A Diterpenoid with a New Skeleton and Cytotoxic Terpenoids Isolated from *Amentotaxus formosana*

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The new diterpenoid 6α-hydroxy-*ent*-kaur-16-en-15-one (**1**) and a diterpenoid with a new skeleton, *i.e.*, amentotaxin BB (**5**), as well as three new lanostanoids, *i.e.*, (3β,23*R*)-3-methoxy-24-methylenelanost-8-en-23-ol (**6**), (23*S*)-23-methoxy-24-methylenelanost-8-en-3-one (**7**), and (3β,24ξ)-3-methoxy-24-methyl-9,19-cyclolanostan-25-ol (**8**), were isolated from the bark and leaves of *Amentotaxus formosana*. Their structures, including the relative configurations, were elucidated from spectroscopic data. Compound **1** and a known compound, *ent*-kaur-16-en-15-one (**2**), showed potent cytotoxic effects against a number of cancer cells *in vitro*.

1. Introduction. – *Amentotaxus formosana* LI (Amentotaxaceae) is an endemic tree of southeastern Taiwan. Recently, we have isolated and characterized two new lanostanoids, '3β,23β-dimethoxycycloartan-24(24^1)-ene' and 3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' (see below for modified names), a novel compound consisting of two diterpenoid substructures, *i.e.*, amentotaxin BA, and two novel terpenoids with a novel skeleton, *i.e.*, amentotaxins WA (**9**) and WB from the *A. formosana* [1][2]. In a continued search for novel cytotoxic constituents from this plant, the new diterpenoid 6α-hydroxy-*ent*-kaur-16-en-15-one (**1**), a diterpenoid with a new skeleton, *i.e.*, amentotaxin BB (**5**), and ten known compounds, as well as three new lanostanoids, *i.e.*, (3β,23*R*)-3-methoxy-24-methylenelanosta-8-en-23-ol (**6**), (23*S*)-23-methoxy-24-methylenelanosta-8-en-3-one (**7**), and (3β,24ξ)-3-methoxy-24-methyl-9,19-cyclolanostan-25-ol (**8**), were isolated from the bark and leaves of *Amentotaxus formosana* (see *Fig.*). In the present paper, we report the structure elucidation of the new compounds **1** and **5**–**8** as well as the cytotoxicity of **1** and of the already known compounds *ent*-kaur-16-en-15-one (**2**), ferrugiol acetate (**3** (acetate derivative)), sugiol (**4**), and amento-

taxin WA (**9**), a terpenoid with a new skeleton [2], also isolated from the bark and heartwood of this plant, against several human-cancer cell lines.

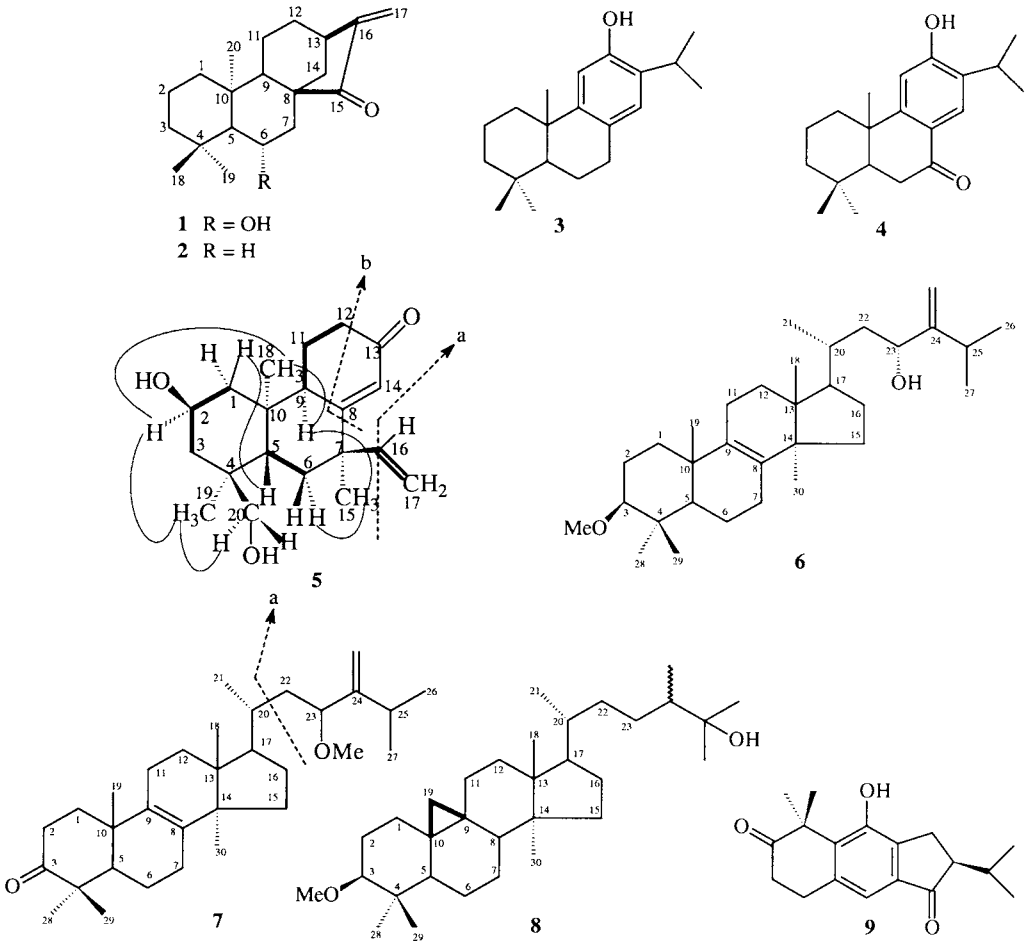

Figure. *Structures of* **1**–**9** *including selected NOESY correlations, relative configurations, and major MS fragmentation pattern*. Arbitrary numbering.

2. Results and Discussion. – The HR-EI-MS of **1** indicated a molecular-ion peak at *m*/*z* 302.2242, which corresponded to a molecular formula of $C_{20}H_{30}O_2$. The IR spectrum showed the presence of an OH group (3543 cm^{-1}) and the partial structure of a cyclopentanone conjugated to an exocyclic methylene group (1704 and 1640 cm^{-1}) [3]. The structure of 6α-hydroxy-*ent*-kaur-16-en-15-one for **1** was deduced from extensive analysis of 1D and 2D NMR data, including those from COSY, HMQC, HMBC, and NOESY experiments in $CDCl_3$.

The ^{1}H-NMR spectrum of **1** displayed signals of three angular Me groups, a cyclic skeletal methine group, an oxymethine proton, a methylene group at C(14), and an exocyclic methylene group, consistent with a kaurane skeleton [4][5]. The ^{13}C-NMR spectrum indicated an oxygenated tertiary C-atom and a carbonyl signal. These results suggested that **1** was a diterpenoid with a hydroxylated *ent*-kauren-15-one structure [3][5]. The COSY cross-peaks of H–C(5)/H–C(6) and H–C(6)/H$_\beta$–C(7), the HMBC correlations of H–C(6)/C(10) and C(8), and the NOESY cross-peaks of H$_\beta$–C(7)/H–C(6), H–C(6)/H–C(5), and H–C(13)/H$_\alpha$–C(14) established the structure of **1**. The relative configurations at C(5), C(6), and C(13) were deduced from the NOESY cross-peaks while H–C(5) and H–C(6), and OH–C(6) and H–C(13) are on the β- and α-sides of **1**, respectively.

The HR-EI-MS of amentotaxin BB (**5**) indicated a molecular-ion peak at *m*/*z* 318.2205, which corresponded to a molecular formula of $C_{20}H_{30}O_3$. The IR spectrum showed the presence of OH groups (3433 cm^{-1}) and an α,β-unsaturatured carbonyl group (1675 cm^{-1}). The structure of (2β,4β,7β)-2,20-dihydro-7-methyl-7-vinylpodocarp-8(14)-en-13-one for **5** was established from a detailed analysis of its spectral data.

The ^{1}H-NMR of **5** (*Table 1*) revealed the presence of three Me groups at δ 0.89, 0.92, and 1.11 (3*s*), a proton geminal to a secondary OH group at δ 3.99 (*dddd*, *J* = 12, 12, 4, 4 Hz) [7], an *AB* system (δ 3.14, 3.36 (*d*, *J* = 10.8 Hz, 2 H)) characteristic of a CH_2OH group, and four olefinic protons. Three of the olefinic protons, attributed to a monosubstituted olefin, constituted an *ABX* system (δ 4.99 (H$_A$), 5.00 (H$_B$), 5.80 (*X*) (J_{AB} = 1.2, J_{AX} = 10.8, J_{BX} = 17.2 Hz)), while one olefinic proton was isolated (δ 6.76 (*t*, *J* = 2.4 Hz)). The ^{13}C-NMR spectrum indicated the presence of three Me, seven CH_2, and five CH groups besides five quaternary C-atoms. The above evidence and analysis of the COSY and HMQC experiments established the connectivities of four ^{1}H,^{1}H and ^{1}H,^{13}C spin systems corresponding to the partial structures represented with bold lines in the *Figure*. The HMBC correlations of H–C(14)/C(9) and C(13), and H$_\alpha$–C(12)/C(13) established the connectivities between C(12) and C(13), and C(8) and C(9). The HMBC correlations of two H–C(17)/C(7) and Me(15)/C(6), C(7), and C(16) confirmed the connectivities between C(6) and C(7), and C(7) and C(16), and the correlations of 2 H–C(20)/C(3) and C(5), and Me(19)/C(3), C(4), and C(20) established the connectivities between C(3) and C(4), and C(4) and C(5) and confirmed the position of C(19) and C(20) at C(4). The HMBC correlations of Me(18)/C(1), C(5), C(9), and C(10) and the quaternary atoms C(7) and C(8) confirmed the linkage of C(1), C(5), C(9), and C(18) to C(10) and the connectivity between C(7) and C(8). The relative configurations at C(2), C(4), C(5), C(7), C(9), and C(10) were deduced from the NOESY cross-peaks H$_\alpha$–C(6)/Me(15), Me(15)/H–C(9), H–C(9)/Me(18), Me(18)/H–C(2), H–C(2)/Me(19), Me(19)/H$_\alpha$–C(20), and H–C(5)/H$_\beta$–C(1), establishing that H–C(2), Me(19), Me(15), H–C(9), and Me(18) are on the α-side and H–C(5) on the β-side of **5**. The EI-MS of **5** showed significant peaks at *m*/*z* 300 ([*M* – H_2O]$^+$), 269 ([300 – CH_2OH]$^+$), 241 ([269 – a – H]$^+$), and 187 ([241 – b – H]$^+$) (see *Figure*), which also supported the structure of **5**.

The HR-EI-MS of **6** indicated a molecular-ion peak at *m*/*z* 470.4109, which corresponded to a molecular formula of $C_{32}H_{54}O_2$. The IR spectrum showed the presence of an OH group (3402 cm^{-1}) and a C=C bond (1654 cm^{-1}). Extensive analysis of the spectral data and comparison with those of the known '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' (= 3β,23*S*)-3,23-dimethoxy-24-methylenelanost-8-ene) [1] established the structure of (3β,23*R*)-3-methoxy-24-methylenelanost-8-en-23-ol for **6**.

The EI-MS spectrum of **6** exhibited significant peaks at *m*/*z* 455 ([M – Me]$^+$), 437 ([455 – H_2O]$^+$), 405 ([437 – MeOH]$^+$), 341 ([437 – (side chain – $CHMe_2$) + H]$^+$), 309 ([405 – (side chain – $CHMe_2$) + H]$^+$). The ^{1}H-NMR spectrum of **6** showed signals for five tertiary and three secondary Me groups as required by the lanostane skeleton [8], two oxymethine signals at δ 2.67 (*dd*, *J* = 11.6, 3.4 Hz, H$_\alpha$–C(3)) [1] and 4.20 (*t*, *J* = 7.2 Hz), a MeO signal at δ 3.49 (*s*), and two olefinic-proton signals at δ 4.92 and 5.02 (2*s*, 1 H each). The ^{13}C-NMR spectrum of **6** showed the presence of two oxygenated C-atoms at δ 74.5 and 88.7 and was almost identical to that of '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene', except for the δ of C(23) and C(24) [1] (*Table 2*) [1]. The ^{13}C-NMR signals were assigned by performing ^{1}H-decoupled, DEPT, and 2D NMR correlation

Table 1. *1D and 2D NMR Data* (δ in ppm, J in Hz) *of* **5** *in* $CDCl_3$

	δ(H)	δ(C)	HMBC (^{1}H)
H_α–C(1)	1.07 (*d*, *J* = 12.4)	47.6	0.92 (Me(18))
H_β–C(1)	2.13 (*ddd*, *J* = 12.4, 4.0, 2.4)		
H_α–C(2)	3.99 (*dddd*, *J* = 12.0, 12.0, 4.0, 4.0)	64.9	
H_α–C(3)	1.45 (*m*)	44.5	0.89 (Me(19))
			3.14 (H_α–C(20))
H_β–C(3)	1.71 (*ddd*, *J* = 12.4, 4.0, 2.4)		3.36 (H_β–C(20))
C(4)		39.4	0.89 (Me(19))
H_β–C(5)	1.80 (*m*)	42.1	0.92 (Me(18))
			3.14 (H_α–C(20))
H_α–C(6)	1.53 (*m*)	34.0	1.11 (Me(15))
H_β–C(6)	1.67 (*m*)		
C(7)		38.7	1.11 (Me(15))
			4.99 (H_a–C(17))
			5.00 (H_b–C(17))
C(8)		134.5	
H_α–C(9)	2.19 (*m*)	50.9	6.76 (H–C(14))
			0.92 (Me(18))
C(10)		37.4	0.92 (Me(18))
H_α–C(11)	1.52 (*m*)	19.1	
H_β–C(11)	1.89 (*dd*, *J* = 14.0, 5.2)		
H_α–C(12)	2.25 (*dd*, *J* = 18.4, 13.6)	36.8	
H_β–C(12)	2.49 (*dd*, *J* = 18.4, 5.2)		
C(13)		199.6	2.49 (H_β–C(12))
			6.76 (H–C(14))
H–C(14)	6.76 (*t*, *J* = 2.4)	145.2	
Me(15)	1.11 (*s*)	25.8	
H–C(16)	5.80 (*dd*, *J* = 17.2, 10.8)	146.2	
H_a–C(17)	4.99 (*dd*, *J* = 10.8, 1.2)	111.9	
H_b–C(17)	5.00 (*dd*, *J* = 17.2, 1.2)		
Me(18)	0.92 (*s*)	15.4	
Me(19)	0.89 (*s*)	18.0	
H_α–C(20)	3.14 (*d*, *J* = 10.8)	70.5	0.89 (H–C(19))
H_β–C(20)	3.36 (*d*, *J* = 10.8)		

experiments and by comparison with those of corresponding data of '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' [1]. In addition to the above evidence, the HMQC and the HMBC correlations of H_α–C(3)/MeO, Me(28) and Me(29)/C(3) confirmed that the MeO group was linked to C(3), and the correlation CH_2=C(24)/C(23) and C(25) established the presence of a CH_2=C(24) bond and an OH group at C(23) of **6**. The ^{1}H-NMR signal of H–C(23) was shifted by 0.61 ppm to lower field as compared to that of H–C(23) of '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' [1].

The HR-EI-MS of **7** indicated a molecular-ion peak at *m*/*z* 468.3956, which corresponded to a molecular formula of $C_{32}H_{52}O_2$. The IR absorptions were indicative of a carbonyl group (1709 cm^{-1}) and a C=C bond (1653 cm^{-1}). The spectral data and their comparison with those of '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' [1] established the structure of (23*S*)-23-methoxy-24-methylenelanost-8-en-3-one for **7**.

The EI-MS of **7** showed significant peaks at *m*/*z* 453 ([*M* – Me]$^+$), 421 ([453 – MeOH]$^+$), and 325 ([421 – (a – MeOH + H)]$^+$). The ^{1}H-NMR spectrum of **7** exhibited signals for five tertiary and three secondary Me groups as required by the lanostane skeleton [8], an oxymethine-proton signal at δ 3.59 (br. *d*, *J* = 10.4 Hz,

Table 2. *^{13}C-NMR Data* ($CDCl_3$) *of* **6**–**8**[a])

	6[b])	**7**[b])	**8**[b])		**6**[b])	**7**[b])	**8**[b])
C(1)	35.5	36.0	31.8	C(18)	15.7	16.0	18.0
C(2)	22.6	34.6	25.4	C(19)	19.6	21.3	29.8
C(3)	88.7	217.9	88.5	C(20)	34.7	33.4	36.7
C(4)	37.0	47.4	40.5	C(21)	19.1	18.6	18.6
C(5)	51.2	51.2	47.7	C(22)	42.9	43.1	35.2
C(6)	18.1	19.4	21.0	C(23)	74.5	81.6	28.4
C(7)	28.4	28.3	28.1	C(24)	159.3	156.6	45.2
C(8)	134.2	133.1	48.0	CH_2=C(24)	108.2	107.4	14.8
C(9)	134.5	135.4	20.0	C(25)	29.9	30.0	73.6
C(10)	38.8	36.9	26.3	C(26)	23.1	22.4	26.2
C(11)	21.0	21.1	26.0	C(27)	23.6	23.5	27.1
C(12)	26.5	26.9	32.9	C(28)	28.0	26.2	25.5
C(13)	44.6	44.6	45.0	C(29)	16.2	18.7	14.8
C(14)	49.8	50.0	48.8	C(30)	24.2	24.3	19.3
C(15)	31.0	31.1	35.5	*Me*O–C(3)		56.4	
C(16)	30.8	30.9	26.5	*Me*O–C(23)	57.6		57.6
C(17)	50.9	51.1	52.2				

[a]) The number of protons directly attached to each C-atom was verified by DEPT experiments. [b]) Signals obtained by ^{1}H,^{1}H COSY, HMBC, HMQC, NOESY techniques.

H_α–C(23)), a MeO signal at δ 3.21 (*s*), and two olefinic-proton signals at δ 4.91 and 4.98 (2*s*, 1 H each). The ^{13}C-NMR spectrum of **7** established the presence of a carbonyl signal at δ 217.9 and of an oxygenated C-atom signal at δ 81.6 and was almost identical to that of '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' [1] except for C(2) to C(4), C(10), C(28), and C(29) (*Table 2*), suggesting that the carbonyl and MeO group of **7** were located at C(3) and C(23), respectively. The ^{13}C-NMR signals of **7** were assigned by performing ^{1}H-decoupled, DEPT, and ^{1}H,^{13}C COSY correlation experiments and by comparison with those of corresponding data of '3β,23β-dimethoxy-5α-lanosta-24(24^1)-ene' [1].

The HR-EI-MS of **8** indicated a molecular-ion peak at *m*/*z* 472.4272, which corresponded to a molecular formula of $C_{32}H_{56}O_2$. The IR absorptions were indicative of an OH group (3483 cm^{-1}). Comparison of the spectral data with those of '3β,23β-dimethoxycycloartan-24(24^1)-ene' (=(3β,23*S*)-3,23-dimethoxy-24-methylene-9,19-cyclolanostane) [1] and 24-methyl-5α-cholestan-3β-ol [9][10] established the structure of (3β,24ξ)-3-methoxy-24-methyl-9,19-cyclolanostan-25-ol for **8**.

The EI-MS of **8** showed significant peaks at *m*/*z* 440 ([*M* – MeOH]$^+$), 425 ([440 – Me]$^+$), and 297 ([440 – side chain]$^+$). The ^{1}H-NMR spectrum of **8** revealed signals of six tertiary and two secondary Me groups of two geminal protons of a cyclopropane ring at δ 0.32 (*d*, *J* = 4.0 Hz) and 0.55 (*d*, *J* = 4.0 Hz), of an oxymethine proton at δ 2.71 (*dd*, *J* = 11.2, 4.4 Hz, H_α–C(3)) [1], and of a MeO group at δ 3.36 (*s*). The ^{13}C-NMR spectrum showed the presence of an oxygenated quarternary and an oxygenated tertiary C-atom at δ 73.6 and 88.5, respectively. The chemical shifts of C(1) to C(21) and C(28) to C(30) of **8** (*Table 2*) were almost identical to the corresponding data of '3β,23β-dimethoxycycloartan-24(24^1)-ene', and those of C(22) to C(27) and *Me*–C(24) of **8** were very similar to the corresponding data of 24-methyl-5α-cholestan-3β-ol [9]. The δs of C(24) to C(27) of **8** revealed a downfield shift of 6.1, 42.0, 8.4, and 6.6 ppm (see *Table 2*) as compared with the corresponding data of 24-methyl-5α-cholestan-3β-ol [9][10], suggesting that an OH group was located at C(25) of **8**. The ^{13}C-NMR signals of **8** were assigned by performing ^{1}H-decoupled and DEPT experiments and by comparison with those of corresponding data of '3β,23β-dimethoxycycloartan-24(24^1)-ene' [1] and 24-methyl-5α-cholestan-3β-ol [9].

The cytotoxicity of **1**–**4** and **9** isolated from bark and heartwood of *A. formosana*, respectively, was studied for a number of cancer cell lines. The results are listed in *Table 3*. Compounds **1** and **2** showed significant cytotoxic activity against Hep G2, Hep 3B, HT-29, and HCT 116. Compounds **3** (acetate derivative) and **9** were marginally active against Hep G2 and Hep 3B, and Hep 3B and HT-29, respectively, while compound **4** did not show significant cytotoxic activity against any of the tested cell lines (see *Table 3*). This clearly indicated that a OH group at C(6) on the α-side enhances the cytotoxicity against several human-cancer cell lines (see **1** in *Table 3*).

Table 3. *Cytotoxicity of Constituents Isolated from* Amentotaxus formosana[a])

	Cell lines			
	Hep G2	Hep 3B	HT-29	HCT 116
1	0.18	0.64	0.48	0.21
2	0.35	1.00	0.60	0.30
3 (acetate derivative)	5.20	6.20	[b])	[b])
4	> 8.00	> 8.00	> 8.00	> 8.00
9	[c])	9.0	5.6	[c])
5-fluorouracil	$3.3 \cdot 10^{-2}$	$7.15 \cdot 10^{-2}$	$7.4 \cdot 10^{-2}$	0.48

[a]) Data are presented as ED_{50} values in μg/ml. For significant activity of the pure compounds, an $ED_{50} \leq 4.0$ μg/ml is required. [b]) No significant activity. [c]) Not determined.

The uncommon structure of amentotaxin BB (**5**) can be biologically derived from 5,8-secopodocarp-5,8-diol and isopropylethylene.

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Experimental Part

General. M.p.: uncorrected. Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *Jasco* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 200*-FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian Unity-400* spectrometer; at 400 and 100 MHz, resp.; δ in ppm, J in Hz. MS: *JMSHX-100*-mass spectrometer; m/z (rel.%).

Plant Material. Whole plants of *A. formosana* (Amentotaxaceae) were collected at Kaohsiung Hsien, Taiwan, R.O.C., during July 2001. A voucher specimen (9001) has been deposited at the Department of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical University.

Extraction and Isolation. The air-dried bark (700 g) of *A. formosana* was chipped and extracted with $CHCl_3$ at r.t. The $CHCl_3$ extract (15 g) was chromatographed (silica gel). Elution with cyclohexane/acetone 150:1 yielded **2** (15 mg), **3** (30 mg), and *ent*-kaur-16-en-6α-ol (5 mg). Elution with CH_2Cl_2 yielded **1** (6 mg), **4** (7 mg), candol A (2 mg), candol B (3 mg), 11α-hydroxy-*ent*-kaur-16-en-15-one (2 mg), and kauran-16α-ol (5 mg). Elution with CH_2Cl_2/acetone 9:1 yielded *ent*-sandaracopinara-8(14),15-diene-2β,18-diol (3 mg) and sandaracopinaradiene-2α,18,19-triol (4 mg). Elution with CH_2Cl_2/MeOH 10:0.5 yielded **5** (0.6 mg).

The air-dried leaves (3.1 kg) were extracted with $CHCl_3$. The $CHCl_3$ extract (79 g) was chromatographed (silica gel). Elution with cyclohexane/CH_2Cl_2 3:5 yielded **6** (3 mg), elution with cyclohexane/CH_2Cl_2 8:2 gave **7** (10 mg), and elution with cyclohexane/CH_2Cl_2/AcOEt 7:2:0.5 furnished **8** (2 mg).

The known compounds were identified by spectroscopic methods and comparison with reported data or authentic samples [1][2][7][11–18].

6α-Hydroxy-ent-*kaur-16-en-15-one* (=*(6α)-6-Hydroxykaur-16(17)-en-15-one*[1]); **1**): Coloress powder. $[\alpha]_D^{25} = -157$ ($c = 0.11$, $CHCl_3$). IR (KBr): 3543, 1704, 1640. ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.98 (*s*, Me(18)); 1.20 (*s*, Me(19)); 1.47 (*s*, Me(20)); 1.51 (*dd*, $J = 12.4$, 6.8, H_α–C(14)); 2.79 (*d*, $J = 12.4$, H_β–C(14)); 3.06 (br. *s*, H–C(13)); 4.56 (br. *s*, H_β–C(6)); 5.27 (*s*, 1 H–C(17)); 5.96 (*s*, 1 H–C(17)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 18.2 (C(2)); 18.7 (C(11)); 18.9 (C(20)); 24.0 (C(19)); 32.3 (C(12)); 33.6 (C(18)); 34.1 (C(4)); 38.0 (C(14)); 38.5 (C(13)); 39.9 (C(10)); 41.6 (C(7)); 42.1 (C(1)); 43.9 (C(3)); 50.8 (C(8)); 52.8 (C(9)); 56.1 (C(5)); 67.2 (C(6)); 114.5 (C(17)); 149.5 (C(16)); 211.1 (C(15)). EI-MS: 302 (90, M^+) , 284 (24, $[M - H_2O]^+$), 269 (39, $[284 - Me]^+$), 109 (96), 91 (100). HR-EI-MS: 302.2242 ($C_{20}H_{30}O_2^+$; calc. 302.2246).

Amentotaxin BB (=*(2β,4β,7β)-2,20-Dihydroxy-7-methyl-7-vinylpodocarp-8(14)-en-13-one* = *(4a*R*, 4b*S*,6*S*,8*S*,8a*S*,10*R*)-10-Ethenyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-6-hydroxy-8-(hydroxymethyl)-4b,8,10-trimethylphenanthren-2(3*H*)-one*; **5**): Colorless powder. $[\alpha]_D^{26} = +4$ ($c = 0.03$, $CHCl_3$). IR (film): 3433, 1675. UV (MeOH): 210 (4.43), 240 (3.50). ^{1}H-NMR: *Table 1*. EI-MS (70 eV): 318 (35, M^+), 300 (21), 269 (15), 241 (6), 201 (31), 162 (44), 149 (50), 133 (100), 121 (69), 105 (91). HR-EI-MS: 318.2205 ($C_{20}H_{30}O_3^+$; calc. 318.2195).

*(3β,23*R*)-3-Methoxy-24-methylenelanost-8-en-23-ol* (**6**): Colorless powder. $[\alpha]_D^{25} = +31.2$ ($c = 0.15$, $CHCl_3$). IR (KBr): 3402, 1654. ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.68 (*s*, Me(18)); 0.79 (*s*, Me(29)); 0.88 (*s*, Me(30)); 0.96 (*d*, $J = 6.4$, Me(21)); 0.98 (*s*, Me(19), Me(28)); 1.08 (*d*, $J = 6.4$, Me(26)); 1.09 (*d*, $J = 6.4$, Me(27)); 2.67 (*dd*, $J =$ 11.6, 3.4, H_α–C(3)); 3.49 (*s*, MeO(23)); 4.20 (*t*, $J = 7.2$, H_β–C(23)); 4.92 (*s*, 1 H, CH_2=C(24)); 5.01 (*s*, 1 H, CH_2=C(24)). ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table 2*. EI-MS (70 eV): 470 (4, M^+), 455 (3), 437 (3), 405 (2), 341 (4), 309 (4), 69 (100). HR-EI-MS: 470.4109 ($C_{32}H_{54}O_2^+$; calc. 470.4124).

*(23*S*)-23-Methoxy-24-methylenelanost-8-en-3-one* (**7**): Colorless powder. IR (KBr): 1709, 1653. $[\alpha]_D^{25} =$ +35.6 ($c = 0.2$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.75 (*s*, Me(18)); 0.88 (*s*, Me(30)); 0.94 (*d*, $J = 6.4$, Me(21)); 1.04 (*d*, $J = 6.4$, Me(27)); 1.06 (*s*, Me(28)); 1.08 (*d*, $J = 6.4$, Me(26)); 1.09 (*s*, Me(19)); 1.11 (*s*, Me(29)); 3.21 (*s*, MeO–C(23)); 3.59 (*d*, $J = 10.4$, H_α–C(23)); 4.91 (*s*, 1 H, CH_2=C(24)); 4.98 (*s*, 1 H, CH_2=C(24)). ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table 2*. EI-MS (70 eV): 468 (2, M^+) , 453 (1), 421 (7), 325 (13), 257 (4), 113 (96), 69 (100). HR-EI-MS: 468.3956 ($C_{32}H_{52}O_2^+$; calc. 468.3967).

(3β,24ξ)-3-Methoxy-24-methyl-9,19-cyclolanostan-25-ol (**8**): Colorless powder. $[\alpha]_D^{25} = +14.6$ ($c = 0.1$, $CHCl_3$). IR (KBr): 3483. ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.32 (*d*, $J = 4.0$, H_{exo}–C(19)); 0.55 (*d*, $J = 4.0$, H_{endo}–C(19)); 0.79 (*s*, Me(29)); 0.89 (*d*, $J = 7.2$, Me(21)); 0.90 (*s*, Me(30)); 0.90 (*d*, $J = 6.8$, Me=C(24)); 0.95 (*s*, Me(28)); 1.00 (*s*, Me(18)); 1.17 (*s*, Me(26)); 1.16 (*s*, Me(27)); 2.71 (*dd*, $J = 11.2$, 4.4, H_α–C(3)); 3.36 (*s*, MeO–C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz): *Table 2*. EI-MS (70 eV): 472 (0.9, M^+), 440 (6), 425 (2), 407 (3), 397 (4), 353 (3), 318 (5), 297 (6), 175 (27), 83 (100). HR-EI-MS: 472.4272 ($C_{32}H_{56}O_2^+$; calc. 472.4280).

Tumor-Cell-Growth-Inhibition Assays. A microassay for cytotoxicity was performed with MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [20][21]. Briefly, $1–3 \cdot 10^3$ cells/100 µl were seeded in 96-well microplates (*Nunck*, Roskilde, Denmark) and preincubated for 6 h to allow cell attachment. The cells were incubated with each drug for 6 days and then pulsed with 10 µl of MTT (5 mg MTT/ml; *Sigma*, St. Louis, MO) and incubated for an additional 4 h at 37°. The microplates were read at 550 nm on a *Multiskan* photometer (*MR5000*; *Dynatech*, McLean, VA) after lysis of cells with 100 µl of 10% SDS (=sodium dodecyl sulfate) in 0.01M HCl. Control wells contained medium and cells (total absorbance) or medium alone (background absorbance). Cell death was calculated as the percentage of MTT inhibition.

Human hepatomacellular carcinoma Hep 3B and Hep G2, human colorectal adenocarcinoma HT-29, and human colorectal carcinoma HCT 116 cells were obtained from *American Type Culture Collection* (*ATCC*; Rockville, MD) and grown in DMEM [21][22], containing 10% FBS, 2 mM L-glutamine, 100 units/ml of penicillin, and 100 µg/ml of streptomycin. For the microassay, the growth medium was supplemented with 10 mM *HEPES* (=4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer pH 7.3 and incubated at 37° in a CO_2 incubator.

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[1]) Systematic name based on the kaurane skeleton used by *Chem. Abstr.*; according to IUPAC, this skeleton is *ent*-kaurane [19].

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