Studies on the Constituents of Leguminous Plants. XII.[1] The Structures of New Triterpenoid Saponins from *Wistaria brachybotrys* SIEB. *et* ZUCC.[2]

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Four new triterpenoid saponins (wistariasaponins A, B_1, B_2 and C) were isolated as methyl ester forms from the knots of *Wistaria brachybotrys* (Leguminosae), and their structures were characterized as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl]wistariasapogenol A, 3-*O*[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl]wistariasapogenol B, 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]wistariasapogenol B and 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl]soyasapogenol B, respectively, on the basis of chemical and physicochemical evidence. The inhibitory effects of these saponins and sapogenols on Epstein–Barr virus activation induced by a tumor promoter were also tested for the primary screening of anti-tumor promoting activities.

Keywords *Wistaria brachybotrys*; Leguminosae; triterpenoid saponin; wistariasaponin A; wistariasaponin B_1; wistariasaponin B_2; wistariasaponin C; 2D-NMR; difference NOE; Epstein–Barr virus activation

In the previous paper,[1] we reported the isolation and the structure determination of several triterpenes, wistariasapogenol A (**1**), wistariasapogenol B (**2**) and soyasapogenol B (**3**), from the knots of *Wistaria brachybotrys* SIEB. *et* ZUCC. (Leguminosae). As a continuation of our chemical studies on the constituents of leguminous plants and biological studies on the potential anti-tumor promoting activities of crude drugs,[3] we have now isolated four new triterpenoid saponins, wistariasaponin A (**4**), wistariasaponin B_1 (**5**), wistariasaponin B_2 (**6**) and wistariasaponin C (**7**), together with known saponins, soyasaponin I (**8**) and soyasaponin II (**9**),[4] from the same source.

In this paper, we describe the structure elucidation and the inhibitory effects on Epstein–Barr virus (EBV) activation by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), of these new triterpenoid saponins.

The crude saponin fraction was fractionated by column chromatography on silica gel to give the fractions I, II and III, and each fraction was methylated with diazomethane to afford I-Me, II-Me and III-Me, respectively. Each methylated fraction was purified by column chromatography and preparative high-performance liquid chromatography (HPLC) repeatedly to give **4**, **5**, **6** and **7** together with **8** and **9** as methyl esters.

The methyl ester of wistariasaponin A (**4**), $C_{48}H_{76}O_{18}$, was obtained as a white powder. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **4** showed the presence of two carbonyl carbons (at δ 216.24 and 170.44) and three anomeric carbons (at δ 105.47, 102.55 and 102.31). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **4** showed three anomeric proton signals at δ 6.37 (d, J=1.2 Hz), δ 5.70 (d, J=7.7 Hz) and δ 5.00 (d, J=7.6 Hz). On acid hydrolysis of **4**, wistariasapogenol A (**1**), D-glucuronic acid, D-xylose and L-rhamnose were obtained. The sapogenol (**1**) was identified by comparison with an authentic sample,[1] and monosaccharides were led to the trimethylsilyl (TMS) ethers of methyl glycosides and identified by comparison with authentic samples on the basis of gas-liquid chromatography (GLC). These facts suggested the presence of β-linked of D-xylose and D-glucuronic acid in **4**. The ^{13}C-NMR spectrum of **4** showed that the signal of C-3 was shifted to lower field (+10 ppm) whereas other signals of hydroxylated carbons were not shifted by comparison with those of **1**. From this glycosylation shift, **4** was deduced to be a 3-*O*-monodesmoside of wistariasapogenol A.

The methyl ester of wistariasaponin B_1 (**5**), $C_{48}H_{78}O_{18}$, and methyl ester of wistariasaponin B_2 (**6**), $C_{49}H_{80}O_{19}$, were each obtained as a white powder. In the ^{13}C-NMR spectra of **5** and **6**, the signals of one carbonyl carbon (at δ 170.46 and 170.39, respectively) and three anomeric carbons (at δ 105.48, 102.58 and 102.36, and 105.48, 101.71 and 102.41, respectively) were seen. The ^{1}H-NMR spectra of **5** and **6** each showed three anomeric proton signals, at δ 6.16 (d, J=1.2 Hz), δ 5.49 (d, J=7.6 Hz) and δ 4.92 (d, J=7.8 Hz), and δ 6.29 (d, J=1.2 Hz), δ 5.78 (d, J=7.3 Hz) and δ 4.97 (d, J=7.8 Hz), respectively. On acid hydrolysis of **5** and **6**, L-rhamnose, D-xylose and D-glucuronic acid, and L-rhamnose, D-galactose and D-glucuronic acid were respectively obtained and identified in the same manner as described above. Wistariasapogenol B (**2**) was obtained from both saponins **5** and **6**, and identified. From the results of the hydrolysis and the ^{1}H- and ^{13}C-NMR spectra, it was deduced that **5** and **6** were also 3-*O*-monodesmosides of **2** having β-linked of D-xylose and D-glucuronic acid, and D-galactose and D-glucuronic acid, respectively. The negative fast atom bombardment mass spectra (negative FAB-MS) of **5** and **6** showed the $M^- - 1$ peaks (at m/z 941 and 971, respectively) and M^- − rhamnose peaks (at m/z 795 and 825, respectively). Therefore, it was ascertained that rhamnose was at the terminal of each sugar moiety.

The methyl ester of wistariasaponin C (**7**), $C_{48}H_{78}O_{17}$, was obtained as a white powder. In the ^{13}C-NMR spectrum of **7**, the signals of one carbonyl carbon (at δ 170.46) and three anomeric carbons (at δ 105.49, 102.58 and 102.33) were seen. The ^{1}H-NMR of **7** showed three anomeric proton signals at δ 6.36 (d, J=1.2 Hz), δ 5.68 (d, J=7.6 Hz) and δ 5.00 (d, J=7.6 Hz). On acid hydrolysis of **7**, soyasapogenol B (**3**), L-rhamnose, D-xylose and D-glucuronic acid were obtained and identified by comparison with authentic samples in the same manner as described above. For the same reason as in the case of **4**, the saponin (**7**) was

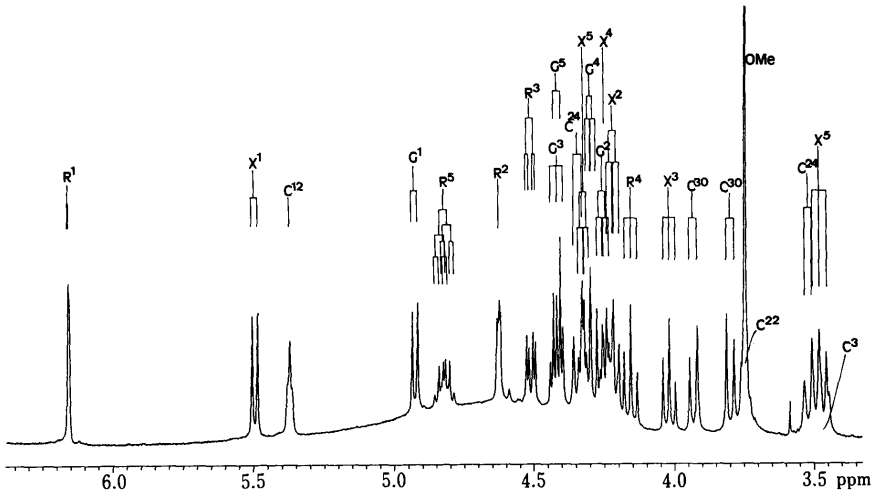

Fig. 1. ^{1}H-NMR Spectrum of Wistariasaponin B_1 (**5**) in Pyridine-d_5 at 95°C (400 MHz)

Abbreviations: R^1—R^5, rhamnose C-1-H—C-5-H; X^1—X^5, xylose C-1-H—C-5-H; G^1—G^5, glucuronic acid C-1-H—C-5-H; C^{12}, C^{24}, C^{30}, due to sapogenol moiety.

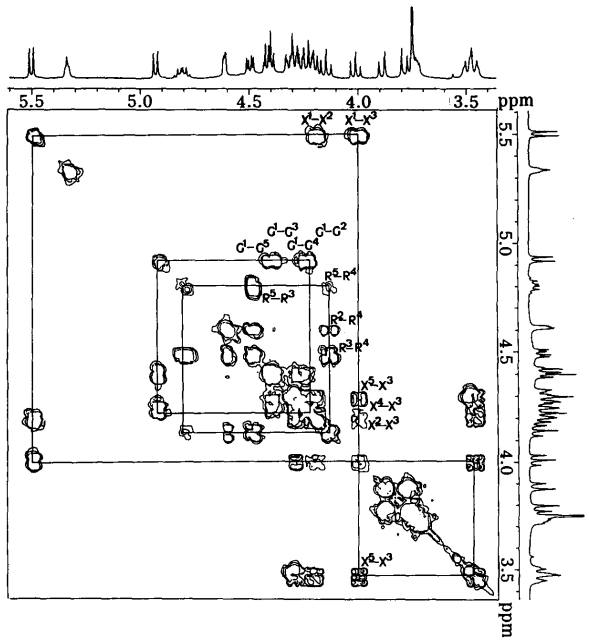

Fig. 2. ^{1}H–^{1}H Relay COSY Spectrum of **5** in Pyridine-d_5 at 95°C (400 MHz)

Abbreviations: R, rhamnose; X, xylose; G, glucuronic acid; X—X, R—R, G—G, mutual relations in each sugar.

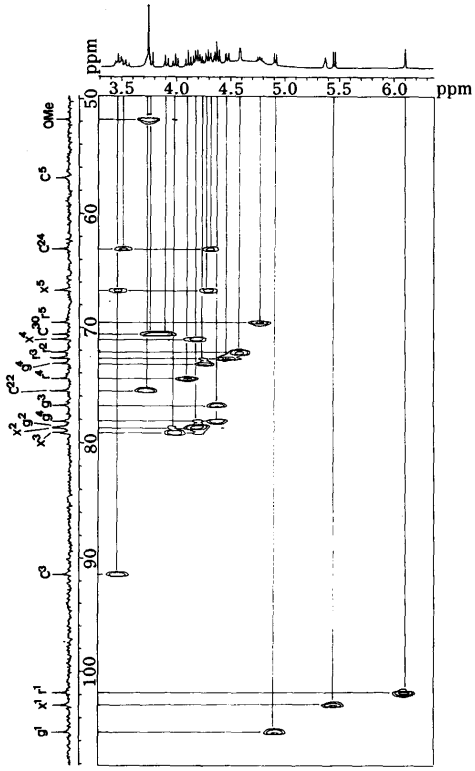

Fig. 3. ^{1}H–^{13}C COSY Spectrum of **5** in Pyridine-d_5 at 95°C (400 MHz)

Abbreviations: r^1—r^5, carbons of rhamnose; x^1—x^5, carbons of xylose; g^1—g^5, carbons of glucuronic acid; $C^{3,5,22,24,30}$, carbons of sapogenol.

considered to be a 3-*O*-monodesmoside of **3** having two β-linked D-xylose and D-glucuronic acid. The structure of this oligosaccharide moiety was determined as follows.

From the ^{1}H-NMR spectrum and two-dimensional (2D) ^{1}H[^{1}H]-correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H[^{13}C]-correlation (^{1}H–^{13}C COSY) and ^{1}H–^{1}H relay COSY experiments on wistariasaponin B_1 (**5**) at 80°C and 95°C, respectively, all proton signals in the sugar moiety could be assigned as shown in Fig. 1. On the basis of these assignments, difference nuclear Overhauser effect (difference NOE) experiments were carried out to determine the position of the glycosyl bonds of each sugar in **5**.

While irradiation at δ 6.16 (anomeric proton of rhamnose) enhanced the signal intensity of xylose 2-H (δ 4.22), irradiation at δ 5.49 (anomeric proton of xylose) enhanced the signal intensity of xylose 3-H (δ 4.02), 5-H (δ 4.32) and the anomeric proton of glucuronic acid (δ 4.92). Irradiation at δ 4.92 (anomeric proton of glucuronic acid) enhanced the signal intensity of glucuronic acid 3-H (δ 4.42) and 5-H (δ 4.43). Furthermore, enhancements of the signal intensity of anomeric protons in glucuronic acid (δ 4.92) and rhamnose (δ 6.16) were observed on irradiation at δ 3.52 (one of the methylene protons at C-24 of the sapogenin) and δ 4.63 (rhamnose 2-H), respectively. Some other significant NOEs are indicated by arrows in Fig. 4.

On the basis of the results of difference NOE experiments and the comparison of ^{13}C chemical shift values of **5** with those of **8** and **9**, the sugar moiety of **5** was characterized as

TABLE I. ^{13}C-NMR Chemical Shift Values (δ) of **4**, **5**, **6** and **7** in C_5D_5N at 75 MHz

	4	5	6	7		4	5	6	7
Sapogenol moiety					Sugar moiety				
						Glu.	Glu.	Glu.	Glu.
C-1	38.76	38.85 (39.26)[d]	38.66	38.88	C-1′	105.47	105.48 (105.23)[d]	105.48	105.49
C-2	26.64	26.69 (26.59)[b]	26.64	26.69	C-2′	78.63	78.63 (78.60)	78.09	78.65
C-3	91.12	91.13 (91.39)	91.33	91.18	C-3′	76.81	76.83 (76.74)	76.43	76.83
C-4	44.30	44.35 (44.54)	43.89	44.35	C-4′	73.61	73.65 (73.09)	72.65	73.64
C-5	56.35	56.40 (56.86)	56.08	56.42	C-5′	77.55	77.58 (78.11)	76.55	77.58
C-6	18.60	18.66 (18.56)	18.51	18.67	C-6′	170.44	170.46 (169.97)	170.39	170.46
C-7	33.13	33.28 (33.56)	33.18	33.26	(OMe)	(52.14)	(52.13) (51.78)	(52.18)	(52.15)
C-8	39.78	39.95 (40.31)	39.90	39.96		Xyl.	Xyl..	Gal.	Xyl.
C-9	47.62	47.76 (48.07)	47.78	47.81	C-1″	102.55	102.58 (102.89)	101.71	102.58
C-10	36.54	36.55 (36.83)	36.43	36.57	C-2″	79.41[a]	79.47[a] (78.69)	77.76	79.45
C-11	23.95	24.00 (24.14)	23.98	24.05	C-3″	78.06[a]	78.17[a] (79.07)	73.56	78.11
C-12	123.62	122.74 (123.00)	122.64	122.43	C-4″	70.84	70.88 (70.96)	71.08	70.86
C-13	142.17	144.63 (144.75)	144.61	144.83	C-5″	66.80	66.86 (66.67)	76.92	66.83
C-14	42.10	42.40 (42.55)[c]	42.32	42.42	C-6″			61.56	
C-15	25.44	26.39 (26.59)[b]	26.38	26.41		Rham.	Rham.	Rham.	Rham.
C-16	27.31	28.71 (28.81)	28.66	28.67	C-1‴	102.31	102.36 (101.81)	102.41	102.33
C-17	47.78	38.08 (38.03)	38.04	38.02	C-2‴	72.34	72.39 (72.10)	72.35	72.37
C-18	47.39	45.17 (45.36)	45.10	45.37	C-3‴	72.67	72.74 (72.62)	72.68	72.71
C-19	42.84	42.07 (42.55)[c]	42.05	46.77	C-4‴	74.30	74.34 (74.40)	74.35	74.34
C-20	38.87	35.90 (35.69)	35.87	30.89	C-5‴	69.38	69.45 (69.45)	69.28	69.42
C-21	46.99	38.74 (38.64)	38.60	42.28	C-6‴	18.91	18.95 (18.94)	18.95	18.93
C-22	216.24	75.22 (75.48)	75.20	75.57					
C-23	22.94	22.99 (23.00)	22.95	22.97					
C-24	62.84	62.88 (63.05)	63.56	62.87					
C-25	15.52	15.60 (15.64)	15.80	15.63					
C-26	16.83	17.01 (17.16)	16.94	17.05					
C-27	25.35	25.79 (25.89)	25.76	25.68					
C-28	21.29	21.25 (21.04)	21.19	28.67					
C-29	26.97	28.52 (28.54)	28.48	33.26					
C-30	68.21	70.25 (70.55)	70.23	21.16					

a) Assignments may be interchangeable within the same column. *b*, *c*) These signals were overlapping under the this conditions used. *d*) Chemical shift values in parentheses were measured at 100 MHz (95 °C).

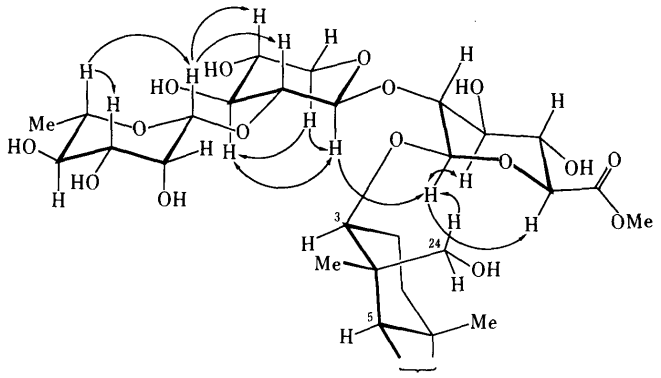

Fig. 4. Enhancements of Signal Intensity in Difference NOE Experiments on **5**

α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranoside. Therefore, wistariasaponin B_1 was characterized as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl]wistariasapogenol B (Chart 1).

As shown in Table I, the ^{13}C chemical shift values of the oligosaccharide moieties of **4**, **5** and **7** were superimposable on each other within ±0.1 ppm. Therefore, it was deduced that **4**, **5** and **7** have the same sugar moieties at the C-3 position of each triterpene.

As a result, wistariasaponin A (**4**) was characterized as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl]wistariasapogenol A, and wistariasaponin C (**7**) was characterized as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl]soyasapogenol B (Chart 1).

Since the chemical shifts of the sugar moiety in **6** were superimposable with those of **8**, wistariasaponin B_2 was characterized as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]wistariasapogenol B (Chart 1).

The inhibitory effects of these triterpenes and triterpenoid saponins on EBV activation induced by TPA are shown in Table II. Wistariasapogenol A (**1**), soyasapogenol B (**3**), wistariasaponin A (**4**), wistariasaponin C (**7**), soyasaponin I (**8**) and soyasaponin II (**9**) exhibited notable inhibitory effects at 1×10^3 mol ratio and preserved high viability of Raji cells at this high dose. In our experiments, the degrees of inhibitory activity of **3**, **4**, **8** and **9** were comparable to that found with oleanolic acid, a known inhibitor of EBV activation.[5] Compounds **4** and **9** showed significant inhibitory effects (20—30%) even at low doses (1×10^2 mol ratio). On the other hand, such effects were not found with **2**, **5** and **6**.

These results suggested that soyasapogenol B (**3**), wistariasaponin A (**4**) and soyasaponin II (**9**) might be effective anti-tumor promoters. Tumor initiation–promotion tests *in vivo* with these compounds are in progress.

Experimental

Melting points were taken on a Yanagimoto micro melting point

TABLE II. Inhibitory Effects of **1**—**9** on Epstein-Barr Virus Activation Induced by TPA in Raji Cells

Compounds	Concentration (mol ratio, compound/TPA)			
	1×10^3	5×10^2	1×10^2	1×10
	% to control (% viability of Raji cell)			
TPA (32 pM)	100 = positive control			
1	47.4±2.5[a] (70.0)	91.3±4.1[a] (>80)	100.0±0.0[a] (>80)	100.0±0.0[a] (>80)
2	75.5±5.1 (70.0)	92.4±3.6 (>80)	100.0±0.0 (>80)	100.0±0.0 (>80)
3	27.6±4.8 (70.0)	45.3±3.1 (>80)	88.5±1.0 (>80)	100.0±0.0 (>80)
4	34.1±2.7 (70.0)	55.5±2.1 (>80)	80.3±5.4 (>80)	96.6±3.0 (>80)
5	50.7±4.2 (70.0)	64.3±4.0 (>80)	89.6±5.7 (>80)	100.0±0.0 (>80)
6	59.2±3.8 (60.0)	81.4±5.5 (>80)	100.0±0.0 (>80)	100.0±0.0 (>80)
7	43.6±4.4 (60.0)	73.6±3.8 (>80)	100.0±0.0 (>80)	100.0±0.0 (>80)
8	43.0±1.0 (50.0)	67.8±2.4 (>80)	90.3±4.1 (>80)	100.0±0.0 (>80)
9	45.2±3.4 (50.0)	51.3±5.4 (>80)	73.0±2.2 (>80)	91.3±1.6 (>80)
10[b]	100.0±0.0 (>80)	100.0±0.0 (>80)	100.0±0.0 (>80)	100.0±0.0 (>80)

a) Each value of this column represents the average of three determinations ± S.D. *b*) This compound was obtained from **4** and **5** by acid hydrolysis as an artifact (22,30-oxide of **2**).[1]

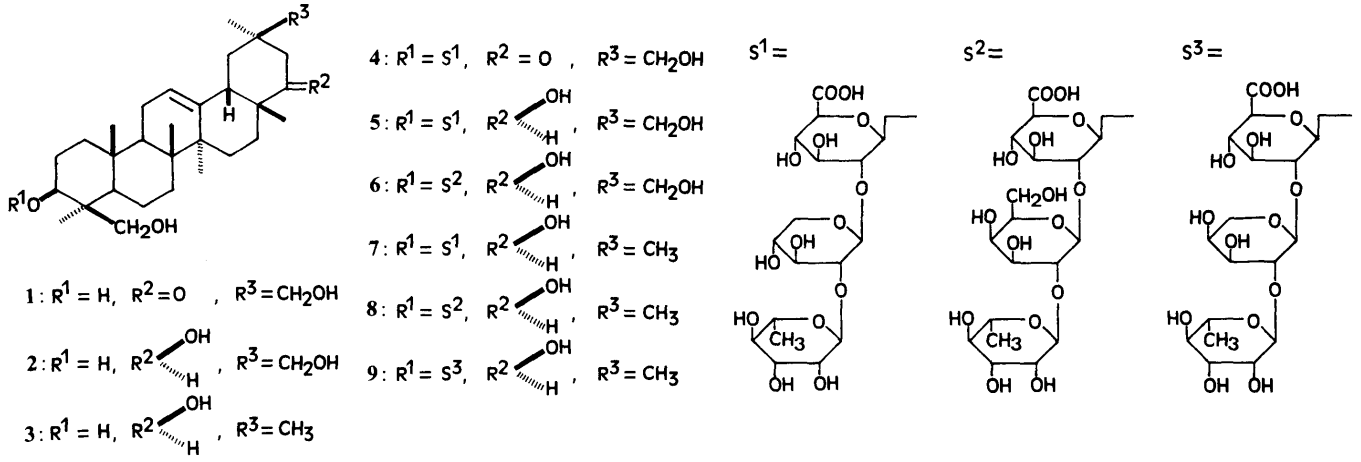

Chart 1

apparatus, and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-408 spectrometer. Unless otherwise stated, ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-300 spectrometer in pyridine-d_5 using TMS as an internal standard. 2D-NMR and difference NOE spectra were recorded on a JEOL JNM GX-400 spectrometer. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Preparative HPLC was carried out on a Nihon Bunseki Kogyo LC-09 using a GPC column (300 mm × 2) with an RI detector. GLC was carried out on a Shimadzu GC-9A [column, ULBON HR-1701 (0.25 mm × 15 m); column temperature, 130—180 °C (2.5 °C/min); injection and detection port temperature, 250 °C, carrier gas, N_2 0.7 ml/min; inlet pressure, 0.4 kg/cm^2; split ratio, 1/60; make-up gas, N_2 40 ml/min].

Isolation of Wistariasaponin A (4), B$_1$ (5), B$_2$ (6), and C (7) The crude saponin fraction (42 g) was obtained from the knots of *W. brachybotrys* as described in a previous paper.[1] This fraction (3.2 g) was repeatedly chromatographed on a silica gel column with $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower layer) to afford three fractions, I (410 mg), II (370 mg) and III (530 mg). Each fraction was methylated with CH_2N_2 in methanol at room temperature and worked up in a usual manner to give I-Me, II-Me and III-Me. Each methylated fraction was chromatographed on silica gel with the same solvent as described above. The major fraction was purified by preparative HPLC on gel permeation chromatography (GPC) (recycled 15 times with MeOH). From fraction I-Me (120 mg), soyasaponin II (9, 45 mg) and wistariasaponin A (**4**, 42 mg) were obtained as methyl esters. From fraction II-Me (180 mg), wistariasaponin B$_1$ (**5**, 83 mg) and wistariasaponin B$_2$ (**6**, 37 mg) were obtained as methyl esters. From fraction III-Me (230 mg), soyasaponin I (**8**, 120 mg) and wistariasaponin C (**7**, 30 mg) were obtained as methyl esters. Methyl esters of soyasaponins I and II were identified by comparison with authentic samples (thin layer chromatography (TLC) and HPLC behavior, and IR and ^{13}C-NMR spectra).

Methyl Ester of Wistariasaponin A (4) Hygroscopic white powder, $[\alpha]_D^{20}$ −18.8° (*c* = 0.65, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1720 ($COOCH_3$), 1695 (>C=O), ^{1}H-NMR δ: 6.37 (1H, d, *J* = 1.2 Hz, rhamnose anomeric H), 5.70 (1H, d, *J* = 7.7 Hz, xylose anomeric H), 5.33 (1H, t like, C_{12}-H), 5.00 (1H, d, *J* = 7.6 Hz, glucuronic acid anomeric H), 3.76 (3H, s, $COOCH_3$), 2.57 (2H, brs, C_{21}-H_2), 1.81 (3H, d, *J* = 6.1 Hz, rhamnose CH_3). *Anal.* Calcd for $C_{48}H_{76}O_{18}\cdot 3/2H_2O$: C, 59.55; H, 8.22. Found: C, 59.74; H, 8.42. ^{13}C-NMR (Table I).

Methyl Ester of Wistariasaponin B$_1$ (5) Hygroscopic white powder, $[\alpha]_D^{20}$ −10.9° (*c* = 0.87, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1710 ($COOCH_3$), ^{1}H-NMR δ (400 MHz, at 95 °C)[6]: 6.16 (1H, d, *J* = 1.2 Hz, rhamnose anomeric H), 5.49 (1H, d, *J* = 7.6 Hz, xylose anomeric H), 5.37 (1H, t like, *J* = 1.0 Hz, C_{12}-H), 4.92 (1H, d, *J* = 7.8 Hz, glucuronic acid anomeric H), 4.82 (1H, dq, *J* = 6.5, 3.2 Hz, R_5-H), 4.63 (1H, dd, *J* = 3.4, 1.7 Hz, R_2-H), 4.51 (1H, dd, *J* = 9.3, 3.4 Hz, R_3-H), 4.43 (1H, d, *J* = 9.5 Hz, G_5-H), 4.42 (1H, t, *J* = 8.9 Hz, G_3-H), 4.30 (1H, dd, *J* = 9.5, 9.0 Hz, G_4-H), 4.26 (1H, dd, *J* = 8.9, 7.8 Hz, G_2-H), 4.32 (1H, dd, *J* = 9.7, 5.6 Hz, one of X_5-H_2), 4.22 (1H, dd, *J* = 9.0, 7.6 Hz, X_2-H), 4.16 (1H, t, *J* = 9.3 Hz, R_4-H), 4.02 (1H, t, *J* = 9.0 Hz, X_3-H), 3.93, 3.80 (2H, ABd, *J* = 10.4 Hz, C_{30}-H_2), 3.77 (3H, s, $COOCH_3$), 4.34, 3.52 (2H, ABd, *J* = 11.7 Hz, C_{24}-H_2), 3.48 (1H, t, *J* = 10.4 Hz, one of X_5-H_2), 1.72 (3H, d, *J* = 6.4 Hz, R_6-H_3), 1.52, 1.35, 1.23, 1.16, 1.05, 0.86 (3H, each s, CH_3 × 6). Negative FAB-MS *m/z*: 941 (M^- − 1), 795 (M^- − rhamnose). *Anal.* Calcd for $C_{48}H_{78}O_{18}\cdot H_2O$: C, 59.98; H, 8.39. Found: C, 60.36; H, 8.46. ^{13}C-NMR (Table I).

Methyl Ester of Wistariasaponin B$_2$ (6) Hygroscopic white powder, $[\alpha]_D^{20}$ −7.9° (*c* = 1.21, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1710 ($COOCH_3$), ^{1}H-NMR δ: 6.29 (1H, d, *J* = 1.2 Hz, rhamnose anomeric H), 5.78 (1H, d, *J* = 7.3 Hz, galactose anomeric H), 5.29 (1H, t like, C_{12}-H), 4.97 (1H, d, *J* = 7.8 Hz, glucuronic acid anomeric H), 3.76 (3H, s, $COOCH_3$), 1.77 (3H, d, *J* = 5.9 Hz, rhamnose CH_3), 1.44, 1.31, 1.23, 1.18, 0.94, 0.70 (3H, each s, CH_3 × 6). Negative FAB-MS *m/z*: 971 (M^- − 1), 825

TABLE III. GLC of TMS Ethers of Methyl Glycoside from **4**, **5**, **6** and **7**[a]

Saponin	Sugars	t_R (min)[b]
4, **5** and **7**	TMS-methyl rhamnoside	8.29, 8.95, 10.80
	TMS-methyl xyloside	9.68, 11.70, 12.22
	TMS-methyl glucuronide	21.65, 22.04, 22.50
6	TMS-methyl rhamnoside	8.29, 8.95, 10.80
	TMS-methyl galactoside	17.26, 17.68, 18.71
	TMS-methyl glucuronide	21.65, 22.04, 22.50

a) Conditions are given in the experimental section. *b*) These t_R values were in agreement with these of authentic TMS-methyl glycosides.

(M^- − rhamnose). *Anal.* Calcd for $C_{49}H_{80}O_{19}\cdot H_2O$: C, 59.38; H, 8.34. Found: C, 59.66; H, 8.39. ^{13}C-NMR (Table I).

Methyl Ester of Wistariasaponin C (7) Hygroscopic white powder, $[\alpha]_D^{20}$ −14.9° (c=0.71, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1710 ($COOCH_3$), ^{1}H-NMR δ: 6.36 (1H, d, J=1.2 Hz, rhamnose anomeric H), 5.68 (1H, d, J=7.6 Hz, xylose anomeric H), 5.32 (1H, t like, C_{12}-H), 5.00 (1H, d, J=7.6 Hz, glucuronic acid anomeric H), 3.76 (3H, s, $COOCH_3$), 1.18 (3H, d, J=6.2 Hz, rhamnose CH_3), 1.53, 1.30, 1.29, 1.22, 1.00, 0.98, 0.78 (3H, each s, $CH_3\times 7$). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot H_2O$: C, 61.00; H, 8.53. Found: C, 61.23; H, 8.65. ^{13}C-NMR (Table I).

General Procedure for Methanolysis of 4, 5, 6 and 7 A solution of the pure saponin in 1 N HCl–dry MeOH was refluxed for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and the inorganic precipitate was filtered off. The filtrate was concentrated to half the initial volume *in vacuo*. The precipitate was filtered and identified by comparison with an authentic sample (TLC behavior and IR spectrum). [Wistariasapogenol A[1] (**1**, 7 mg) was obtained from **4** (30 mg), wistariasapogenol B[1] (**2**, 4 mg) was obtained from **5** (20 mg) and **6** (20 mg), and soyasapogenol B[4] (**3**, 6 mg) was obtained from **7** (20 mg)]. After identification of each sapogenol, each methanolic filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in dry pyridine and treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide for 1.5 h. The product was subjected to GLC to identify the trimethylsilyl (TMS) derivatives of the methyl monosaccharides. The results are shown in Table III.

Biological Activities The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer), EBV genome-carrying human lymphoblastoid cells, which were cultivated in 8% fetal bovine serum (FBS) RPMI 1640 medium (Nissui). The indicator cells (Raji) (1×10^6/ml) were incubated at 37 °C for 48 h in 1 ml of the medium containing *n*-butyric acid (4 mM, inducer), 2 μl of TPA (20 ng/ml in dimethylsulfoxide (DMSO)), and a known amount of a test compound in DMSO. Smears were made from the cell suspension. The activated cells were stained with high-titer EBV-EA positive sera from nasopharyngeal carcinoma (NPC) patients and detected by a conventional indirect immunofluorescence technique. In each assay, at least 500 cells were counted, and the experiments were repeated three times. The average EA induction was compared with that of positive control experiments with *n*-butyric acid (4 mM) plus TPA (20 ng/ml) in which EA induction was ordinarily around 30%. In this screening method, the cell viability required for the judgement of inhibitory effects was more than 60%.[3]

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References and Notes

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6) Abbreviations for the signal assignments are as follows: R_{2-6}, X_{2-5} and G_{2-5}, carbon numbers of rhamnose, xylose and glucuronic acid, respectively. The signals of X_4-H and C_3-H were detected by ^{1}H–^{13}C COSY experiments.