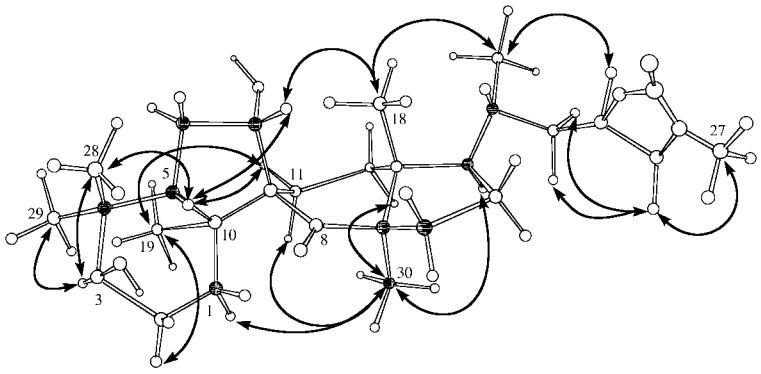
The $^1H,^1H$-COSY NMR spectra of **1a** and **2a** showed that H–C(7) was related only to H_α–C(6) and H_β–C(6), in agreement with a 3,7-dihydroxy-8-oxo-7(8→9)*abeo*-lanost-24-en-26,23-olide structure (*Fig. 3*). The difference between **1a** and **2a** was assumed to lie in the configuration at the spiro center (C(9)). We arrived at this conclusion based on the observations that H–C(5) in **1a** appeared at δ_H 1.84 (*m*), whereas it appeared at 2.80 (*dd*) in **2a** ($\Delta\delta_H$ 0.96), and that Me(19) appeared at δ_H 1.44 in **1a**, whereas it appeared at δ_H 0.93 in **2a** ($\Delta\delta_H$ 0.51). In addition, acetylation of **1a** and **2a** in Ac_2O/pyridine at room temperature under identical conditions afforded, in the case of the former, the monoacetate **1b**, while the latter gave the diacetate **2b**, indicating that there was steric hindrance near C(7)–OH in **1**, but not in **2**.

In the NOESY NMR spectra of **1a** (*Fig. 4*), significant NOEs were observed between *1*) H_{ax}–C(3) and both Me(28) and Me(29); *2*) H_{ax}–C(7) and both H_α–C(12) and Me(30); *3*) H_β–C(1) and both Me(18) and Me(19); *4*) H_β–C(11) and Me(18), corroborating that the C(9) spiro center in compound **1a** had the (*R*)-configuration, while C(3) and C(7) were (*R*)- and (*S*)-configured, respectively, forcing the cyclohexanone C-ring into a deformed chair conformation. In the case of **2a**, significant NOEs (*Fig. 4*) were observed between *1*) H_{ax}–C(3) and both Me(28) and Me(29); *2*) H_α–C(2) and Me(19); *3*) H_{ax}–C(7) and both $H_{ax,\beta}$–C(5) and Me(18); *4*) $H_{eq,\alpha}$–C(1) and Me(30). Thus, the absolute configuration of **2a** was (3*R*,7*S*,9*S*), with the C-ring

adopting a deformed boat conformation. Consequently, the structures of **1a** and **2a** were established as (3*R*,7*S*,9*R*,23*R*)- (**1a**) and the epimeric (3*R*,7*S*,9*S*,23*R*)-3,7-dihydroxy-8-oxo-7(8 → 9)*abeo*-lanosta-24-en-26,23-olide (**2a**), the former corresponding to C(3)–*O* methylated spiroveitchionolide (**6**) [11].



1a

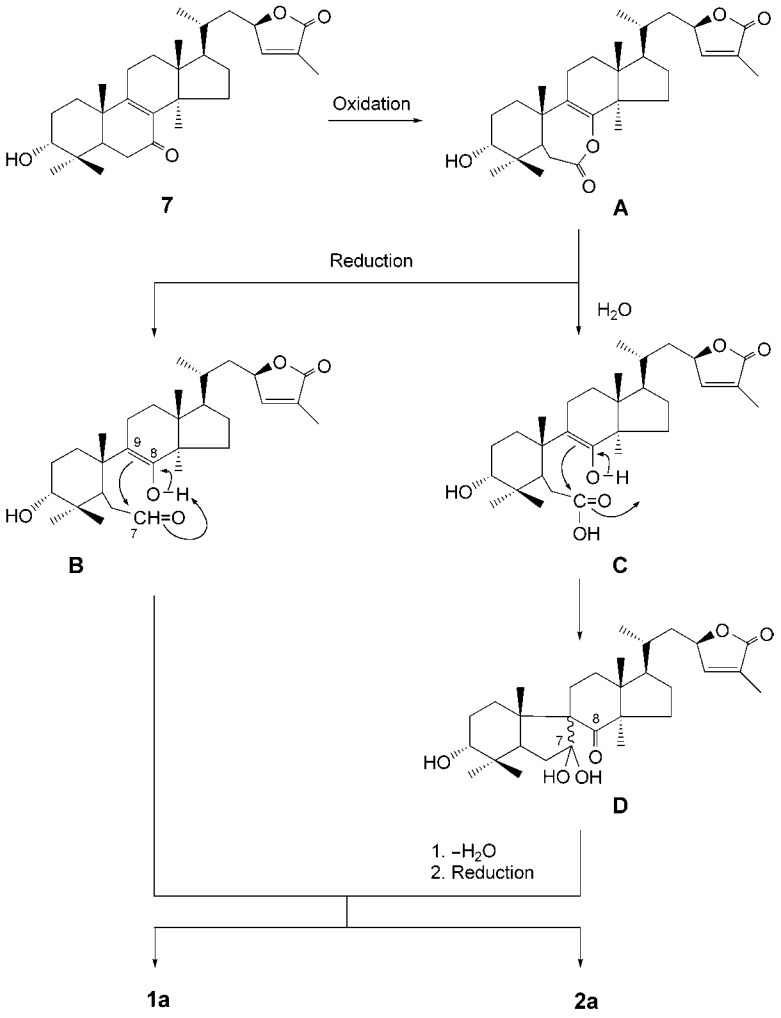


2a

Fig. 4. *NOESY Correlations of* **1a** *and* **2a**

It is noteworthy that the two epimers **1a** and **2a** occur concurrently in the stem bark of *Abies mariesii*. They seem to be biosynthesized from (23*R*)-3α-hydroxy-7-oxolanosta-8,24-dien-26,23-olide (**7**). A plausible biogenesis is proposed in the *Scheme*. Formal *Baeyer–Villiger* oxidation of **7** furnishes the lactone **A**, which then either gives rise to the B-*seco*-enol-aldehyde **B** by reductive cleavage, or to the B-*seco*-enol-carboxylic acid **C** by hydrolysis. Rearrangement of **B** may lead to the spiro[4.5] ring system, generating **1a** or **2a**. Alternatively, an intermediate **D**, derived from **C**, may be dehydrated to form a 7,8-dione, which then gives the target compounds after C(7)=O reduction.

Scheme. *Proposed Mechanism for the Biogenesis of Spiromarienonols A* (**1a**) *and B* (**2a**)



The cancer cell growth inhibitory properties of both abiesenonic acid methyl ester (**3**) and abieslactone (**4**) were evaluated against 39 human cancer cell lines at the *Cancer Chemotherapy Center* of the *Japanese Foundation for Cancer Reseach* [8]. The experimental delta and range values of **3** were 0.82 and 1.25, respectively (effective values: delta > 0.5, range > 1.0; for definitions, see the corresponding footnotes in *Table 2*). Hence, **3** showed moderate cytotoxic activity, while **4** was completely inactive (MG-MID $= -4.01$, delta $= 0.43$, range $= 0.44$) [8]. In addition, evaluation of the pattern of differential cytotoxicities with the COMPARE program suggested the possibility that the mode of action for **3** might be different from that shown by any other anticancer drug developed to date. Compound **3** displayed chemopreventive as well as

Table 2. *Cytotoxicities of Compound* **3** *Against a Panel of 39 Human Cancer Cell Lines*

Type of cancer	Cell line	log GI_{50} [M][a])
Breast	HBC-4	– 4.15
	BSY-1	– 4.46
	HBC-5	– 4.69
	MCF-7	– 4.62
	MDA-MB-231	– 4.58
Central nervous system	U251	– 4.64
	SF-268	– 4.02
	SF-295	– 4.46
	SF-539	– 4.18
	SNB-75	– 4.39
	SNB-78	– 4.00
Colon	HCC2998	– 4.39
	KM-12	– 4.53
	HT-29	– 4.37
	HCT-15	– 4.49
	HCT-116	– 4.51
Lung	NCI-H23	– 4.53
	NCI-H226	– 4.66
	NCI-H522	– 4.89
	NCI-H460	– 5.25
	A549	– 4.58
	DMS273	– 4.55
	DMS114	– 4.89
Melanoma	LOX-IMVI	– 4.72
Ovary	OVCAR-3	– 4.22
	OVCAR-4	– 4.33
	OVCAR-5	– 4.00
	OVCAR-8	– 4.43
	SK-OV-3	– 4.00
Kidney	RXF-631L	– 4.38
	ACHN	– 4.00
Stomach	St-4	– 4.50
	MKN1	– 4.64
	MKN7	– 4.58
	MKN28	– 4.29
	MKN45	– 4.12
	MKN74	– 4.45
Prostate	DU-145	– 4.18
	PC-3	– 4.05
MG-MID[b])		– 4.43
Delta[c])		0.82
Range[d])		1.25

[a]) Logarithmic conc. of compound for inhibition of cell growth at 50% rel. to control. [b]) Mean value of log GI_{50} over all cell lines tested. [c]) Difference in log GI_{50} value between the most sensitive cell and the MG-MID value. [d]) Difference in log GI_{50} value between the most and least sensitive cells.

moderate anti-cancer activities. The biological activities of compounds **1a** and **2a** are now under investigation.

Experimental Part

General. Column chromatography (CC) and medium-pressure liquid chromatography (MPLC) were carried out with silica gel (70–230 mesh, *Merck*). Anal. and prep. thin-layer chromatography (TLC): silica gel *60 F_{254}*, *Merck* (prep. plates: 0.5 mm). Melting points (m.p.) were measured with a *Yanagimoto* micro-melting point apparatus, uncorrected. Optical rotations were determined with a *JASCO DIP-1000* digital polarimeter. IR Spectra: on a *Perkin-Elmer 1720X* FT-IR spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a *Varian INOVA-500* spectrometer (standard pulse sequences), operating at 500 and 125 MHz, resp.; δ in ppm rel. to $SiMe_4$ as internal standard, J in Hz. Mass spectra (EI, 70 eV) were recorded on a *Hitachi 4000 H* double-focusing mass spectrometer; in m/z.

Plant Material. The stem bark of *Abies mariesii* Master was collected in the mountainous terrain under the control of the *National Yamaguchi Forestry Office*, Fukushima Prefecture, Japan, in July 1994. A voucher specimen (AM-9407-1) was deposited at the *Herbarium of the Laboratory of Medicinal Chemistry*, Osaka University of Pharmaceutical Sciences. The extraction was carried out in April 1997.

Extraction and Isolation. Extraction and chromatographic prepurification of the $CHCl_3$ extract (1.36 kg) of the stem bark of *A. mariesii* has been reported before [3]. From the five main fractions obtained, residue C_2 (fraction No. 164–166) were rechromatographed (CC, SiO_2) to afford a solid, which was recrystalized from hexane/$CHCl_3$ to afford *spiromarienonol A* (**1a**; 27.0 mg). The same procedure was applied to residue C_3 (fraction No. 167–170), which afforded *spiromarienonol B* (**2a**; 124.0 mg).

*(3*R*,7*S*,9*R*,23*R*)-3,7-Dihydroxy-8-oxo-7(8→9)*abeo-*lanost-24-eno-26,23-lactone* (*Spiromarienonol A*; **1a**). Colorless prisms. M.p. 184–187° (hexane/$CHCl_3$). $[\alpha]_D^{23}$ −3.5 (c = 0.11, $CHCl_3$). IR (KBr): 3480, 2922, 2851, 1745, 1679, 1462, 1383, 1339, 1055, 878. ^1H- and ^{13}C-NMR: see *Table 1*. EI-MS: 486 (2, M^+), 468 (9, $[M-H_2O]^+$), 453 (5, $M-H_2O-Me]^+$), 450 (3, $[M-2\,H_2O]^+$), 397 (10), 357 (2), 333 (100), 273 (2), 233 (7), 154 (9), 136 (36), 129 (2), 121 (43), 111 (12), 97 (52), 95 (63), 55 (76). HR-EI-MS: 486.3343 (M^+, $C_{30}H_{46}O_5^+$; calc. 486.3343).

*(3*R*,7*S*,9*R*,23*R*)-3-Acetoxy-7-hydroxy-8-oxo-7(8→9)*abeo-*lanost-24-eno-26,23-lactone* (*Spiromarienonol A 3-Acetate*; **1b**). *Spiromarienonol A* (**1a**; 2.3 mg) was reacted with Ac_2O/pyridine 1:1 (2 ml) at r.t. for 24 h to yield an amorphous solid, which was purified by prep. TLC ($CHCl_3$/MeOH 19:1) to afford pure **1b** (2.0 mg). ^1H-NMR: 0.71 (*s*, Me(18)); 0.82 (*s*, Me(28)); 1.03 (*s*, Me(29)); 1.06 (*d*, J = 6.0, Me(21)); 1.22 (*s*, Me(30)); 1.44 (*s*, Me(19)); 1.95 (*t*, J = 1.5, Me(27)); 2.09 (*s*, AcO); 4.28 (*m*, H–C(7)); 4.75 (*t*, J = 2.8, H–C(3)); 4.98 (*ddd*, J = 10.5, 2.0, 1.5, H–C(23)); 6.99 (*quint.*, J = 1.5, H–C(24)). EI-MS: 528 (6, M^+), 510 (6, $[M-H_2O]^+$), 468 (12, $[M-AcOH]^+$), 450 (14, $[M-AcOH-H_2O]^+$), 355 (55), 333 (100), 273 (4), 233 (12), 164 (34), 136 (50), 121 (31).

*(3*R*,7*S*,9*S*,23*R*)-3,7-Dihydroxy-8-oxo-7(8→9)*abeo-*lanost-24-eno-26,23-lactone* (*Spiromarienonol B*; **2a**). Colorless prisms. M.p. 238–241° (hexane/$CHCl_3$). $[\alpha]_D^{23} = -43.0$ (c = 0.34, $CHCl_3$). IR (KBr): 3443, 2957, 2874, 1745, 1685, 1458, 1388, 1350, 1056, 878. ^1H- and ^{13}C-NMR: see *Table 1*. EI-MS: 486 (1, M^+), 468 (4, $[M-H_2O]^+$), 453 (1, $[M-H_2O-Me]^+$), 450 (9, $[M-2\,H_2O]^+$), 425 (1), 397 (3), 355 (3), 333 (100), 273 (8), 233 (3), 154 (6), 136 (20), 129 (1), 121 (15), 111 (2), 97 (7), 95 (11), 55(7). HR-EI-MS: 486.3345 (M^+, $C_{30}H_{46}O_5^+$; calc. 486.3343).

*(3*R*,7*S*,9*S*,23*R*)-3,7-Diacetoxy-8-oxo-7(8→9)*abeo-*lanost-24-eno-26,23-lactone* (*Spiromarienonol B 3,7-Diacetate*; **2b**). *Spiromarienonol B* (**2a**; 5.0 mg) was reacted with Ac_2O/pyridine 1:1 (2 ml) at r.t. for 24 h to yield a crystalline solid, which was purified by prep. TLC ($CHCl_3$/MeOH 19:1) to afford pure **2b** (4.9 mg). M.p. 237–240° (MeOH/$CHCl_3$). $[\alpha]_D^{28}$ −33.1 (c = 0.20, $CHCl_3$). IR (KBr): 1745, 1735, 1239, 1685. ^1H- and ^{13}C-NMR: see *Table 1*. EI-MS: 570 (1, M^+, $C_{34}H_{50}O_7^+$), 510 (57, $[M-AcOH]^+$), 468 (45, $[M-AcOH-CH_2CO]^+$), 450 (88, $[M-2\,AcOH]^+$), 374 (31), 333 (68), 273 (5), 233 (17), 187 (22), 148 (47), 136 (16), 121 (59), 97 (35).

Cell Lines, Human Cancer Cell Line Panel, and Database. Human breast cancer cells MDA-MB-231 were purchased from *American Type Culture Collection* (Rockville, MD). The following human cancer cell lines [12] were generously provided by the *National Cancer Institute* (Frederick, MD): lung cancer, NCI-H23, NCI-H226, NCI-H522, NCI-H460, DMS273, and DMS-114; colon cancer, HCC-2998, KM-12, HT-29, HCT-15, and HCT-116; ovarian cancer, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; breast cancer, MCF-7; renal cancer, RXF-631 L, and ACHN; melanoma, LOX-IMVI; brain tumor, U251, SF-268, SF-295, SF-539, SNB-75, and SNB-78. Cells of human stomach cancer (St-4, MKN-1, MKN-7, MKN-28, MKN-45, MKN-74) and human

breast cancer (HBC-4, BSY-1, HBC-5) were obtained as described in [13]. The cells were cultured in RPMI 1640, supplemented with 5% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 mg/ml) at 37° in humidified air containing 5% of CO_2. To evaluate drugs for the cell-growth inhibition profile, we established a human cancer cell line panel combined with a database. The system as a whole was developed according to the method of the *National Cancer Institute* [14], with some minor modifications.

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