Structures and Antiproliferative Activity of Saponins from *Sechium pittieri* and *S. talamancense*

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Six bisdesmosidic bayogenin saponins, named tacacosides A_1, A_2, B_1, B_2, B_3 and C, were isolated from the fruit and aerial parts of *Sechium pittieri* (COGN.) C. JEFFREY and *S. talamancense* (WUNDERLIN) C. JEFFREY, Costa Rican cucurbitaceae plants. Their structures were elucidated based on spectral and chemical evidence as follows. Tacacoside A_1: 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]bayogenin 28-*O*-{α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} ester, tacacoside A_2: 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]bayogenin 28-*O*-{α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} ester, tacacoside B_1: 3-*O*-[β-D-glucopyranosyl]bayogenin 28-*O*-{α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} ester, tacacoside B_2: 3-*O*-[β-D-glucopyranosyl]bayogenin 28-*O*-{α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} ester, tacacoside B_3: 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]bayogenin 28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, and tacacoside C: 3-*O*-[β-D-glucopyranosyl]bayogenin 28-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester. These saponins showed moderate antiproliferative activity (ED_{50}: 10—74 μg/ml) against MK-1, HeLa and B16F10 cells.

Key words *Sechium*; cucurbitaceae; tacacoside; bayogenin glycoside; antiproliferative activity

Sechium species are cucurbitaceous vines distributed in the tropical and subtropical regions of the world, and the fruits of some of them are eaten as vegetables. In Costa Rica, there grow several *Sechium* species, *Sechium edule* (JACQ.) SW., *S. tacaco* (PITT.) C. JEFFREY, *S. pittieri* (COGN.) C. JEFFREY and *S. talamancense* (WUNDERLIN) C. JEFFREY. The former two are cultivated, and the fruit of *S. edule*, locally called "chayote," and the fruit of *S. tacaco* called "tacaco" are edible; they are both popularly eaten as cooked vegetables in Costa Rica. The latter two species are wild ones, and *S. talamancense* is supposed to be the wild precursor of *S. tacaco*. The fruits of *Sechium pittieri* and *S. talamancense* are also called "tacaco", but people do not ordinarily eat these fruits because they are too bitter. The fruits and the aerial parts of the wild species have an unpleasant bitter taste, they irritate the throat and make one feel like vomiting. Ground fruits and leaves form stable foams when shaken vigorously in water, suggesting the presence of saponins. Six saponins having a disgusting taste were isolated from the fruits and the aerial parts of *S. pittieri* and *S. talamancense*, and they were named tacacosides A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), B_3 (**5**) and C (**6**). The present paper describes the isolation, structural elucidation and antiproliferative activity of these saponins against some tumor cells.

The fruits were homogenized in MeOH and the saponin fraction was separated using a styrene polymer MCI gel, Diaion HP-20. The analytical scale high-performance liquid chromatography (HPLC) of the saponin fraction showed three peaks, and the corresponding fractions were separated by preparative scale HPLC, giving frs. A, B and C. Fraction C was shown to be a pure compound and was named tacacoside C. Fractions A and B were mixtures of several saponins judging from FAB-MS and NMR spectra. These two fractions were acetylated and the component acetates were separated by preparative HPLC. The original saponins, tacacosides A_1 (**1**) and A_2 (**2**) from the fr.A acetate, and tacacosides B_1 (**3**), B_2 (**4**) and B_3 (**5**) from the fr.B acetate, were obtained by treatment of the corresponding acetate with sodium methoxide.

The dried aerial parts of both species were percolated with MeOH and the MeOH extracts were suspended in water. After removing the water-insoluble materials by centrifugation, the water solutions were treated with Diaion HP-20 in the same manner as for the fruit extracts. The saponins were isolated in the same way. The yield of each saponin in the fruits and the aerial parts of *S. pittieri* and *S. talamancense* are given in Table 1.

Tacacoside A_1 (**1**) was isolated as a white amorphous powder. The positive-ion FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1523, while the negative-ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 1499, indicating the molecular weight to be 1500. The high-resolution (HR) FAB-MS gave the molecular formula $C_{69}H_{112}O_{35}$. On acid hydrolysis, **1** gave L-arabinose, D-xylose, D-apiose, L-rhamnose and D-glucose. The ^{1}H-NMR spectrum showed signals of six tertiary methyl groups (δ 0.91, 1.01, 1.13, 1.23, 1.33, 1.55), one trisubstituted olefinic proton (δ 5.46, br s) (Table 2) and seven sugar anomeric protons at δ 5.10 (d, $J=7$ Hz), 5.20 (d, $J=8$ Hz), 5.26 (d, $J=8$ Hz), 5.57 (d, $J=2$ Hz), 5.88 (d, $J=5$ Hz), 6.13 (d, $J=2$ Hz), and 6.54 (br s) (Table 3).

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The ^{13}C-NMR spectrum showed signals of six C–C bonded quaternary carbons (δ 30.9, 36.9, 40.0, 42.2, 42.8, 47.3), one ester carbonyl carbon (δ 176.2), a pair of olefinic carbons (δ 123.0, 144.1) (Table 4) and seven anomeric carbons (δ 92.9, 100.9, 102.7, 104.9, 105.4, 105.9, 112.0) (Table 3). These spectral data suggested that **1** is a glycoside of an oleanene-type triterpene carboxylic acid in which the sugar moiety is linked to the carboxyl group with an ester linkage. The aglycone was identified as bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid) (**7**) by comparison of the ^{13}C-NMR chemical shifts of the carbon signals of the aglycone moiety of **1** with those of bayogenin[1] and 3-*O*-[β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl]bayogenin 28-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, the deacylation product (**8b**) of lobatoside E (**8a**), a bayogenin glycoside isolated from the aerial part of *Actinostemma lobatum* MAXIM. (Cucurbitaceae).[2a)]

The positive-ion FAB-MS of **1** showed fragment ions at *m/z* 835 ([prosapogenin + Na]$^+$), 789 ([835 − HCOOH]$^+$), and at 711. The last fragment ion ($C_{27}H_{44}NaO_{20}$) is attributed to an ester-linked sugar moiety, and the com-

Table 1. Yields (mg) of Tacacosides from the Fruits and the Aerial Parts of *Sechium pittieri* and *S. talamancense*

	Sechium pittieri		*S. talamancense*	
	Fruit	Aerial part	Fruit	Aerial part
Tacacoside A_1 (**1**)	252	8	1830	221
Tacacoside A_2 (**2**)	1078	6	—	—
Tacacoside B_1 (**3**)	47	12	227	554
Tacacoside B_2 (**4**)	113	11	—	—
Tacacoside B_3 (**5**)	113	5	1059	148
Tacacoside C (**6**)	11	12	70	362
Total yield of saponins[a)]	1622	54	3186	1285
Content (%)	0.324	0.054	0.637	1.285

a) Yields from 500 g fresh fruits and 100 g dried aerial parts.

Table 2. ^{1}H-NMR Chemical Shifts (Pyridine-d_5) of **7** and the Aglycone Moieties of **1**, **2**, **9**, **10** and **8a**[a)]

	8a	**1**	**2**	**9**	**10**	**7**
H2	4.78 br s	*ca.* 4.76	4.76 br s	4.76 dd (3, 6)	4.80 dd (4, 6)	4.52 br s
H3	4.1 d (3)	*ca.* 4.30	*ca.* 4.30	*ca.* 4.33	4.31 d (4)	4.27 d (4)
H12	*ca.* 5.50	5.46 br s	5.45 br s	5.49 t (3)	5.49 t (4)	5.52 t-like
H18	3.23 dd (4, 14)	3.30 dd (4, 14)	3.28 dd (5, 14)	3.29 dd (4, 13)	3.29 dd (4, 13)	3.31 dd (4, 14)
H23	3.66 d (11)	3.68 d (10)	3.67 d (10)	3.69 d (11)	3.68 d (11)	3.72 d (11)
	4.35 d (11)	*ca.* 4.30	*ca.* 4.27	4.34 d (11)	4.35 d (11)	4.17 d (11)
H24	1.34 s	1.33 s	1.33 s	1.33 s	1.34 s	1.37 s
H25	1.51 s	1.55 s	1.55 s	1.55 s	1.55 s	1.61 s
H26	1.13 s	1.13 s	1.15 s	1.08 s	1.08 s	1.12 s
H27	1.25 s	1.23 s	1.24 s	1.26 s	1.27 s	1.27 s
H29	0.92 s	0.91 s	0.90 s	0.93 s	0.93 s	0.93 s
H30	0.98 s	1.01 s	1.00 s	1.00 s	1.00 s	1.01 s

a) The ^{1}H-NMR chemical shifts of the aglycone moieties of **3**, **4**, **5**, and **6** were almost the same as those of **1** and **2**.

Table 3. NMR Chemical Shifts (Pyridine-d_5) of Anomeric Protons and Carbons of the Component Sugars of Tacacosides

	A_1 (**1**)		A_2 (**2**)		B_1 (**3**)	
	^{1}H (J_{H1-H2})	^{13}C (J_{H1-C1})	^{1}H (J_{H1-H2})	^{13}C (J_{H1-C1})	^{1}H (J_{H1-H2})	^{13}C
in. Glc	5.10 d (7)	105.4 (159)	5.09 d (8)	105.3 (159)	5.13 d (8)	105.6
out. Glc	5.20 d (8)	105.9 (160)	5.20 d (8)	105.9 (160)		
Ara	6.54 br s	92.9 (172)	6.51 d (2)	93.2 (171)	6.53 s	92.9
in. Rha	5.57 d (2)	100.9 (173)	5.68 br s	100.7 (171)	5.56 d (2)	100.9
out. Rha	6.13 d (2)	102.7 (171)	6.16 d (2)	102.6 (170)	6.13 d (2)	102.7
in. Xyl	5.26 d (8)	104.9 (159)	5.38 d (8)	104.7 (159)	5.26 d (8)	104.9
out. Xyl			5.07 d (8)	105.8 (160)		
Api	5.88 d (5)	112.0 (172)			5.88 d (4)	111.9

	B_2 (**4**)		B_3 (**5**)		C (**6**)	
	^{1}H (J_{H1-H2})	^{13}C	^{1}H (J_{H1-H2})	^{13}C (J_{H1-C1})	^{1}H (J_{H1-H2})	^{13}C
in. Glc	5.12 d (8)	105.6	5.09 d (8)	105.3 (160)	5.12 d (8)	105.6
out. Glc			5.21 d (8)	105.9 (160)		
Ara	6.50 d (2)	93.2	6.44 d (3)	93.4 (171)	6.44 d (3)	93.4
in. Rha	5.68 d (2)	100.7	5.76 br s	101.0 (169)	5.76 s	101.0
out. Rha	6.16 d (2)	102.6	6.18 d (2)	102.6 (169)	6.17 d (2)	102.6
in. Xyl	5.37 d (8)	104.7	5.04 d (8)	106.8 (156)	5.08 d (8)	106.8
out. Xyl	5.07 d (8)	105.8				

in., inner; out., outer.

Table 4. ^{13}C-NMR Chemical Shifts (Pyridine-d_5) of **7** and the Aglycone Moieties of **1**, **2**, **9**, **10** and **8b**[a]

C	**8b**[2b]	**1**	**2**	**9**	**10**	**7**[2b]
1	44.0	44.1	44.1	44.1	44.0	44.8
2	70.4	70.7	70.6	70.7	70.5	71.5
3	82.6	82.9	82.9	82.8	83.0	73.1
4	42.7	42.8	42.8	42.7	42.7	42.3
5	48.5	47.6	47.6	47.6	47.7	48.5
6	18.1	17.9	18.0	17.9	18.0	18.3
7	33.0	32.9	33.0	33.1	33.2	33.1
8	40.0	40.0	40.0	39.8	39.9	39.9
9	48.0	48.4	48.5	48.5	48.5	48.2
10	36.9	36.9	36.9	36.9	36.9	27.2
11	24.0	24.0	24.0	23.6	23.6	23.9
12	123.1	123.0	123.1	122.7	122.7	122.7
13	144.2	144.1	144.1	144.8	144.8	144.8
14	42.3	42.2	42.2	42.3	42.3	42.3
15	28.3	28.1	28.1	28.2	28.2	28.2
16	23.2	23.1	23.1	23.9	23.9	23.7
17	47.3	47.3	47.3	46.6	46.6	46.6
18	41.7	41.6	41.7	41.9	42.0	42.0
19	46.3	46.1	46.2	46.3	46.4	46.4
20	30.8	30.9	30.8	30.8	30.9	30.9
21	34.2	34.1	34.1	34.1	34.2	34.2
22	32.7	32.7	32.7	32.9	32.9	33.1
23	65.4	65.1	65.1	65.0	65.5	67.8
24	14.9	15.0	15.0	14.9	14.9	14.4
25	17.3	17.2	17.2	17.1	17.2	17.2
26	17.5	17.6	17.6	17.4	17.4	17.5
27	26.1	26.1	26.1	26.2	26.2	26.2
28	176.2	176.2	176.2	180.0	180.1	180.1
29	33.1	33.1	33.1	33.1	33.2	33.1
30	23.7	23.7	23.7	23.7	23.7	23.7

a) The ^{13}C-NMR chemical shifts of the aglycone moieties of **3**, **4**, **5** and **6** were almost the same as those of **1** and **2**.

position of the ion suggested that this sugar moiety is composed of one mole each of L-arabinose, D-xylose, D-apiose and two moles of L-rhamnose.

Mild acid hydrolysis of **1** with methanolic trifluoroacetic acid gave two hydrolysis products. The FAB-MS of the less polar one (**9**) showed an $[M+Na]^+$ ion at *m/z* 835, and the molecular composition $C_{42}H_{68}O_{15}$ was given by HR FAB-MS. The NMR spectra indicated that this compound is a bayogenin glucobioside. The position of the glucobiose linkage was determined to be located at the C_3-OH of bayogenin from the chemical shifts of C_2 (δ 70.7), C_3 (δ 82.8) and C_{23} (δ 65.2). The ^{13}C-NMR chemical shifts of the carbon signals due to the sugar moiety are almost the same as those of methyl β-laminaribioside [methyl β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside].[3] The fully methylated compound **9** gave methyl glycosides of 2,3,4,6-tetra-*O*-methyl glucopyranose and 2,4,6-tri-*O*-methyl glucopyranose on methanolysis. Thus, **9** was determined to be 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl bayogenin.

A more polar hydrolysis product showed in the positive-ion FAB-MS an $[M+Na]^+$ ion at *m/z* 1391, which indicates the loss of one pentosyl unit from **1**. The MS and NMR spectra, lacking signals due to an apiosyl group, indicated it to be a deapiosyl A_1, and was identified to be tacacoside B_3 (**5**) by NMR spectral comparison.

When **1** was treated with cellulase, a demonoglucosyl compound was obtained. It was identified as tacacoside B_1 (**3**). Therefore, **1** and **3** have the same ester-linked sugar, and it was also suggested that deapiosyl B_1 and demonoglucosyl B_3 would be tacacoside C (**6**). Enzymatic hydrolysis of **5** with cellulase gave **6**, as expected, and mild acid hydrolysis of **3** gave **6** and a prosapogenin (**10**), which was identified as 3-*O*-β-D-glucopyranosyl bayogenin.

On selective cleavage of the ester glycoside linkage,[4] **1** gave a prosapogenin (**9**) and an anomeric mixture (A_1S) of methyl glycosides, and **3** gave a prosapogenin (**10**) and an anomeric mixture (B_1S) of methyl glycosides, which is identical with A_1S. The anomers were separated by preparative HPLC. The faster eluting anomer, A_1S_1 (**11**), $C_{28}H_{48}O_{21}$, and the later eluting anomer, A_1S_2 (**12**), showed in the negative-ion FAB-MS an $[M-H]^-$ ion at *m/z* 719 and fragment ions at *m/z* 587, 573, 441, 309, and 163 given by the stepwise splitting of the sugar units from the terminals. The fragment ions at *m/z* 587 ($[M-H-132]^-$) and 573 ($[M-H-146]^-$) indicate that the methyl glycoside is a branched-chain pentasaccharide which has a rhamnosyl group and a pentosyl group at two terminals, and the last fragment ion (*m/z* 163) indicates that the methylated sugar is pentose.

Compound **1** gave **5** on mild acid hydrolysis, and the enzymatic hydrolysis of **1** gave **3**. This means that the ester-linked sugar moiety of **3** is the same as that of **1**, and the main structure of the ester-linked sugar moiety of **1** (and **3**) is the same as that of **5**, differing only in that **1** has an apiosyl group linked to the one sugar of the inner triose unit of the ester-linked sugar moiety of **5**. Therefore, the structure of the ester-linked sugar moiety of **5** was investigated first.

On the selective cleavage of the ester linkage, **5** gave a prosapogenin (**9**) and two anomers, B_3S_1 (**13**) and B_3S_2 (**14**), of methyl glycosides. The negative-ion FAB-MS of **13** showed an $[M-H]^-$ ion at *m/z* 587 and fragment ions at *m/z* 441 ($[M-H-146]^-$), 309 ($[M-H-146-132]^-$), and 163 ($[M-H-146-132-146]^-$). The fragmentation pattern indicates that the sugar is a linear tetrasaccharide and the sugar sequence is MeO–arabinose–rhamnose–xylose– rhamnose or MeO–xylose–rhamnose–arabinose–rhamnose.

In order to determine the sugar sequence and the sites of the sugar linkages, ^{1}H-NMR spectra of both **13** and **14** were examined (Table 5). The ^{1}H-NMR spectrum of **13** showed anomeric proton signals of rhamnosyl groups at δ 5.13 (d, J=2 Hz) [R1: C_1-H of a rhamnosyl group (R)] and δ 5.04 (d, J=2 Hz) (R′1), and those of two pentosyl groups at δ 4.54 (d, J=8 Hz) (P1) and δ 4.20 (d, J=6 Hz) (P′1). The ^{1}H-NMR spectrum of **14** showed anomeric proton signals at δ 5.13 (d, J=2 Hz) (R1), δ 4.85 (d, J=2 Hz) (R′1), δ 4.78 (d, J=3 Hz) (P′1) and δ 4.55 (d, J=8 Hz) (P1). The chemical shifts of the P′1 signal of **14** shifted downfield, while the signal of R′1 shifted upfield, and the signals of R1 and P1 stayed at almost the same magnetic fields compared with the corresponding anomeric proton signals of **13**. These differences indicate that P′1 is the anomeric proton signal of the methyl pentoside, whereas R′1 would be that of the rhamnosyl group which is linked to the methyl pentoside. It is necessary to know the coupling constant of the C_3-H signal of the pentosyl group to differentiate the arabinosyl group from the

Table 5. ^{1}H-NMR and ^{13}C-NMR Chemical Shifts (CD_3OD) of B_3S_1 (**13**) and B_3S_2 (**14**)

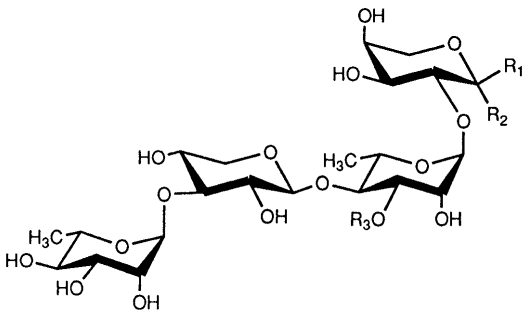
	B_3S_1 (**13**)		B_3S_2 (**14**)	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
MeO-Ara (P') 1	4.20 d (6)	105.0	4.78 d (3)	101.5
2	3.60—3.66	78.5	*ca.* 3.78	79.0
3	3.60—3.66	75.2	*ca.* 3.83	69.4
4	3.76 br s	70.5	3.85—3.88	71.0
5	3.48—3.53	66.9	3.55—3.59	63.7
	3.80—3.87		3.75—3.80	
Xyl (P) 1	4.54 d (8)	107.2	4.55 d (8)	106.3
2	*ca.* 3.30	77.0	3.31 t (8)	76.1
3	3.46 t (9)	85.0	3.46 t (9)	84.1
4	3.49—3.53	70.7	3.49—3.53	69.9
5	3.22 dd (10, 12)	67.9	3.22 dd (10, 11)	67.1
	3.80—3.87		3.87—3.91	
in. Rha (R') 1	5.04 d (2)	103.1	4.85 d (2)	104.3
2	3.90—3.91	69.3	3.96—3.97	72.0
3	3.80—3.89	73.4	3.80—3.90	72.5
4	3.55 t (10)	84.5	3.56 t (10)	83.4
5	3.88—3.95	73.0	3.75—3.80	68.6
6	1.25 d (6)	18.6	1.27 d (6)	18.2
out. Rha (R) 1	5.13 d (2)	103.4	5.13 d (2)	102.5
2	3.94—3.95	73.1	3.94—3.95	72.3
3	3.70 dd (3, 10)	73.1	3.70 dd (3, 10)	72.3
4	3.39 t (10)	74.9	3.39 t (10)	74.0
5	3.95—4.02	70.9	3.95—4.02	70.1
6	1.25 d (6)	18.7	1.25 d (6)	17.9
MeO	3.44 s	57.5	3.35 s	55.6

in., inner; out., outer.

xylosyl one. The signal of C_2-H (P'2) of the methyl pentoside group of **13**, located at around δ 3.63, was overlapped by the P'3 signal, and the splitting pattern of the P'3 signal could not be clarified. In the spectrum of **14**, the P'2 signal was also overlapped by other signals and the P'3 signal could not be traced by the ^{1}H–^{1}H chemical shift correlation spectroscopy (COSY) technique. On the other hand, the P3 signal of **14** appeared as a typical triplet (δ 3.46, t, J=9 Hz), although the P2 signal was masked by the signal of MeOH. From the splitting pattern of the P3 signal, P1 was assigned to the anomeric proton of the xylosyl group, and thus, P'1, to the anomeric proton of methyl arabinoside. Therefore, the sugar sequence was determined to be MeO–arabinose(P')–rhamnose(R')–xylose(P)–rhamnose(R).

The sites of sugar linkages were investigated by checking the nuclear Overhauser effect difference (NOED) spectra obtained by irradiation at the anomeric proton signal of each component sugar of **13**.

When the anomeric proton signals, P'1, P1, R'1 and R1 of **13** were irradiated, NOEs were observed on the signals at δ 3.44 (s), 3.55 (t, J=10 Hz), *ca.* 3.64 (perturbed signal)[5] and 3.46 (t, J=9 Hz), respectively, and they were assigned as signals of the methoxyl proton, R'4 , P'2 or P'3, and P3, respectively. The latter three signals were assigned by checking the ^{1}H–^{1}H COSY spectrum. Therefore, the sugar linkages in **13** and **14** were determined to be MeO–arabinopyranosyl-(2 or 3←1)–rhamnopyranosyl-(4←1)-xylopyranosyl-(3←1)-rhamnopyranosyl. The coupling constant (6 Hz) of the arabinosyl group of **13**, and that (3 Hz) of **14** indicate that the former is an α-anomer and the latter, a β-anomer.



	R_1	R_2	R_3
11	OMe	H	β-D-Apiofuranosyl
12	H	OMe	β-D-Apiofuranosyl
13	OMe	H	H
14	H	OMe	H
15	OMe	H	β-D-Xylopyranosyl
16	H	OMe	β-D-Xylopyranosyl

Compounds **11** and **12** have an apiosyl unit linked to one of the inner triose units of **13** and **14**. The ^{1}H-NMR spectrum of **11** showed anomeric proton signals at δ 5.24 (d, J=4 Hz) (Ap1), 5.12 (d, J=2 Hz) (R1), 5.03 (d, J=2 Hz) (R'1), 4.66 (d, J=8 Hz) (P1) and 4.20 (perturbed signal) (P'1) (Table 6). The first one was assigned to that of the apiofuranosyl group, the second to a terminal rhamnopyranosyl group, the third to an inner rhamnopyranosyl group, the fourth to a xylopyranosyl group, and the last to a methyl arabinopyranosyl group using ordinary NMR techniques and by comparison of the NMR chemical shifts of the anomeric proton signals with those of **12** [δ 5.26 (d, J=4 Hz) (Ap1), 5.12 (d, J=2 Hz (R1), 4.86 (d, J=2 Hz) (R'1), 4.78 (d, J=3 Hz) (P'1), 4.67 (d, J=8 Hz) (P1)].

On irradiation of the signal at δ 5.24 (Ap1 of **11**), NOE was observed on the signal at δ 3.84 (dd, J=4, 10 Hz), which was assigned to the signal (R'3) of the C_3-H of the inner rhamnopyranosyl group. This result shows that the apiofuranosyl group is linked to the C_3-OH of the inner rhamnopyranosyl group of **13** (and **14**).

The site of the inner rhamnopyranosyl group linkage could not be determined because the signals of P'2 and P'3 of the methyl arabinoside are overlapped in the spectrum of **11**. However, in the NOED spectrum of **12**, NOE was observed on the double doublet signal (J=3, 10 Hz) at δ 3.77 when the anomeric proton signal of the inner rhamnopyranosyl group was irradiated. This signal was assigned to P'2 of the methyl arabinoside, indicating that the inner rhamnopyranosyl group is linked to the C_2-OH of the arabinopyranosyl group. Therefore, the sugar linkages in **11** and **12** were established to be methyl α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-L-arabinopyranoside. The optical rotations of **11** (−76.3°) and **12** (−17.9°) indicated that the former is an α-anomer and the latter a β-anomer.

Compound **12** was fully methylated and the component methylated sugars were examined by gas chromatography–

Table 6. ^{1}H-NMR and ^{13}C-NMR Chemical Shifts (CD_3OD) of A_1S_1 (**11**) and A_1S_2 (**12**)

		A_1S_1 (**11**)		A_1S_2 (**12**)	
		^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
MeO-Ara (P')	1	4.20	104.1	4.78 d (3)	101.4
	2	3.64—3.65	74.3	3.77 dd (3, 10)	79.4
	3	3.64—3.65	77.9	3.75—3.78	79.4
	4	3.76 br s	69.7	3.80—3.90	71.0
	5	3.50—3.56	66.1	3.56 dd (2, 12)	63.7
		3.84—3.88		3.75—3.78	
Xyl (P)	1	4.66 d (8)	104.9	4.67 d (8)	104.9
	2	3.27 dd (8, 9)	75.8	3.27 dd (8, 9)	75.8
	3	3.45 t (9)	84.0	3.45 t (9)	83.9
	4	3.51 dd (2, 12)	70.2	3.50—3.56	70.2
	5	3.19 dd (10, 12)	66.9	3.19 dd (10, 11)	66.9
		3.84—3.88		3.80—3.90	
Api (Ap)	1	5.24 d (4)	112.0	5.26 d (4)	112.0
	2	4.04 d (4)	78.2	4.04 d (4)	78.2
	3	—	80.1	—	80.1
	4	3.75 d (10)	74.9	3.76 d (10)	74.9
		4.11 d (10)		4.11 d (10)	
	5	3.58 br s	65.0	3.58 br s	65.0
in. Rha (R')	1	5.03 d (2)	102.2	4.86 d (2)	104.2
	2	4.06 dd (2, 3)	72.1	4.10 br s	71.8
	3	3.84 dd (4, 10)	82.1	3.92 dd (3, 10)	82.0
	4	3.69 t (10)	78.5	3.69 t (10)	78.4
	5	3.93 m	68.6	3.79—3.84	71.0
	6	1.23 d (6)	18.0	1.27 d (6)	18.3
out. Rha (R)	1	5.12 d (2)	102.6	5.12 d (2)	102.6
	2	3.93 dd (2, 3)	72.3	3.93 dd (2, 3)	72.3
	3	3.70 dd (3, 10)	72.3	3.70 dd (3, 10)	72.3
	4	3.39 t (10)	74.0	3.69 t (10)	74.0
	5	3.99 m	70.0	3.99 m	70.1
	6	1.25 d (6)	17.9	1.25 d (6)	17.9
MeO		3.43 s	56.6	3.34 s	55.5

in., inner; out., outer.

chemical ionization mass spectrometry (GC-CI-MS) after methanolysis. Methyl glycosides of 2,3,4-tri-*O*-methylrhamnopyranose, 2,3,5-tri-*O*-methylapiofuranose, 2,4-di-*O*-methylxylopyranose, 2-*O*-methylrhamnopyranose, and 3,4-di-*O*-methylarabinopyranose were identified. These results support the above structures, as determined by spectral analyses.

Compounds **13** and **14** are, therefore, corresponding deapiosyl derivatives, *viz.*, methyl α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and its β-anomer, respectively.

The configuration of the apiofuranosyl group in **12** (and **11**) was determined to be β by comparison of the difference ($\Delta[M]_D$ −128.3°) of the molecular rotations of **12** ($[M]_D$ −128.9°) and **14** ($[M]_D$ −0.6°) with those of methyl α- and β-D-apiofuranosides ($[M]_D$ +239° and −163°, respectively).[6] The coupling constants of C_1-H (d, J=8 Hz), C_2-H (dd, 8, 9 Hz) and C_3-H (t, 9 Hz) of the xylopyranosyl group undoubtedly indicate the conformation and configuration to be $^4C_1$ and β, respectively. The anomeric proton signals of two rhamnopyranosyl groups appeared to be doublets with a J value of *ca.* 2 Hz, and those of C_3-H to be double doublets with J values of 3 and 10 Hz. These coupling constants show that the rhamnopyranosyl groups take the $^1C_4$ conformation, but the configuration (α or β) of the linkages could not be deduced from these coupling constants. Bock and Pedersen[7] reported that the $J_{C1\text{-}H1}$ of sugars which have an axial anomeric proton is smaller than that of sugars having an equatorial anomeric proton by *ca.* 10 Hz. The $J_{C1\text{-}H1}$ value of the β-D-xylopyranosyl group which has the axial anomeric proton is 157 Hz. On the other hand, the $J_{C1\text{-}H1}$ values of two rhamnopyranosyl groups which take a $^1C_4$ conformation are both 171 Hz, *ca.* 14 Hz larger than that of the β-D-xylopyranosyl group. This value indicates that each rhamnopyranosyl group has an equatorial anomeric proton, thus indicating the α configuration.

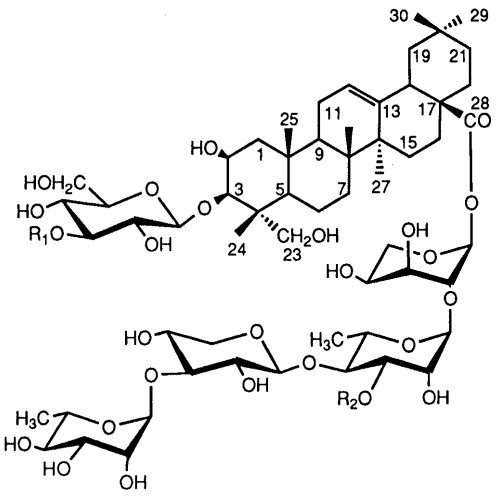
The combination of prosapogenins and ester-linked sugar moieties comprises the total structures of saponins; however, the configuration and conformation of the ester-linked arabinosyl group remains to be determined. The signals of C_1-H and C_2-H of the arabinosyl group in **1** appeared at δ 6.54 (br s) and δ *ca.* 4.5 (br s), and these small coupling constants clearly exclude the α and β configurations in the $^4C_1$ conformation. The $J_{C1\text{-}H1}$ value of the α-arabinopyranosyl group of **11** is 157 Hz. This coupling constant indicates that the α-arabinopyranosyl group in **11** has an axial anomeric hydrogen, and thus, takes the $^4C_1$ conformation. On the other hand, the $J_{C1\text{-}H1}$ value of the arabinopyranosyl group of **1** is 172 Hz, 15 Hz larger than that of **11**. This coupling constant shows that the arabinopyranosyl group in **1** has an equatorial anomeric hydrogen, and thus, the conformation and configuration of the arabinosyl group in **1** were concluded to be $^1C_4$ and α, respectively.

The $J_{C1\text{-}H1}$ value (171 Hz) of the arabinopyranosyl group in **5** indicates that the configuration and conformation of the ester-linked arabinopyranosyl groups in both **5** and **6** would also be α and $^1C_4$, and the total structures of **1**, **3**, **5** and **6** were elucidated as shown.

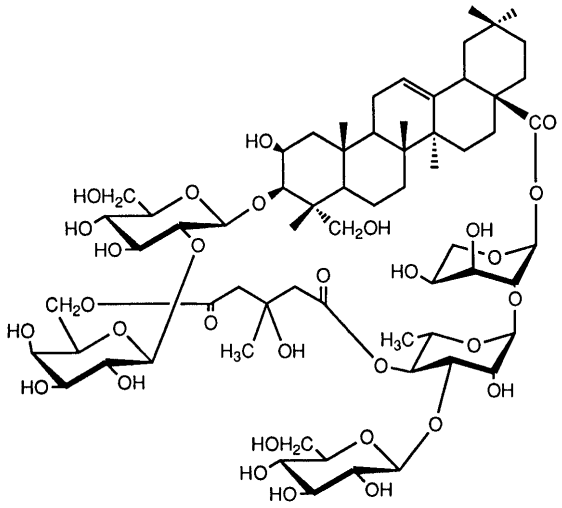
Tacacoside A_2 (**2**), $C_{69}H_{112}O_{35}$, gave L-arabinose, L-rhamnose, D-xylose and D-glucose as component sugars on acid hydrolysis, and it gave a prosapogenin (**9**) and an anomeric mixture [A_2S_1 (**15**) and A_2S_2 (**16**)] of methyl glycosides on selective cleavage of the ester linkage. On enzymatic hydrolysis with cellulase, **2** gave tacacoside B_2 (**4**). Identity was confirmed by comparison of the NMR spectra. The negative-ion FAB-MS of **16** showed the same FAB-MS as that of **12**, showing that it is also a branched-chain pentasaccharide having one pentosyl unit and one rhamnosyl unit at terminals, and the sugar sequence of the straight-chain part would be MeO–pentose–rhamnose–pentose–rhamnose.

The fully methylated **16** gave, on methanolysis, methyl glycosides of 2,3,4-tri-*O*-methylrhamnose, 2,3,4-tri-*O*-methylxylose, 3,4-di-*O*-methylarabinose, 2,4-di-*O*-methylxylose and 2-*O*-methylrhamnose, showing that **16** is a methyl pentasaccharide branched at the inner rhamnosyl uint, having a rhamnosyl unit and a xylosyl unit at the terminals.

The ^{1}H-NMR spectrum (Table 7) of **15** depicted signals of anomeric protons at δ 5.13 (d, J=2 Hz, R1), 5.04 (d, J=2 Hz, R'1), 4.74 (d, J=9 Hz, P1), 4.53 (d, J=8 Hz, P'1) and 4.21 (perturbed signal, P''1), while that of **16** showed anomeric proton signals at δ 5.14 (d, J=2 Hz, R1), 4.85 (overlapped with H_2O signal, R'1), 4.77 (d, J=3 Hz, P''1),



	R_1	R_2
tacacoside A_1 (1)	β-D-Glucopyranosyl	β-D-Apiofuranosyl
A_2 (2)	β-D-Glucopyranosyl	β-D-Xylopyranosyl
B_1 (3)	H	β-D-Apiofuranosyl
B_2 (4)	H	β-D-Xylopyranosyl
B_3 (5)	β-D-Glucopyranosyl	H
C (6)	H	H



8a : lobatoside E

8b : deacylation product of **8a**

4.74 (d, $J=8$ Hz, P1) and 4.55 (d, $J=7$ Hz, P′1). The signals of R1, R′1 and P″1 may be safely assigned to those of anomeric protons of the terminal rhamnosyl unit, the inner rhamnosyl unit and the methyl pentoside, respectively. As shown in Fig. 1, irradiation at the signals of R1, R′1, P″1, P1, and P′1 of **16** brought about NOEs on the signals at δ 3.41 (t, $J=13$ Hz, P3), 3.78 (dd, $J=4$, 10 Hz, P″2), 3.34 (s, OCH_3), 3.69 (t, $J=10$ Hz, R′4) and 3.95 (dd, $J=3$, 10 Hz, R′3), respectively. The splitting pattern of the P3 signal (t, $J=13$ Hz) indicates that P3 is the C_3-H of the xylosyl unit. Therefore, taking the J values of the anomeric proton signals into consideration, **16** is concluded to be methyl α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-L-arabinopyranoside, and **15** its α-anomer.

Table 7. ^{1}H-NMR and ^{13}C-NMR Chemical Shifts (CD_3OD) of A_2S_1 (**15**) and A_2S_2 (**16**)

	A_2S_1 (**15**)		A_2S_2 (**16**)	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
MeO-Ara (P″) 1	4.21	104.2	4.77 d (3)	101.4
2	3.64—3.70	77.7	3.78 dd (3, 10)	79.1
3	3.64—3.70	74.4	*ca.* 3.84	69.4
4	3.77 br s	69.8	*ca.* 3.86	71.0
5	3.50—3.54	66.2	3.56 dd (2, 12)	63.7
	3.83—3.87		3.73—3.80	
in. Xyl (P) 1	4.74 d (9)	104.3	4.74 d (8)	104.7
2	3.21 t (9)	76.1	3.17—3.23	76.1
3	3.41 t (9)	84.5	3.41 t (13)	84.2
4	3.46—3.54	71.1	3.48—3.52	71.0
5	*ca.* 3.20	67.0	*ca.* 3.30	67.0
	3.82—3.88		*ca.* 3.84	
out. Xyl (P′) 1	4.53 d (8)	106.0	4.55 d (7)	105.9
2	*ca.* 3.30	75.3	*ca.* 3.30	75.3
3	*ca.* 3.30	78.2	*ca.* 3.30	78.2
4	3.46—3.52	70.3	3.46—3.52	70.2
5	*ca.* 3.20	66.9	*ca.* 3.30	67.0
	3.83—3.87		*ca.* 3.84	
in. Rha (R′) 1	5.04 d (2)	102.1	*ca.* 4.85	104.1
2	4.10 dd (2, 3)	72.2	4.16 dd (2, 3)	71.8
3	3.90 dd (3, 10)	82.5	3.95 dd (3, 10)	82.4
4	3.68 t (10)	79.0	3.69 t (10)	78.8
5	3.94—3.98	68.5	3.75—3.82	68.6
6	1.23 d (6)	18.0	1.26 d (7)	18.4
out. Rha (R) 1	5.13 d (2)	102.6	5.14 d (2)	102.6
2	3.94 dd (2, 3)	72.3	3.94 dd (2, 4)	72.2
3	3.70 dd (3, 10)	72.3	3.70 dd (4, 10)	72.3
4	3.40 t (10)	74.0	3.39 t (10)	74.0
5	3.99—4.03	70.0	3.90—4.03	70.0
6	1.25 d (6)	17.9	1.24 d (6)	17.9
MeO	3.43 s	56.6	3.34 s	55.5

in., inner; out., outer.

Combination of the structures of the prosapogenin and the ester-linked sugar moiety makes up the full structure of **2**. The configuration and conformation of the ester-linked arabinosyl group were determined to be α and $^1C_4$ by the same reasons for those of **1**.

One of the authors isolated from the aerial part of *Actinostemmma lobatum* (Cucurbitaceae) some bayogenin bisdesmosides which have a 3-hydroxy-3-methyl glutarate (dicrotaric acid ester) bridge between two sugar moieties,[2] and some of these cyclic bisdesmosides have shown potent antiproliferative activity against some of the human tumor cell lines.[8] The deacylated compound (**8b**) obtained by alkaline hydrolysis of lobatoside E (**8a**) showed no antiproliferative activity; therefore, the macrocyclic structure seems to play an important role in exhibiting the activity.

Tacacosides, the normal type bayogenin bisdesmosides, were presumed to show no cell growth inhibition; however, they were subjected to test to make sure of it. Against our surmise, they showed antiproliferative activity, though not so potently as the cyclic bisdesmosides. The results are shown in Fig. 2 and Table 8.

Experimental[9]

Plant Materials Fruits and the aerial parts of *Sechium pittieri* were collected at Tres Rios, San Jose, Costa Rica, and those of *Sechium talamancense* at San Gerardo de Dota, San Jose in 1995. The plants were

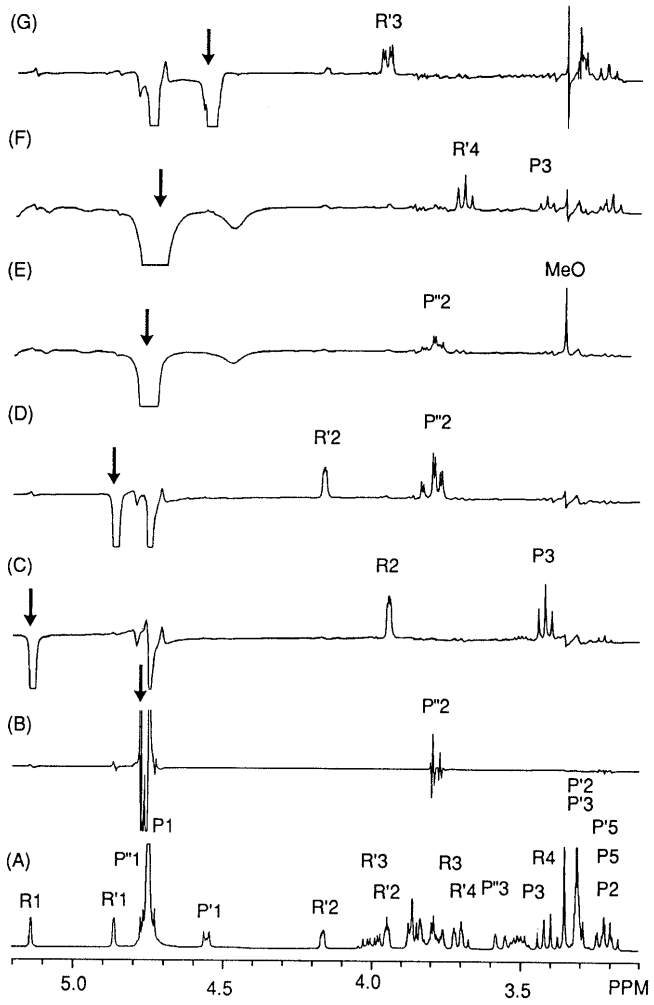


Fig. 1. ^{1}H-NMR Spectrum (A), Decoupling Difference Spectrum (B) and NOED Spectra (C—G) of **16** (CD_3OD)

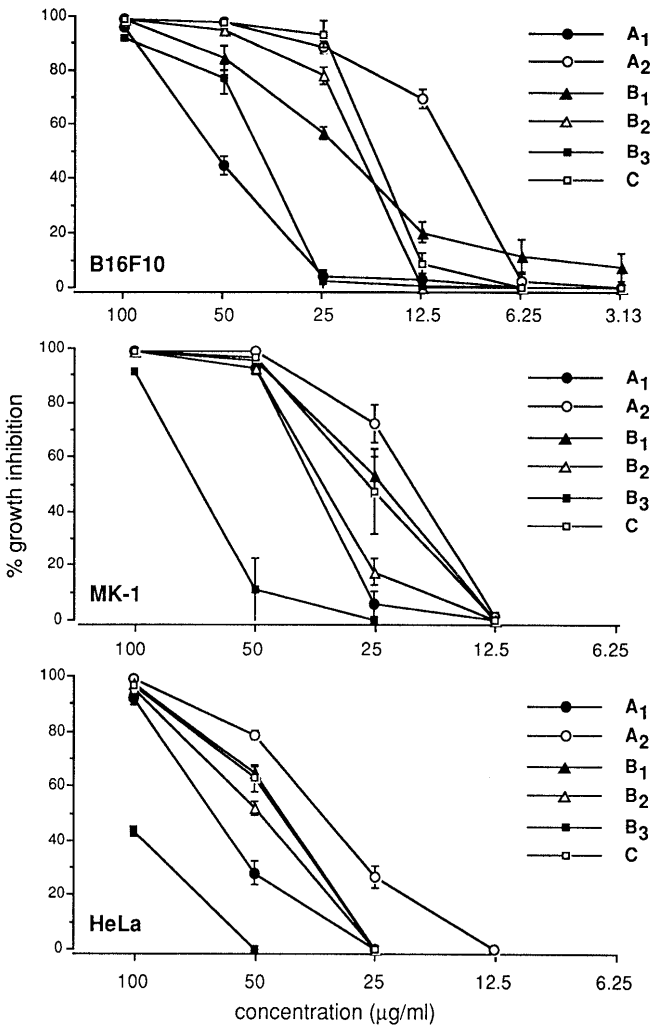


Fig. 2. Growth Inhibition of Tacacosides against Tumor Cell Lines

identified by Dr. Michael Nee of the New York Botanical Garden.

Extraction and Fractionation of the Saponin Fractions from the Fruits of *Sechium* Species The fresh fruit (SPF) (500 g) of *Sechium pittieri* was homogenized in MeOH (500 ml) and centrifuged. The precipitates were washed with MeOH (400 ml). The supernatant and the MeOH washing were combined and concentrated *in vacuo* to give an aqueous solution, which was passed through a column (100 ml) of MCI gel Diaion HP-20, then the column was washed successively with water (200 ml), 50% MeOH (200 ml), MeOH (300 ml) and AcOEt (200 ml). The eluates by 50% MeOH, MeOH and AcOEt were concentrated and dried *in vacuo* to give a 50% MeOH eluate (SPF-1, 0.39 g), a MeOH eluate (SPF-2, 2.37 g) and an AcOEt eluate (SPF-3, 0.20 g), respectively.

The fruit (STF) (500 g) of *Sechium talamancense* were also extracted and the extracts were fractionated in the same way to give a 50% MeOH eluate (STF-1, 0.61 g), a MeOH eluate (STF-2, 4.35 g) and an AcOEt eluate (STF-3, 0.22 g), respectively. The 50% MeOH and MeOH eluates from the Diaion HP-20 column showed a foaming property, and analytical HPLC (30% acetonitrile) showed three peaks, A, B and C, in the order of increasing t_R value.

Extraction and Fractionation of the Saponin Fractions of the Aerial Parts of *Sechium* Species The dried and powdered aerial part (SPL) (100 g) of *Sechium pittieri* (SP) was percolated with MeOH (500 ml), and the MeOH solution was concentrated and dried *in vacuo*. The residue (7.59 g) was suspended in water (200 ml) and centrifuged to remove the water-insoluble materials. The supernatant was passed through a column (100 ml) of MCI gel Diaion HP-20, and the column was then washed successively with water (200 ml), 50% MeOH (200 ml), MeOH (200 ml) and AcOEt (200 ml). The eluates by 50% MeOH and MeOH were concentrated and dried *in vacuo* to give SPL-1 (0.3 g) and SPL-2 (0.37 g), respectively. The water-insoluble materials from the original MeOH extract were extracted with AcOEt (50 ml), and the AcOEt solution was combined with the AcOEt washing of the column and concentrated *in vacuo* to give a dark green resinous residue (SPL-3, 1.39 g).

Table 8. Antiproliferative Activity (ED_{50}, μg/ml) of Tacacosides against Tumor Cell Lines *in Vitro*

	MK-1	HeLa	B16F10
Tacacoside A_1 (**1**)	35.7±1.04	67.3±2.52	54.5±4.24
Tacacoside A_2 (**2**)	20.0±1.80	33.7±2.84	9.0±0.50
Tacacoside B_1 (**3**)	24.7±2.52	43.2±0.76	22.3±0.58
Tacacoside B_2 (**4**)	33.3±0.29	49.2±1.44	18.9±1.16
Tacacoside B_3 (**5**)	73.8±3.88	>100	39.6±1.21
Tacacoside C (**6**)	25.8±4.07	43.7±2.47	17.2±0.29

$n=3$.

The dried aerial part (STL) (100 g) of *Sechium talamancense* (ST) was also extracted and fractionated in the same way to give a 50% MeOH eluate (STL-1, 0.73 g), a MeOH eluate (STL-2, 1.82 g) and an AcOEt eluate (STL-3, 1.52 g). Among these fractions, the 50% MeOH eluates and MeOH eluates formed foams when they were shaken in water.

The analytical HPLC (30% acetonitrile) of the STL-1 and STL-2 showed peaks A, B and C, corresponding to those from the fruits, but the relative areas of peak C were much bigger than those from the fruit.

Fractionantion and Isolation of Saponins (Tacacosides). From the Fruit of *Sechium pittieri* SPF-1 (0.39 g) and SPF-2 (2.37 g) were combined and repeatedly subjected to preparative HPLC (33% acetonitrile) to give three fractions (SPF-fr.A, 1.574 g; SPF-fr.B, 308 mg; SPF-fr.C, 8 mg). SPF-fr.C was proven to be a pure compound, and was named tacacoside C.

Tacacoside C (**6**): A white powder. $[\alpha]_D^{26}$ −4.5° (c=1.24, MeOH).

Positive-ion HR FAB-MS *m/z*: 1229.5953 ($[M+Na]^+$, $C_{58}H_{94}NaO_{26}$ requires 1229.5935), 673.3932 ([prosapogenin + Na]$^+$, $C_{36}H_{58}NaO_{10}$ requires 673.3926), 627.3878 ([prosapogenin + Na − HCOOH]$^+$, $C_{35}H_{56}NaO_8$ requires 627.3871). Negative-ion FAB-MS *m/z*: 1205 ($[M-H]^-$), 1059 ([M − H − rhamnose]$^-$), 1043 ([M − H − glucose]$^-$), 649 ([prosapogenin − H]$^-$), 487 ([aglycone − H]$^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are almost the same as those of **1** and **2** shown in Tables 2 and 4. As for the sugar moiety, only chemical shifts of anomeric H and C signals are shown in Table 3.

SPF-fr.A (1.483 g) and SPF-fr.B (308 mg) were respectively dissolved in the pyridine–Ac_2O (1 : 1) mixture (100 mg in 1 ml) and heated at 80 °C for 20 h. After adding MeOH, the solvents were evaporated off in a N_2 stream and dried *in vacuo* to give SPF-fr.A-Ac (2.57 g) and SPF-fr.B-Ac (494 mg). SPF-fr.A-Ac showed two peaks in analytical HPLC (85% MeOH): (SPF-A_2-Ac and SPF-A_1-Ac in the order of increasing t_R value), while SPF-fr.B-Ac showed three peaks (SPF-B_3-Ac, -B_2-Ac, -B_1-Ac). Acetates were separated by preparative HPLC (90% MeOH) and SPF-A_2-Ac (1.576 g), -A_1-Ac (524 mg), -B_3-Ac (175 mg), -B_2-Ac (174 mg) and -B_1-Ac (72 mg) were obtained. All acetates were respectively dissolved in 0.5 N MeONa solution (100 mg acetate in 2 ml MeONa solution) and refluxed for 4 h. After neutralizing the mixture with acetic acid, the MeOH was evaporated off. The residue was passed through a Diaion HP-20 column (10 ml) and the column was washed with water. The saponin was eluted out with MeOH. The MeOH eluate was purified by preparative HPLC (35% acetonitrile) to give a pure saponin.

SPF-A_1 (Tacacoside A_1, **1**): A white powder. $[\alpha]_D^{26}$ −36.6° (c = 1.16, MeOH). Positive-ion HR FAB-MS *m/z*: 1523.6897 ($[M+Na]^+$, $C_{69}H_{112}NaO_{35}$ requires 1523.6887), 789.4409 ([prosapogenin + Na − HCOOH]$^+$, $C_{41}H_{66}NaO_{13}$ requires 789.4400), 711.2361 ([ester-linked sugar ($C_{27}H_{46}O_{21}$) + Na − H_2O]$^+$, $C_{27}H_{44}NaO_{20}$ requires 711.2308). Negative-ion FAB-MS *m/z*: 1499 ($[M-H]^-$), 1367 ([M − H − apiose]$^-$), 1353 ([M − H − rhamnose]$^-$), 1337 ([M − H − glucose]$^-$), 1221 ([1353 − apiose]$^-$), 1175 ([1337 − glucose]$^-$), 811 ([prosapogenin − H]$^-$), 765 ([811 − HCOOH]$^-$), 649 ([prosapogenin − H − glucose]$^-$), 631 ([649 − H_2O]$^-$), 487 ([aglycone − H]$^-$), 469 ([aglycone − H − H_2O]$^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are shown in Tables 2 and 4. As for the sugar moiety, only chemical shifts of anomeric H and C signals are shown in Table 3.

SPF-A_2 (Tacacoside A_2, **2**): A white powder. $[\alpha]_D^{26}$ −28.3° (c = 1.12, MeOH). Positive-ion HR FAB-MS *m/z*: 1523.6887 ($[M+Na]^+$, $C_{69}H_{112}NaO_{35}$ requires 1523.6883), 835.4448 ([prosapogenin + Na]$^+$, $C_{42}H_{68}NaO_{15}$ requires 835.4458), 789.4398 ([prosapogenin + Na − HCOOH]$^+$, $C_{41}H_{66}NaO_{13}$ requires 789.4402), 711.2354 ([ester-linked sugar ($C_{27}H_{46}O_{21}$) + Na − H_2O]$^+$, $C_{27}H_{44}NaO_{20}$ requires 711.2312. Negative-ion FAB-MS *m/z*: 1499 ($[M-H]^-$), 1367 ([M − H − xylose]$^-$), 1353 ([M − H − rhamnose]$^-$), 1337 ([M − H − glucose]$^-$), 1221 ([1353 − xylose]$^-$), 1175 ([1337 − glucose]$^-$), 811 ([prosapogenin − H]$^-$), 765 ([811 − HCOOH]$^-$), 649 ([prosapogenin − H − glucose]$^-$), 487 ([aglycone − H]$^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are shown in Tables 2 and 4. As for the sugar moiety, only chemical shifts of anomeric H and C signals are shown in Table 3.

SPF-B_1 (Tacacoside B_1, **3**): A white powder. $[\alpha]_D^{26}$ −36.7° (c = 1.10, MeOH). Positive-ion HR FAB-MS *m/z*: 1361.6353 ($[M+Na]^+$, $C_{63}H_{102}NaO_{30}$ requires 1361.6354), 711.2361 ([ester-linked sugar ($C_{27}H_{46}O_{21}$) + Na − H_2O]$^+$, $C_{27}H_{44}NaO_{20}$ requires 711.2309), 673.3925 ([prosapogenin + Na]$^+$, $C_{36}H_{58}NaO_{10}$ requires 673.3929), 627.3874 ([prosapogenin + Na − HCOOH]$^+$, $C_{35}H_{56}NaO_8$ requires 627.3872). Negative-ion FAB-MS *m/z*: 1337 ($[M-H]^-$), 1205 ([M − H − apiose]$^-$), 1191 ([M − H − rhamnose]$^-$), 1175 ([M − H − glucose]$^-$), 1059 ([M − apiose − rhamnose]$^-$), 649 ([prosapogenin − H]$^-$), 487 ([aglycone − H]$^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are almost the same as those of **1** and **2** shown in Tables 2 and 4. As for the sugar moiety, only chemical shifts of anomeric H and C signals are shown in Table 3.

SPF-B_2 (Tacacoside B_2, **4**): A white powder. $[\alpha]_D^{26}$ −26.6° (c = 1.15, MeOH). Positive-ion HR FAB-MS *m/z*: 1361.6353 ($[M+Na]^+$, $C_{63}H_{102}NaO_{30}$ requires 1361.6354), 711.2332 ([ester-linked sugar ($C_{27}H_{46}O_{21}$) + Na − H_2O]$^+$, $C_{27}H_{44}NaO_{20}$ requires 711.2309), 673.3934 ([prosapogenin + Na]$^+$, $C_{36}H_{58}NaO_{10}$ requires 673.3929), 627.3868 ([prosapogenin + Na − HCOOH]$^+$, $C_{35}H_{56}NaO_8$ requires 627.3872). Negative-ion FAB-MS *m/z*: 1337 ($[M-H]^-$), 1205 ([M − H − apiose]$^-$), 1191 ([M − H − rhamnose]$^-$), 1175 ([M − H − glucose]$^-$), 1059 ([M − apiose − rhamnose]$^-$), 649 ([prosapogenin − H]$^-$), 487 ([aglycone − H]$^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are almost the same as those of **1** and **2** shown in Tables 2 and 4. As for the sugar moiety, only chemical shifts of anomeric H and C signals are shown in Table 3.

SPF-B_3 (Tacacoside B_3, **5**): A white powder. $[\alpha]_D^{26}$ −16.7° (c = 1.18, MeOH). Positive-ion HR FAB-MS *m/z*: 1391.6454 ($[M+Na]^+$, $C_{64}H_{104}NaO_{31}$ requires 1391.6458), 835.4463 ([prosapogenin + Na]$^+$, $C_{42}H_{68}NaO_{15}$ requires 835.4455), 789.4404 ([prosapogenin + Na − HCOOH]$^+$, $C_{41}H_{66}NaO_{13}$ requires 789.4401), 579.1904 ([ester-linked sugar ($C_{22}H_{38}O_{17}$) + Na − H_2O]$^+$, $C_{22}H_{36}NaO_{16}$ requires 579.1899). Negative-ion FAB-MS *m/z*: 1367 ($[M-H]^-$), 1221 ([M − H − rhamnose]$^-$), 1205 ([M − H − glucose]$^-$), 1043 ([M − H − glucose − glucose]$^-$), 811 ([prosapogenin − H]$^-$), 649 ([prosapogenin − H − glucose]$^-$), 487 ([aglycone − H]$^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are almost the same as those of **1** and **2** shown in Tables 2 and 4. As for the sugar moiety, only chemical shifts of anomeric H and C signals are shown in Table 3.

From the Aerial Parts of *Sechium pittieri* SPL-1 (0.3 g) and SPL-2 (0.37 g) were combined and repeatedly subjected to preparative HPLC (33% acetonitrile), and three fractions (SPL-fr.A, 55 mg; SPL-fr.B, 61 mg; SPL-fr.C, 36 mg) were obtained. SPL-fr.C was again subjected to preparative HPLC for purification to give **6** (12 mg).

SPL-fr.A and SPL-fr.B were acetylated and chromatographed in the same way described above to give SPL-fr.A-Ac (81 mg) and SPL-fr.B-Ac (81 mg). Both acetylated fractions were subjected to preparative HPLC (90% MeOH) in the same way described above to give SPL-A_2-Ac (=SPF-A_2-Ac, 10 mg), -A_1-Ac (=SPF-A_1-Ac, 12 mg), B_3-Ac (=SPF-B_3-Ac, 7 mg), B_2-Ac (=SPF-B_2-Ac, 17 mg) and B_1-Ac (=SPF-B_1-Ac, 18 mg). Each acetate was treated with MeONa to give the original saponin.

From *Sechium talamancense* The 50% MeOH and MeOH eluates of the fruit and the aerial parts of *Sechium talamancense* were fractionated, and saponins were isolated in the same way described above. The saponins and their yields are shown in Table 1.

Identification of the Component Sugars of Tacacosides Tacacoside (3—5 mg each) was dissolved in 0.9 N HCl–MeOH (0.5 ml) and heated at 75—85 °C for 1 h, diluted with MeOH (1 ml), and the HCl was neutralized with Ag_2CO_3. After centrifugation of the precipitates, the supernatant was bubbled with a H_2S gas and evaporated. *N*-Trimethylsilylimidazole (0.1 ml) was added to the residue and the mixture was heated at 80 °C for 20 min. Hexane (1 ml) and water (1 ml) were added to the reaction mixture and shaken vigorously. After centrifugation, the hexane layer was checked by GC. The standard sugar samples were also treated in the same way.

GC Conditions: Column, Shimadzu DB-1 (0.25 mm i.d. × 30 m, liquid film, 0.25 μm); carrier gas, He (flow rate, 50 ml/min); split ratio, 53; column oven temperature, 175 °C (13 min)→200 °C (5 min), elevation rate 5 °C/min; injection port temperature, 250 °C; detector temperature, 250 °C.

t_R Values (min) of TMS Ethers of the Methanolysates of Standard Sugars and Apiin: L-Arabinose methanolysate: 7.65, 7.89; L-rhamnose methanolysate: 8.50, 8.93; D-glucose methanolysate: 19.80, 20.74; apiin methanolysate: 7.01, 7.18, 7.39, 7.76, 19.80, 20.74.

t_R Values (min) of TMS Ethers of the Saponins Methanolysates: Methanolysate of **1**: 7.01, 7.18, 7.38, 7.76 (Api), 7.65, 7.91 (Ara), 10.75, 11.53 (Xyl), 8.47, 8.92 (Rha), 19.80, 20.74 (Glc). Methanolysate of **2**: 7.70, 7.96 (Ara), 10.84, 11.62 (Xyl), 8.47, 8.92 (Rha), 19.89, 20.83 (Glc). Methanolysate of **3**: 7.70, 7.24, 7.44 (Api), 7.72, 7.98 (Ara), 10.85, 11.64 (Xyl), 8.55, 9.01 (Rha), 19.91, 20.84 (Glc). Methanolysate of **4**: 7.72, 7.97 (Ara), 10.85, 11.63 (Xyl), 8.54, 9.00 (Rha), 19.90, 20.84 (Glc). Methanolysate of **5**: 7.72, 7.97 (Ara), 10.85, 11.64 (Xyl), 8.54, 9.00 (Rha), 19.93, 20.86 (Glc). Methanolysate of **6**: 7.72, 7.98 (Ara), 10.85, 11.64 (Xyl), 8.54, 9.01 (Rha), 19.92, 20.86 (Glc).

Determination of the Absolute Configurations of the Component Sugars Determination of the absolute configurations was performed according to the method reported by Hara *et al.*[10] A glycoside (10—20 mg) was hydrolyzed in 2 N HCl (1 ml) at 95 °C for 2 h. The acidic solution was neutralized by passing it through a column of Amberlite MB-3 (20 ml). One half of the neutral eluate was concentrated and the residue was dissolved in pyridine (0.2 ml). After the addition of a pyridine solution (0.4 ml) of L-cysteine methyl ester hydrochloride (0.06 mol/l), the mixture was warmed at 60 °C for 1 h. The solvent was blown off

under a N_2 stream, and the residue was trimethylsilylated and checked by GC. The t_R values of the samples were compared with those of the standard sugars. The absolute configuration of the component apiose was determined by comparison of the t_R value of the sample with that of apiin hydrolysate. Retention times (min) of the hydrolysates and standard sugar samples are as follows. Tacacoside A_1 hydrolysate: 10.8, 12.8, 18.2; tacacoside A_2 hydrolysate: 10.8, 12.8, 18.2; apiin hydrolysate: 10.8, 18.2; D-glucose: 18.2; L-glucose: 18.8; D-rhamnose: 13.0; L-rhamnose: 12.8; D-xylose: 10.7; L-xylose: 11.3; D-arabinose: 11.5; L-arabinose: 10.8.

GC Conditions: Column: Shimadzu CBP-1 (0.22 mm i.d. × 50 m, liquid film, 0.25 μm); carrier gas: He, (linear velocity: 30 cm/s); split ratio: 30; column oven temperature: 240 °C; injection port temperature: 290 °C; detector temperature: 250 °C.

Enzymatic Hydrolysis of 1, 2 and 5 Compound **1** (105 mg) and cellulase (type II, Sigma) were dissolved in 30% EtOH (10 ml) and the mixture was shaken at 40 °C overnight. After evaporating EtOH, the residue was dissolved in 50% MeOH (10 ml) and passed through a column of Diaion HP-20 (20 ml). The column was washed successively with 50% MeOH and MeOH. The MeOH eluate (101 mg) was subjected to preparative HPLC (33% acetonitrile) and a hydrolysis product (79 mg) was obtained. The *Rf* value in TLC, t_R value in HPLC, FAB-MS and NMR spectra of the hydrolysis product were the same as those of **3**. The same treatment of **2** and **5** gave hydrolysis products, **4** (81 mg) from **2** (113 mg), and **6** (61 mg) from **5** (109 mg).

Partial Hydrolysis of 1 and 3 with Trifluoroacetic Acid Compound **1** (100 mg) was dissolved in 2 N CF_3COOH–MeOH solution (3 ml) and warmed at 60 °C for 6 h. After evaporating the solvents, the residue was dissolved in 50% MeOH (3 ml) and passed through a column of Diaion HP-20 (10 ml). The column was washed successively with 50% MeOH (20 ml) and MeOH (20 ml). The MeOH eluate showed on TLC two spots of methanolysis products accompanied by the spot of **1**. The product was subjected to preparative HPLC (34% acetonitrile) to give **1** (59 mg), a more polar methanolysis product (27 mg) and a less polar methanolysis product (5 mg). The more polar methanolysis product showed the same *Rf* value on TLC, and the same t_R value on HPLC, as those of **5**. The identity was confirmed by comparison of the NMR spectra.

The Less Polar Methanolysis Product (**9**): A white powder. $[\alpha]_D^{26}$ +30.6° (c=1.76, MeOH). Positive-ion HR FAB-MS m/z: 835.4475 ($[M+Na]^+$, $C_{42}H_{68}NaO_{15}$ requires 835.4456). Negative-ion FAB-MS m/z: 811 ($[M-H]^-$), 649 ($[M-H-glucose]^-$), 487 ($[aglycone-H]^-$). The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are shown in Tables 2 and 4. Sugar moiety: The ^{1}H-NMR spectrum showed anomeric proton signals at δ 5.22 (d, J= 8 Hz) and δ 5.12 (d, J=7 Hz). Other proton signals were not assigned. ^{13}C-NMR δ: 105.9, 105.3, 88.7, 78.6, 78.2, 77.8, 75.4, 74.0, 71.6, 69.6, 62.4, 62.2.

Compound **3** was treated in the same manner as described for **1** to give **6** (35 mg) and monoglucosyl bayogenin (**10**) (11 mg).

Compound **10**: A white powder. $[\alpha]_D^{26}$ +40.2° (c=1.30, MeOH). Positive-ion HR FAB-MS m/z: 673.3936 ($[M+Na]^+$, $C_{36}H_{58}NaO_{10}$ requires 673.3928). Negative-ion FAB-MS m/z: 649 ($[M-H]^-$), 487 ($[M-H-glucose]^-$). The chemical shifts of ^{1}H-NMR and ^{13}C-NMR signals of the aglycone moiety are shown in Tables 2 and 4. Sugar moiety: The ^{1}H-NMR spectrum showed signals at δ 5.15 (d, J=9 Hz, H1), 4.02 (t, J=9 Hz, H2), 4.15 (t, J=9 Hz, H3), 4.20 (t, J=9 Hz, H4), 3.90 (ddd, J=2, 6, 9 Hz, H5), 4.45 (dd, J=2, 12 Hz, H6a), 4.31 (dd, J=6, 12 Hz, H6b). ^{13}C-NMR δ: 105.6 (C1), 75.4 (C2), 78.5 (C3), 71.6 (C4), 78.2 (C5), 62.6 (C6).

Selective Cleavage of the Ester Glycoside Linkages of 1, 2, 3 and 5 Compound **1** (620 mg) and LiI (740 mg) were dissolved in a mixture of 2,6-lutidine (6 ml) and MeOH (3.6 ml), and the mixture was heated at 170 °C for 15 h. The reaction mixture was diluted with water (8 ml) and passed through a column of Amberlite MB-3 (50 ml). The eluate was passed through a column (20 ml) of Diaion HP-20 and the column was washed with 50% MeOH (100 ml) and then with MeOH (150 ml). The 50% MeOH eluate was concentrated and dried *in vacuo* to give a crude anomeric mixture (323 mg) of a methyl glycoside. The MeOH eluate was concentrated and dried *in vacuo* to give a crude prosapogenin (343 mg) of **1**. The prosapogenin fraction was purified by preparative HPLC (85% MeOH) to give thin-layer chromatographically and HPL chromatographically homogeneous **9** (246 mg).

Compounds **2** (540 mg), **3** (255 mg) and **5** (100 mg) were treated in the same manner as described for **1**, and as a result, methyl glycoside anomeric mixtures (351 mg from **2**; 186 mg from **3**; 73 mg from **5**) and prosapogenins [**9** (257 mg) from **2**; **10** (84 mg) from **3**; **9** (35 mg) from **5**] were obtained.

Separation of Anomers of Methyl Glycosides Derived from 1, 2, 3 and 5 The anomeric mixture (323 mg) of a methyl glycoside which originated from the ester-linked sugar moiety of **1** was subjected to preparative HPLC (20% MeOH) to give two fractions (**11**, 84 mg; **12**, 107 mg in the order of increasing t_R value). The same treatment of the sugar moiety (351 mg) from **2** gave **15** (81 mg) and **16** (122 mg); the sugar moiety (186 mg) from **3** gave **11** (44 mg) and **12** (61 mg); and the sugar moiety (73 mg) from **5** gave **13** (10 mg) and **14** (14 mg).

Compound **11**: A white powder. $[\alpha]_D^{26}$ −76.3° (c=0.76, MeOH). Positive-ion HR FAB-MS m/z: 743.2589 ($[M+Na]^+$, $C_{28}H_{48}NaO_{21}$ requires 743.2586). Negative-ion FAB-MS m/z: 719 ($[M-H]^-$), 587 ($[M-H-apiose]^-$), 573 ($[M-H-rhamnose]^-$), 441 ($[M-H-apiose-rhamnose]^-$), 309 ($[M-H-apiose-rhamnose-xylose]^-$), 163 ($[Methyl\ arabinoside-H]^-$). ^{1}H-NMR and ^{13}C-NMR spectral data are summarized in Table 6.

Compound **12**: A white powder. $[\alpha]_D^{26}$ −17.9° (c=0.76, MeOH). Positive-ion HR FAB-MS m/z: 743.2596 ($[M+Na]^+$, $C_{28}H_{48}NaO_{21}$ requires 743.2586). The positive-ion and negative-ion FAB-MS spectra are the same as those of **11**. ^{1}H-NMR and ^{13}C-NMR spectral data are summarized in Table 6.

Compound **15**: A white powder. $[\alpha]_D^{26}$ −70.1° (c=0.99, MeOH). Positive-ion HR FAB-MS m/z: 743.2588 ($[M+Na]^+$, $C_{28}H_{48}NaO_{21}$ requires 743.2586).The positive-ion and negative-ion FAB-MS spectra are the same as those of **11**. ^{1}H-NMR and ^{13}C-NMR spectral data are summarized in Table 7.

Compound **16**: A white powder. $[\alpha]_D^{26}$ −13.4° (c=1.00, MeOH). Positive-ion HR FAB-MS m/z: 743.2596. $C_{28}H_{48}NaO_{21}$ requires 743.2586. The positive-ion and negative-ion FAB-MS spectra are the same as those of **11**. ^{1}H-NMR and ^{13}C-NMR spectral data are summarized in Table 7.

Compound **13**: A white powder. $[\alpha]_D^{26}$ −53.6° (c=0.44, MeOH). Positive-ion HR FAB-MS m/z: 611.2166 ($[M+Na]^+$, $C_{23}H_{40}NaO_{17}$ requires 611.2164). Negative-ion FAB-MS m/z: 587 ($[M-H]^-$), 441 ($[M-H-rhamnose]^-$), 309 ($[M-H-rhamnose-xylose]^-$), 163 ($[methyl\ arabinoside-H]^-$). ^{1}H-NMR and ^{13}C-NMR spectral data are summarized in Table 5.

Compound **14**: A white powder. $[\alpha]_D^{26}$ −0.1° (c=0.63, MeOH). Positive-ion HR FAB-MS m/z: 611.2166 ($[M+Na]^+$, $C_{23}H_{40}NaO_{17}$ requires 611.2164). Positive-ion and negative-ion FAB-MS spectra are the same as those of **13**. ^{1}H-NMR and ^{13}C-NMR spectral data are summarized in Table 5.

Permethylation of Apiin, 9, 12 and 16 by Hakomori's Method,[11] and the Identification of Component Methylated Sugars Each sample (10—20 mg) was dissolved in dimethyl sulfoxide (DMSO) (0.5 ml). To the sample solution, 0.75 ml of dimethylsulfinyl carbanion solution, prepared by heating of the suspension of NaH (oil suspended, 200 mg) in DMSO (5 ml) at 80 °C for 30 min, was added, and the mixture was stirred for 30 min. CH_3I (1 ml) was added to the reaction mixture and the whole was stirred at room temperature for 13 h. Water (1 ml) was added and stirred for a while and the top aqueous layer was removed. The bottom layer was washed with water twice, dried over Na_2SO_4, concentrated and dried *in vacuo*. The residue was chromatographed on silica gel (1 g) and eluted first with benzene (10 ml) and then with 40% acetone–benzene (10 ml). The 40% acetone–benzene eluate was dissolved in 1 N HCl–MeOH (1 ml) and heated at 95 °C for 2 h in a sealed test tube. The reaction mixture was treated with Ag_2CO_3, and H_2S, and the solvent was evaporated off. A mixture (0.5 ml) of acetic anhydride–pyridine (1 : 1) was added to the residue and the mixture was stirred overnight. MeOH (2 ml) was added to the reaction mixture and the solution was checked by GC-CI-MS for methylated sugars compared with the standard methylated sugar samples. Identification of methylated sugars was performed by comparison of the t_R values and CI-MS fragmentation patterns of the sample sugars with those of the known standard methylated sugar samples. The methylated sugars identified are shown in the text. Methyl 2,3,5-tri-*O*-methyl α- and β-apiofuranosides were identified by comparison of the t_R values and CI-MS spectra with those obtained from apiin permethylate.

Measurement of Antiproliferative Activity against Tumor Cell Lines Nude mouse-transplantable human gastric adenocarcinoma cells (MK-1), human uterus carcinoma cells (HeLa) and murine melanoma cells (B16F10) were maintained in RPMI 1640 containing 10% fetal calf

serum (FCS). Cellular growth was determined using the MTT-microculture tetrazolium assay described previously.[12] Briefly, 50 μl of tumor cells (5×10^4 cells/ml) and 50 μl of the test solution were plated in flat-bottomed microtiter wells and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. After cultivation, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mg/ml) was added to the microculture wells. After 2-h incubation at 37 °C, 100 μl was removed from each well, to which 100 μl of DMSO was added to solubilize the MTT-formazan product. Absorbance at 590 nm was measured with a UVmax kinetic microplate reader (Molecular Devices, Menlo Park, Calif.). Percent cell growth inhibition $=(1-A/B)\times 100$ (A: OD_{590} of the tumor cells in RPMI 1640 with the test sample, B: OD_{590} of tumor cells in RPMI 1640 without the test sample).

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9) The instruments and materials used in this work were as follows: JASCO DIP-360 digital polarimeter (specific rotations), JEOL JNM GX-400 and JNM A-500 spectrometers (^{1}H-NMR and ^{13}C-NMR spectra), JEOL JMS HX-110 mass spectrometer (MS), Shimadzu GC-17A gas chromatograph (GC), Shimadzu GC-MS QP-5000 gas chromatograph/mass spectrometer with CI-50 controller (GC-CI-MS). For analytical scale HPLC, a Capcell Pak C_{18} column (Shiseido), 4.6 mm i.d. × 250 mm long, 5 μm, and UV detector (220 nm) were used. The flow rate of the solvent was set at 0.6 ml/min. The solvents were noted in the text. For preparative scale HPLC, a LiChrosphere RP-18 (e) column (Cica Merck), 25 mm × 250 mm, 5 μm, and a Shodex RI detector SE-31 were used. The solvent was noted in the text, and the flow rate of the solvent was set at 6 ml/min. Kieselgel 60, 70—230 mesh (E. Merck), MCI Gel HP-20 (Mitsubishi Chemical Industries, Ltd.), LiChroprep RP-18 (E. Merck). GC-CI-MS: GC conditions; column, DB-1 (J & W Scientific, U.S.A.), 0.25 mm × 30 m long i.d.; carrier gas, He, linear velocity in column, 35 cm/s; column oven temperature, 120→190 °C (2 °C/min); injection port temperature, 250 °C; split ratio, 20 : 1. CI-MS condition; reagent gas, isobutane; ionization voltage, 70 eV; interface temperature, 210 °C; scan range, m/z 100→400; scan interval, 0.5 s.
The NMR spectra were measured in pyridine-d_5 or CD_3OD, and chemical shifts were shown on the δ-scale using tetramethylsilane as an internal standard. The signal assignments were essentially based on the reported data, and were confirmed by the aid of the ^{1}H–^{1}H COSY, nuclear Overhauser enhancement and exchange spectroscopy (NOESY), ^{1}H–^{13}C COSY and ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) techniques.
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