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Kaurane-Type Diterpenes from *Adenostemma lavenia* O. KUNTZE

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Ten 11-oxygenated kauran-19-oic acids, *ent*-11α,15α-dihydroxykaur-16-en-19-oic acid, *ent*-11α-hydroxy-15α-acetoxykaur-16-en-19-oic acid, *ent*-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid and adenostemmoic acids A–G and their nine glycosides, paniculosides II and III and adenostemmosides A–G were characterized from *Adenostemma lavenia* O. KUNTZE. *ent*-11α-Hydroxy-15-oxo-kaur-16-en-19-oic acid and adenostemmoic acid B showed cytotoxic activity against L-5178Y cultured cell and prolonged the survival of mice.

Keywords *Adenostemma lavenia*; diterpene; kaurane; adenostemmoic acid; adenostemmoside; cytotoxicity; antitumor activity

In connection with a study on the terpenic glycosides of some plants in Compositae, we investigated *Adenostemma lavenia* O. KUNTZE (Syn. *A. viscosum* FORST.). From Taiwanian *A. lavenia* four *ent*-kaurane-type diterpenes **1**, **3**, **5** and dihydroderivative of **5** were isolated by Cheng *et al.*,[1] but there is no report on the glycosides.

We now wish to report the isolation and structure of nineteen kaurane-type diterpenes and their anticancer activity.

The suspension of methanolic extract of fresh whole plants in water was extracted with ether and the water layer was passed through an Amberlite XAD-2 column. The ether extract was partitioned between hexane–benzene (1 : 1) and methanol–water (8 : 2). The hypophilic layer afforded seven lesser polar diterpenes (**1**, **3**, **5**, **7**, **9**, **16**, **18**) and the methanol eluate of the Amberlite XAD-2 column afforded twelve more polar diterpenes (**2**, **4**, **6**, **8**, **10**, **11**—**15**, **17**, **19**) after repeated chromatography. Five known compounds (**1**—**3**, **5**, **6**) were identified by comparison with reported data[1-6]: [proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, $[\alpha]_D$, circular dichroism (CD) and melting points].

Adenostemmoside A (**4**), $C_{28}H_{42}O_{10}\cdot 1/4\,H_2O$, $[\alpha]_D$ −97.0° was obtained as an amorphous powder. Its ^{1}H- and ^{13}C-NMR spectra were very similar to those of **3**, except for the presence of six more carbon signals due to a glucopyranosyl residue. Acid hydrolysis gave glucose as the sugar moiety, while enzymatic hydrolysis gave **3** as an aglycone. In the ^{13}C-NMR spectrum of **4**, C-19 was shifted upfield by 3.1 ppm compared with that of **3**, but the other carbons due to an aglycone moiety remained almost unshifted and an anomeric carbon appeared at δ 95.8, suggesting that **4** was an ester-type glucoside.

Adenostemmoic acid A (**9**), $C_{20}H_{30}O_4\cdot 1/2\,H_2O$, $[\alpha]_D$ −110.0° was obtained as colorless needles, mp 198—201 °C. The ^{1}H- and ^{13}C-NMR spectra were very similar to those of **1**, but a carbinyl proton signal due to H-15 and long-range coupled with H_2-17, was shifted downfield by 1.01 ppm more than that of **1** causing the effect of β-hydroxyl group at C-11 and suggesting that the hydroxyl group at C-15 was α. Furthermore, the difference of the γ-effects of the C-15 hydroxyl group between **9** and **1** (**9**: C-7; −3.5 ppm, C-8; +2.4 ppm, C-9; +8.4 ppm, C-16; +2.9 ppm, C-17; +1.2 ppm) resembled those between *ent*-15α-hydroxykaur-16-en-19-oic acid and β-isomer.[7]

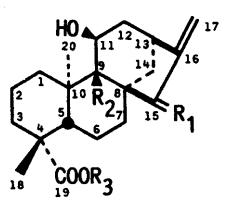
Adenostemmoic acid B (**7**). $C_{20}H_{28}O_5$, $[\alpha]_D$ −124.4° was obtained as colorless needles, mp 273—274 °C. The ultraviolet (UV) spectrum showed an absorption maximum at 243 (3.78) nm. The ^{1}H-NMR spectrum showed the presence of two tertiary methyl at δ 1.32 and 1.36, a carbinyl at δ 4.14 (d, J=4 Hz) and *exo* methylene protons conjugated with a carbonyl group at δ 5.18 (s) and 5.96 (s). In the ^{13}C-NMR spectrum, a quarternary carbon bearing an oxygen atom was observed at δ 79.5 and C-1, C-5, C-7 and C-14 were shifted upfield by 1.7, 6.6, 4.7 and 3.9 ppm, respectively, and C-8, C-9 and C-10 were shifted downfield by 5.9, 16.2 and 5.1 ppm, respectively, compared with those of **5**. A hydroxyl group was therefore found at C-9. The CD spectrum of **7** showed a negative Cotton effect $[\theta]_{348}$ −1550, suggesting that **7** had an *ent*-kaur-16-en skeleton like **5**.[1,8]

Adenostemmoside B (**8**), $C_{26}H_{38}O_{10}\cdot H_2O$, $[\alpha]_D$ −64.3° was obtained as colorless needles, mp 172—173 °C. The ^{13}C-NMR spectrum suggested that **8** was an ester-type glucoside of **7**. This assumption was confirmed by acid and enzymatic hydrolysis to afford glucose and **7**, respectively.

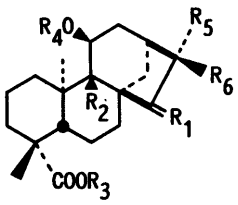
Adenostemmoic acid C (**10**), $C_{20}H_{32}O_6\cdot 1/4\,H_2O$, $[\alpha]_D$ −97.8° was obtained as colorless needles, mp 289—292 °C. In the ^{13}C-NMR spectrum of **10**, four oxygenated carbons were observed at δ 64.9 (d), 70.1 (t), 79.1 (s), and 82.8 (d). Acetylation of **10** afforded the triacetate (**10a**) and its ^{1}H-NMR spectrum showed the presence of two secondary acetoxyl [δ 4.75 (s); 5.37 (d, J=3 Hz)] and a primary acetoxyl group [δ 4.36 (d, J=11 Hz); 4.76 (d, J=11 Hz)]. In the ^{1}H-NMR spectrum of **10**, two carbinyl methine protons were assigned to H-11α [δ 4.14 (br d, J=5 Hz)] and H-15α [δ 3.60 (s)] by comparison with those of **1**, and the nuclear Overhauser effect (NOE) was observed at H-15 by irradiation of the hydroxymethyl proton signal at δ 3.90 (2H, s) in the difference NOE spectrum. **10b** was prepared from **1** by OsO_4 oxidation (attacked from the α side of the double bond due to the steric hindrance of the hydroxyl group at C-11),[9] and the C-16 (δ 64.9) was shifted upfield by 5.2 ppm in comparison to that (δ 70.1) of **10** by steric interaction in the ^{13}C-NMR spectrum. These data led us to conclude that the structure of adenostemmoic acid C is **10**.

Adenostemmoside C (**11**), $C_{26}H_{42}O_{11}\cdot H_2O$, $[\alpha]_D$ −57.7° was obtained as colorless needles, mp 172—176 °C. Acid and enzymatic hydrolysis afforded glucose and **10**, respectively. The ^{13}C-NMR spectrum showed that **11** was an ester-type glucoside of **10**.

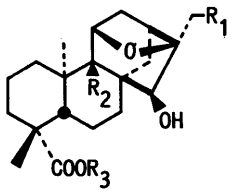
Adenostemmoic acid D (**12**), $C_{20}H_{30}O_6\cdot 1/2H_2O$, $[\alpha]_D$ −92.7 °C was obtained as colorless needles, mp 250—253 °C. The ^{13}C-NMR spectrum showed the presence of three oxygenated carbons at δ 65.0 (d), 65.8 (t), and 83.3 (s)



	R_1	R_2	R_3
1		H	H
2		H	Glc
3		H	H
4		H	Glc
5	O	H	H
6	O	H	Glc
7	O	OH	H
8	O	OH	Glc
9		H	H



	R_1	R_2	R_3	R_4	R_5	R_6
10		H	H	H	CH_2–OH	OH
10a		H	H	Ac	CH_2–OAc	OH
10b		H	H	H	OH	CH_2–OH
11		H	Glc	H	CH_2–OH	OH
12	O	H	H	H	CH_2–OH	OH
12a	O	H	H	Ac	CH_2–OAc	OH
12b	O	H	H	H	OH	CH_2–OH
13	O	H	Glc	H	CH_2–OH	OH
14	O	OH	H	H	CH_2–OH	OH
14a	O	OH	H	Ac	CH_2–OAc	OH
14b	O	OH	H	H	OH	CH_2–OH
15	O	OH	Glc	H	CH_2–OH	OH



	R_1	R_2	R_3
16	H	OH	H
16a	H	H	H
17	H	OH	Glc
18	OH	H	H
19	OH	H	Glc

Chart 1

TABLE I. ^{1}H-NMR Chemical Shifts and Coupling Constants

Proton No.	11	15	17	18	20	Anomeric H	Ac
4		5.14 (1H, br s)	5.06 (1H, br s) 5.44 (1H, br s)	1.22 (3H, s)	1.10 (3H, s)	6.10 (1H, d, J=8 Hz)	2.18 (3H, s)
7	4.14 (1H, d, J=4 Hz)		5.18 (1H, s) 5.96 (1H, s)	1.36 (3H, s)	1.32 (3H, s)		
8			5.16 (1H, s) 5.91 (1H, s)	1.38 (3H, s)	1.28 (3H, s)	6.12 (1H, d, J=8 Hz)	
9	4.18 (1H, br d, J=4 Hz)	5.05 (1H, br s)	5.32 (1H, br s) 5.52 (1H, br s)	1.36 (3H, s)	1.12 (3H, s)		
10	4.14 (1H, br d, J=5 Hz)	3.60 (1H, s)	3.90 (2H, s)	1.32 (3H, s)	1.11 (3H, s)		
10a	5.37 (1H, d, J=3 Hz)	4.75 (1H, s)	4.36 (1H, d, J=11 Hz) 4.76 (1H, d, J=11 Hz)	1.32 (3H, s)	1.10 (3H, s)		2.08 (3H, s) 2.17 (3H, s)
10b	4.13 (1H, d, J=6.5 Hz)	4.16 (1H, s)	4.60 (1H, d, J=11 Hz) 4.85 (1H, d, J=11 Hz)	1.32 (3H, s)	1.17 (3H, s)		2.25 (3H, s)
11			3.90 (2H, s)	1.26 (3H, s)	1.20 (3H, s)	6.12 (1H, d, J=8 Hz)	
12	4.24 (1H, br d, J=4 Hz)		4.14 (2H, s)	1.30 (3H, s)	1.16 (3H, s)		
12a	5.20 (1H, m)		4.40 (1H, d, J=12 Hz) 4.60 (1H, d, J=12 Hz)	1.28 (3H, s)	1.15 (3H, s)		1.95 (3H, s) 2.00 (3H, s)
12b	4.23 (1H, d, J=5.5 Hz)		4.73 (1H, d, J=12 Hz) 5.17 (1H, d, J=12 Hz)	1.31 (3H, s)	1.21 (3H, s).		
13				1.26 (3H, s)	1.20 (3H, s)	6.10 (1H, d, J=8 Hz)	
14	4.06 (1H, d, J=4 Hz)		4.12 (2H, s)	1.35[a]	1.35[a]		
14a	5.42 (1H, d, J=4 Hz)		4.48 (1H, d, J=12 Hz) 4.64 (1H, d, J=12 Hz)	1.35[a]	1.35[a]		2.00 (6H, s)
14b	4.13 (1H, d, J=5.5 Hz)		4.65 (1H, d, J=11.5 Hz) 5.09 (1H, d, J=11.5 Hz)	1.40 (3H, s)	1.36 (3H, s)		
15				1.45 (3H, s)	1.28 (3H, s)	6.14 (1H, d, J=8 Hz)	
16	4.30 (1H, br s)	3.32 (1H, s)	1.51 (3H, s)	1.36[a]	1.36[a]		
17		3.32 (1H, s)	1.48 (3H, s)	1.42 (3H, s)	1.32 (3H, s)	6.30 (1H, d, J=7 Hz)	
18	4.49 (1H, br s)	3.58 (1H, s)	4.08 (2H, s)	1.28 (3H, s)	1.18 (3H, s)		
19		3.52 (1H, s)		1.22[a]	1.22[a]	6.10 (1H, d, J=7 Hz)	

Run at 89.55 MHz in pyridine-d_5 solution. *a*) Overlapping.

and a ketonic carbon at δ 221.9. Acetylation of **12** afforded the diacetate (**12a**) and its ^{1}H-NMR spectrum showed the presence of a secondary acetoxyl [δ 5.20 (m)] and a primary acetoxyl group [δ 4.40 (d, J=12 Hz); 4.60 (d, J=12 Hz)]. In the ^{13}C-NMR spectrum, C-17 (δ 65.8) was shifted downfield by *ca.* 1.8 ppm in comparison with that of **12b**, as obtained from OsO_4 oxidation of **5**. So, the structure of adenostemmoic acid D was determined to be **12**.

Adenostemmoside D (**13**), $[\alpha]_D$ −80.9° was obtained as colorless needles, mp 178—180 °C. The fast atom bombardment mass spectrum (FAB-MS) showed ion peaks at m/z 529 $(C_{26}H_{40}O_{11}+H)^+$ and 551 $(C_{26}H_{40}O_{11}+Na)^+$. Acid and enzymatic hydrolysis afforded glucose and **12**, respectively. Anomeric signals in the NMR spectra showed **13** to be an ester-type glucoside of **12**.

Adenostemmoic acid E (**14**), $C_{20}H_{30}O_7\cdot 1/4\,H_2O$, $[\alpha]_D$ −81.3° was obtained as colorless needles, mp 268—272 °C. Acetylation of **14** afforded the diacetate (**14a**) and its ^{1}H-NMR spectrum showed the presence of a secondary acetoxyl [δ 5.42 (d, J=4 Hz)] and a primary acetoxyl group [δ 4.48 (d, J=12 Hz); 4.64 (d, J=12 Hz)]. The ^{13}C-NMR spectrum showed the presence of four oxygenated carbons at δ 65.8 (t), 66.2 (d), 78.5 (s), 82.6 (s) and a ketonic carbon at δ 220.5. The C-8 and C-10 signals were shifted down-field by 5.2 ppm and 4.7 ppm, respectively, compared with those of **12**, suggesting that C-9 was oxygenated. The stereochemistry at C-16 was determined as in **12**.

Adenostemmoside E (**15**), $[\alpha]_D$ −56.7° was obtained as colorless needles, mp 178.5—180 °C. The FAB-MS showed ion peaks at m/z 545 $(C_{26}H_{40}O_{12}+H)^+$ and 567 $(C_{26}H_{40}O_{12}+Na)^+$. From the ^{13}C-NMR data and the results of acid and enzymatic hydrolysis, **15** was determined to be a glucoside of **14**.

Adenostemmoic acid F (**16**), $C_{20}H_{30}O_5\cdot 1/4\,H_2O$, $[\alpha]_D$ −41.5° was obtained as colorless plates, mp 250—253 °C. The ^{1}H-NMR spectrum showed the presence of three singlet methyl at δ 1.36 (6H), and 1.51 and two carbinyl proton signals at δ 3.32 (s), and 4.30 (br s). The ^{13}C-NMR spectrum showed the presence of four oxygenated carbons at δ 78.9, 81.1, 83.8, and 84.7; C-5 was shifted upfield by 7.0 ppm and C-8 and C-10 were shifted downfield by 2.3 ppm and 6.1 ppm, respectively, compared with those of *ent*-11α-16α-epoxy-15α-hydroxy-16*S*-kauran-19-oic acid (**16a**).[10] C-9 was therefore oxygenated. The NOE was observed at H-15 by irradiation at the methyl proton signal (δ 1.51) in the difference NOE spectrum, suggesting that the methyl group at C-16 was α.

Adenostemmoside F (**17**), $[\alpha]_D$ −43.3° was obtained as colorless plates, mp 158—161 °C. The FAB-MS showed an ion peak at m/z 535 $(C_{26}H_{40}O_{10}+Na)^+$. From the ^{13}C-NMR data and the results of hydrolyses, **17** was a glucoside of **16**.

Adenostemmoic acid G (**18**), $[\alpha]_D$ −49.7° was obtained as colorless needles, mp 217—220 °C. The high-resolution

Table II. ^{13}C-NMR Chemical Shifts

	4	7	8	9	10	10a	10b	11	12	12a	12b	13	14	14a	14b	15	16	17	18	19
Aglycone moiety																				
1	40.6	38.6	38.5	41.0	41.0	40.9	40.9[e]	40.7	40.5	40.0	40.1	40.1	36.4	33.9	37.8	36.2	39.8	39.8	41.8	41.6
2	19.7	19.8	19.6	20.1	20.0	19.9	20.0	19.7	19.8	19.5	19.6	19.3	19.7	19.5	19.8	19.3	19.8	19.5	19.9	19.6
3	38.4	38.6	38.5	38.9	38.7	38.6	38.9	38.4	38.7	39.3	38.5	38.2	38.5	38.3	38.8	38.1	38.6	38.3	38.6	38.5
4	44.3	44.5	44.6	44.2	43.9	44.1	44.1	44.4	44.2	44.0	43.9	44.1	44.6[j]	44.0	44.3[k]	44.4[l]	44.0	44.3	43.9	44.1
5	57.1	50.0	50.7	57.6	56.5	56.3	56.7[f]	57.1	56.7	56.0	56.3	56.9	49.8	49.1	49.8	50.4	50.0	50.6	57.3	57.6
6	21.7	21.2	21.0	22.2	22.2	21.8	22.7	21.8	20.8	21.3	21.1	20.2	20.9	21.0	21.4	20.5	22.5	22.1	22.2	21.7
7	36.4	32.7	32.6	36.8	36.0	33.7[c]	36.8	35.9	35.4	34.6	35.9[g]	35.1	34.8	33.6	35.8	34.5	32.8	32.5	35.4	35.5
8	45.0	56.9	57.0	48.0	46.1	45.2	48.1	46.1	51.1	51.1	51.8	50.9	56.3	56.0	57.1	56.2	48.1	48.1	46.4	46.3
9	57.1	79.5	79.2[b]	64.0	56.7	54.3	57.3[f]	56.4	63.4	59.4	64.1[h]	63.2	78.5	77.3	79.0	78.5	78.9	78.8	51.9	52.0
10	39.6[a]	44.4	44.6	39.3	38.7	39.2	38.8	38.7	39.5	40.0	39.2	39.3	44.2[j]	44.5	44.2[k]	44.3[l]	43.1	43.1	37.3	37.3
11	66.3	66.3	66.4	66.1	64.9	68.2	64.9	64.9	65.0	67.0	64.7	64.9	66.2	69.0	65.5	66.1	83.8	84.7	76.9	76.7
12	43.1	42.7	42.8	43.1	34.4	33.4[c]	34.7	34.1	33.8	32.2	37.0[i]	33.2	32.7	32.5	32.4	32.4	37.4	37.3	38.8	38.5
13	39.6[a]	37.4	37.4	42.2	40.4	40.3[d]	44.1	40.7	34.8	32.6	34.5[g]	34.6	37.0	37.5	40.3	36.9	41.7	41.7	39.3	39.1
14	39.0[a]	31.0	31.0	36.5	41.0	40.4[d]	41.4[e]	40.4	37.5	37.6	40.5[i]	37.3	30.7	30.6	32.0	30.6	32.2	32.2	39.1	39.1
15	82.6	207.3	207.7	81.8	82.8	81.7	83.4	79.2	221.9	221.9	219.5	221.8	220.5	220.6	219.1	220.6	81.1	81.1	79.4	79.2
16	153.8	151.6	151.7	162.5	79.1	69.9	91.2	82.9	83.3	80.9	81.1	83.1	82.6	81.1	81.0	82.6	84.7	83.8	88.1	88.1
17	108.1	111.1	111.4	107.1	70.1	77.1	64.9	70.1	65.8	68.1	64.0[h]	65.8	65.8	67.0	63.9	65.7	21.0	20.9	63.5	63.1
18	28.7	29.7	29.1	29.6	29.4	29.5	29.6	28.8	29.5	29.9	29.2	28.5	29.7	29.3	29.8	28.8	30.0	29.3	29.6	28.9
19	176.7	180.4	177.3	180.4	180.6	180.6	181.1	176.8	179.9	180.0	180.0	176.6	180.4	180.1	181.5	177.0	180.7	177.3	180.3	177.0
20	15.5	17.6	17.6	16.1	15.7	15.7	15.9	15.7	16.1	16.0	15.9	15.8	17.6	17.4	17.5	17.3	19.8	19.9	18.3	18.3
Sugar moiety																				
1	95.8		95.9					95.8				95.8				95.8		95.7		95.8
2	74.1		74.2					74.2				74.1				74.2		74.2		74.1
3	79.2		79.6[b]					79.2				79.1				79.1		79.1		79.2
4	71.2		71.4					71.2				71.2				71.2		71.3		71.2
5	79.0		79.3[b]					79.2				79.3				79.3		79.2		79.2
6	62.3		62.5					62.4				62.3				62.3		62.4		62.4
Ac	170.8					168.7				169.4				168.8						
	21.2					170.7				170.6				170.5						
						171.3				20.6				20.5						
						21.1				20.6				20.5						
						21.2														
						21.7														

Run at 22.5 MHz in pyridine-d_5 solution. *a—l*) Assignments may be interchanged in each column.

Table III. Cytotoxicity and Prolongation of Survival in Mice

Compound	ID_{50} (μg/ml)	%	(Dose)
5	2.8	163	(100 mg/kg × 5 d)
7	4.2	178	(100 mg/kg × 5 d)
6	>100	107	(150 mg/kg × 5 d)
8	>100	110	(150 mg/kg × 5 d)
Mitomycin C	0.05	205	(0.8 mg/kg × 5 d)

mass spectrum (high-MS) showed the molecular formula $C_{20}H_{30}O_5$. The ^{1}H-NMR spectrum was similar to that of **16**, except for the appearance of hydroxymethyl proton signals at δ 4.08 (2H, s). The NOE was observed at H-15 by irradiation at the hydroxymethyl proton signal in the difference NOE spectrum. The structure of adenostemmoic acid G was thus determined to be **18**.

Adenostemmoside G (**19**), $C_{26}H_{40}O_{10}\cdot H_2O$, $[\alpha]_D$ −50.0° was obtained as colorless needles, mp 154—156 °C and determined to be a glucoside of **18**.

The absolute configuration of compounds **5** and **7** was found to be *ent*-kauranoic acid type from the CD curve (n–π*) (negative Cotton effect at 343 and 348 nm, respectively). The other compounds have no spectroscopic evidence of *ent*-type, but they are also presumed to be so by the occurrence of *ent*-kauranoic acid type compounds **5** and **7**.

The cytotoxic activities of related glycosides and aglycones having an exocyclic α-methylane ketone structure, which is thought to be a cytotoxic activator, were compared in the L-5178Y cultured cell system.[11] The results are summarized in Table III. These data suggested that the glycosides have lower cytotoxic activity towards L-5178Y cells as in the case of sesquiterpene lactone glycosides.[12] Aglycones **5** and **7** have the effect of prolonging the survival of mice inoculated with Sarcoma-180.

Experimental

Melting points were taken on a Yanaco MP-500 micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. UV spectra were taken on a Shimadzu UV-360 recording spectrophotometer and CD spectra were recorded on a JASCO 20A spectropolarimeter. MS and FAB-MS were measured on JEOL JMS-D100 and JEOL JMS-SX102 mass spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX 90Q (89.55 and 22.5 MHz, respectively). Chemical shifts are given on the δ scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was done on a Hitachi G-3000 gas chromatograph. High-performance liquid chromatography (HPLC) was done on a JASCO medel 800 instrument.

Isolation Whole plants of fresh *A. lavenia* O. Kuntze (7 kg) collected in October 1988, in Shizuoka, Japan, were extracted twice with methanol under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with ether to give a gum (54 g). The water layer was passed through an Amberlite XAD-2 column and the absorbed material was eluted with methanol to give an amorphous powder (32 g). The gum was partitioned between hexane–benzene (1:1) and methanol–water (8:2) to give a lipophilic fraction (40 g) and a hypophilic fraction (13 g). The hypophilic fraction and the amorphous powder were chromatographed on a silica gel column and semi-preparative HPLC to give compounds **1** (500 mg), **3** (1.1 g), **5** (1.9 g), **7** (1.0 g), **9** (20 mg), **16** (25 mg), **18** (27 mg) and **2** (4.0 g), **4** (3.0 g), **6** (5.3 g), **8** (1.8 g), **10** (100 mg), **11** (180 mg), **12** (88 mg), **13** (15 mg), **14** (130 mg), **15** (8 mg), **17** (6 mg), **19** (160 mg), respectively.

Adenostemmoside A (4) Amorphous powder, $[\alpha]_D^{22}$ −97.0° (c=1.00, MeOH). *Anal.* Calcd for $C_{28}H_{42}O_{10}\cdot 1/4\,H_2O$: C, 61.92; H, 7.89. Found: C, 62.11; H, 7.90. FAB-MS m/z: 561 $(M+Na)^+$, 539 $(M+H)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid B (7) Recrystallized from chloroform–methanol as colorless needles, mp 273—274 °C, $[\alpha]_D^{22}$ −124.4° (c=0.80, MeOH). *Anal.* Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.69; H, 8.12. UV λ_{max}^{MeOH} nm (log ε): 243 (3.78). MS m/z: 348 (M^+, 18), 330 (M^+-H_2O, 15), 302 (29), 219 (21), 109 (100). CD (c=0.054, MeOH) [θ] (nm): −1550 (348), +6440 (245), −18300 (216). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoside B (8) Recrystallized from methanol as colorless needles, mp 172—173 °C, $[\alpha]_D^{22}$ −64.3° (c=1.57, MeOH). *Anal.* Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.69; H, 8.12. UV λ_{max}^{MeOH} nm (log ε): 243 (3.84). FAB-MS m/z: 533 $(M+Na)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid A (9) Recrystallized from methanol as colorless needles, mp 198—201 °C, $[\alpha]_D^{22}$ −110.0° (c=0.35, MeOH). *Anal.* Calcd for $C_{20}H_{30}O_4\cdot 1/2\,H_2O$: C, 69.94; H, 9.10. Found: C, 69.78; H, 8.83. MS m/z: 334 (M^+, 7), 316 (M^+-H_2O, 15), 301 ($M^+-H_2O-CH_3$, 9), 298 (9), 84 (100). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid C (10) Recrystallized from methanol as colorless needles, mp 289—292 °C, $[\alpha]_D^{22}$ −97.8° (c=0.68, MeOH). *Anal.* Calcd for $C_{20}H_{32}O_6\cdot 1/4\,H_2O$: C, 64.41; H, 8.78. Found: C, 64.74; H, 8.65. MS m/z: 368 (M^+, 1), 332 (20), 319 (21), 304 (29), 93 (100). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoside C (11) Recrystallized from methanol–water as colorless needles, mp 172—176 °C, $[\alpha]_D^{22}$ −57.7° (c=0.26, MeOH). *Anal.* Calcd for $C_{26}H_{42}O_{11}\cdot H_2O$: C, 56.92; H, 8.08. Found: C, 56.67; H, 7.89. FAB-MS m/z: 553 $(M+Na)^+$. 531 $(M+H)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid D (12) Recrystallized from chloroform–methanol as colorless needles, mp 250—253 °C, $[\alpha]_D^{22}$ −92.7° (c=1.03, MeOH). *Anal.* Calcd for $C_{20}H_{30}O_6\cdot 1/2\,H_2O$: C, 63.98; H, 8.32. Found: C, 63.81; H, 8.40. MS m/z: 348 (M^+-H_2O, 1), 320 (38), 289 (40), 215 (38), 93 (100). CD (c=0.061, MeOH) [θ] (nm): +1200 (307). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoside D (13) Recrystallized from methanol–water as colorless needles, mp 178—180 °C, $[\alpha]_D^{22}$ −80.9° (c=0.34, MeOH). FAB-MS m/z: 551 $(M+Na)^+$, 529 $(M+H)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid E (14) Recrystallized from chloroform–methanol as colorless needles, mp 268—272 °C, $[\alpha]_D^{22}$ −81.3° (c=0.16, MeOH). *Anal.* Calcd for $C_{20}H_{30}O_7\cdot 1/4\,H_2O$: C, 62.08; H, 7.94. Found: C, 62.25; H, 7.90 MS m/z: 364 (M^+-H_2O, 1), 109 (100). CD (c=0.021, MeOH) [θ] (nm): +5090 (320), −8730 (221). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoside E (15) Recrystallized from methanol–water as colorless needles, mp 178.5—180 °C, $[\alpha]_D^{22}$ −56.7° (c=0.52, MeOH). FAB-MS m/z: 567 $(M+Na)^+$, 545 $(M+H)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid F (16) Recrystallized from chloroform–methanol as colorless needles, mp 250—253 °C, $[\alpha]_D^{22}$ −41.5° (c=0.94, MeOH). *Anal.* Calcd for $C_{20}H_{30}O_5$: C, 67.67; H, 8.66. Found: C, 67.52; H, 8.47. MS m/z: 350 (M^+, 13), 335 (M^+-CH_3, 8), 332 (M^+-H_2O, 9), 322 (11), 97 (100). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoside F (17) Recrystallized from methanol–water as colorless plates, mp 158—161 °C, $[\alpha]_D^{22}$ −43.3° (c=0.60, MeOH). FAB-MS m/z: 535 $(M+Na)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoic Acid G (18) Recrystallized from chloroform–methanol as colorless needles, mp 217—220 °C, $[\alpha]_D^{22}$ −49.7° (c=1.68, MeOH). MS m/z: 350 (M^+, 3), 320 (9), 304 (6), 149 (17), 69 (100). High-MS m/z: $C_{20}H_{30}O_5$, 350.2076 (Calcd for $C_{20}H_{30}O_5$: 350.2093). ^{1}H- and ^{13}C-NMR: Tables I and II.

Adenostemmoside G (19) Recrystallized from methanol–water as colorless needles, mp 154—156 °C, $[\alpha]_D^{22}$ −50.0° (c=0.84, MeOH). *Anal.* Calcd for $C_{26}H_{40}O_{10}\cdot H_2O$: C, 58.85; H, 7.98. Found: C, 58.80; H, 7.68. FAB-MS m/z: 535 $(M+Na)^+$, 513 $(M+H)^+$. ^{1}H- and ^{13}C-NMR: Tables I and II.

Acetylation of 10, 12 and 14 **10** (20 mg), **12** (5 mg) and **14** (5 mg) were acetylated in the usual manner using acetic anhydride and pyridine to give the acetate **10a** (13 mg), **12a** (4 mg) and **14a** (4 mg) as an amorphous powder, respectively. ^{1}H- and ^{13}C-NMR: Tables I and II.

Osmium Tetraoxide Oxidation of 1, 5 and 7 To a solution of **1** (30 mg) in pyridine (2 ml) and anhydrous ether (1 ml) was added OsO_4 (50 mg). The reaction mixture was stirred for 12 h at room temperature and the excess OsO_4 was destroyed with a solution of $NaHSO_3$ (200 mg) in water (3 ml) and pyridine (1 ml). The product was extracted with ethyl acetate after addition of water (5 ml) to the reaction mixture and purified by preparative thin layer chromatography [Kiesel gel PF_{254}, benzene–acetone–acetic acid (70:30:1)] as colorless amorphous powder **10b** (11 mg), **5** (17 mg) and **7** (30 mg) gave **12b** (8 mg) and **14b** (10 mg), respectively, in the same manner. ^{1}H- and ^{13}C-NMR: Tables I and II.

Enzymatic Hydrolysis of 4, 8, 11, 13, 15, 17 and 19 Each glycoside (*ca.* 0.5 mg) was dissolved in water (0.2 ml) and the solution was treated with

cellulase (Sigma) (1 mg) overnight at 37 °C with stirring; then the reaction mixture was extracted with ethyl acetate. Aglycones **3**, **7**, **10**, **12**, **14**, **16** and **18** were detected by thin layer chromatography [Kiesel gel HF_{254}: benzene–acetone–acetic acid (70 : 30 : 3): *Rf* 0.63, 0.58, 0.09, 0.16, 0.18, 0.52 and 0.31, respectively] from the corresponding glycosides.

Acid Hydrolysis of 4, 8, 11, 13, 15, 17 and 19 A solution of a glycoside (*ca.* 0.5 mg) in 5% H_2SO_4 (2 drops) was heated in a boiling water bath for 30 min. The solution was passed through an Amberlite IRA-45 column and concentrated to give a residue, which was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the residue was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (1 drop each) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. From each glycoside, glucitol acetate was detected by GC. Conditions: column, Spelco SPB 35 capillary column (0.75 mm × 30 m); column temperature, 230 °C; carrier gas, N_2; t_R, 8.2 min.

Test for Antitumor Activity (i) Sarcoma 180 ascites carcinoma (10^6 cells/mouse) was inoculated intraperitoneally (i.p.) into female ICR strain mice (five mice were used for each test sample). A suspension of test sample in normal saline was injected i.p. once daily for five consecutive days, starting 24 h after the tumor implantation. The effect was evaluated as the ratio of survival days in treated and control groups (%, treated/control). (ii) L-5178Y leukemia cells were cultured in a stoppered tube in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C. The growth inhibitory effect was determined as the ratio of cell numbers, which were counted visually with a microscope in treated and control groups (%, treated/control) after incubation of 10^5 cells/ml for 48 h with various concentrations of each test sample. To express the results, the ID_{50} (50% inhibiting dose) value was calculated by probit diagramming analysis.

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