Saponins and *C*-Glycosyl Flavones from the Seeds of *Abrus precatorius*

Chao-mei MA, Norio NAKAMURA, and Masao HATTORI*

Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930–0194, Japan. Received December 4, 1997; accepted February 16, 1998

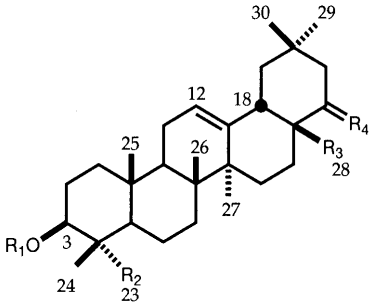
Two new saponins, 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl]hederagenin (named abrus-saponin I) and 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic acid 28-β-D-glucopyranosyl ester (abrus-saponin II), and three new flavones, 6-*C*-β-D-glucopyranosyl-4′,5-dihydroxy-7,8-dimethoxyflavone (precatorin I), 6-*C*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl]-4′,5-dihydroxy-7,8-dimethoxyflavone (precatorin II), 6-*C*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl]-4′,5-dihydroxy-7-methoxyflavone (precatorin III), were isolated from the seeds of *Abrus precatorius* L. together with twelve known compounds including a naturally new saponin, 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic acid. Their structures were determined on the basis of chemical and spectroscopic methods. In addition, the unusual NMR spectral behavior of the flavone *C*-glycosides is also discussed.

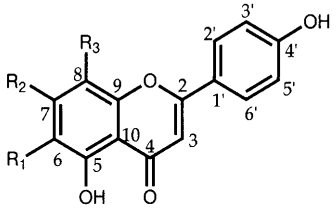
Key words *Abrus precatorius*; Leguminosae; saponin; *C*-glycosyl flavone

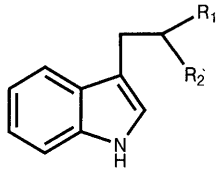
Abrus (*A.*) *precatorius* L. (Leguminosae) is widely distributed in South China, South East Asia and Africa. Its seeds, Xiang-si-zi (相思子) in Chinese, have been used as an insecticide and for skin diseases since ancient times.[1] In our extensive screening of medicinal plants for anti-human immunodeficiency virus type-1 (HIV-1) activity, the seeds of *A. precatorius* showed relatively potent activity,[2] which stimulated us to investigate the active principles. As regards the chemical constituents, alkaloids,[3] triterpenoids,[3] and flavonoids[4] have previously been reported from the seeds. The present paper deals with the isolation and structural elucidation of two new saponins, named abrus-saponins I (**1**) and II (**3**), three new *C*-glycosyl flavones named precatorins I (**9**), II (**10**), and III (**11**) and twelve known compounds, including a naturally new saponin (**2**) from the seeds. In addition, the unusual NMR spectral behavior of the *C*-glycosyl flavones is also described.

Results and Discussion

A MeOH extract of the seeds of *A. precatorius* was passed through a porous polymer gel (Diaion HP-20) column. The MeOH eluate was repeatedly chromatographed over normal and reversed-phase silica-gel to yield eight saponins (**1**—**8**) and six flavonoids (**9**, **10**, **12**—**15**). The MeOH–H_2O (1 : 1) eluate was subjected to repeated chromatography to afford two alkaloids (**16** and **17**) and three flavonoids (**10**, **11** and **13**). The known compounds







	R_1	R_2	R_3	R_4
1	glc^2-glcA	CH_2OH	COOH	H, H
2	glc^2-glcA	Me	COOH	H, H
3	glc^2-glcA	Me	COOglc	H, H
4	$glcA^2$-gal	Me	Me	H, β-OH
5	$glcA^2$-gal^2-xyl	Me	Me	H, β-OH
6	$glcA^2$-gal^2-rha	Me	Me	H, β-OH
7	$glcAMe^2$-gal^2-rha	Me	Me	H, β-OH
8	$glcA^2$-gal^2-rha	Me	Me	O

	R_1	R_2	R_3
9	glc	OMe	OMe
10	glc^2-apiose	OMe	OMe
11	glc^2-apiose	OMe	H
12	OMe	OMe	glc
13	OMe	OMe	glc^2-apiose
14	OMe	OMe	H
15	OMe	OH	H

	R_1	R_2
16	$NHCH_3$	COOH
17	$N^+(CH_3)_3$	COO^-

Chart 1. Structures of Isolated Compounds

* To whom correspondence should be addressed.

were identified as 3-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl]oleanolic acid (**2**),[5] kaikasaponin I (**4**),[6] 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl] sophoradiol (**5**),[7] kaikasaponin III (**6**),[8] kaikasaponin III methyl ester (**7**),[3] phaseoside IV (**8**),[8] abrusin (**12**),[4] abrusin 2″-*O*-β-D-apioside (**13**),[4] 4′,5-dihydroxy-6,7-dimethoxyflavone (**14**),[9] 4′,5,7-trihydroxy-6-methoxyflavone (**15**),[10] abrine (**16**),[3] and *N*,*N*,*N*-trimethyl tryptophan (**17**)[3] by comparing their spectral data with those previously reported. Compound **2** has been synthesized as a strongly protective compound against carbon tetrachloride (CCl_4)-induced hepatotoxicity *in vivo*.[5] However, this is the first report of this compound from natural sources.

Compound **1** (abrus-saponin I) showed a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 833 in the positive ion atmospheric pressure ionization (API) MS, and $[M-H]^-$ at *m/z* 809 in the negative ion API-MS. The ^{1}H-NMR spectrum of **1** exhibited signals characteristic of six singlet methyls at δ 0.82, 0.88, 0.94, 0.95, 1.12, 1.19, one trisubstituted olefinic proton at δ 5.42, and two anomeric protons at δ 5.14 and 5.38. The ^{13}C-NMR spectrum showed signals for two anomeric carbons at δ 104.1 and 106.9, a pair of olefinic carbons at δ 122.4 and 144.7, and two carbonyl carbons at δ 172.2 and 180.1. On acid hydrolysis, **1** afforded an aglycone which was identified as hederagenin by comparing its ^{13}C-NMR data with that previously reported,[11] and the monosaccharide units obtained were identified by co-TLC with authentic samples as glucose and glucuronic acid; their absolute configurations were determined as the D-form by the method developed by Hara *et al.*[12] In the homonuclear Hartmann–Hahn (HOHAHA) spectrum of **1**, correlations were observed between the anomeric signal at δ 5.38 and signals at δ 4.23, 4.27, 4.59 as well as 4.58. This series of signals was considered to be due to the protons of a glucuronic acid, because correlations were found between the carbonyl signal at δ 172.2 (glcA-C-6) and two of the proton signals at δ 4.58 (glcA-H-5) and 4.59 (glcA-H-4) in the heteronuclear multiple bond coherence (HMBC) spectrum. Similarly, the spin system for a glucose moiety was assigned, since the anomeric proton signal at δ 5.14 was found to correlate with the signals at δ 4.11—4.15, 3.75, 4.33 and 4.45. On the basis of spectroscopic evidence obtained by ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H-detected multiple quantum coherence (HMQC) experiments, the sugar protons and carbons were assigned as shown in Tables 1 and 2, respectively. The sugar linkages were determined from the HMBC spectrum. Long-range coupling ($^3J_{HCOC}$) observed between the proton signal at δ 5.14 (glc-H-1) and the carbon signal at δ 81.8 (C-3 of the aglycone), and between the proton signal at δ 5.38 (glcA-H-1) and the carbon signal at δ 85.3 (glc-C-2) confirmed glycosylation at C-3 with a glcA(1→2)glc

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**3** in C_5D_5N (500 MHz)

	1	**2**	**3**
Aglycon			
3	4.25[a)]	3.26 (d, *J*=12.4, 3.9 Hz)	3.24 (dd, *J*=4.0, 11.3 Hz)
12	5.42 (t-like)	5.44 (t-like)	5.39 (t-like)
18	3.24 (dd, *J*=4.0,14.0 Hz)	3.26 (dd, *J*=12.4, 3.9 Hz)	3.16 (dd, *J*=4.1, 13.6 Hz)
23	3.78 (d, *J*=11.0 Hz)	1.37 (s)	1.35 (s)
	4.55 (d, *J*=11.0 Hz)		
24	1.12 (s)	1.16 (s)	1.16 (s)
25	0.82 (s)	0.73 (s)	0.76 (s)
26	0.94 (s)	0.93 (s)	1.04 (s)
27	1.19 (s)	1.25 (s)	1.21 (s)
29	0.88 (s)	0.93 (s)	0.88 (s)
30	0.95 (s)	0.98 (s)	0.85 (s)
Glc			
1′	5.14 (d, *J*=7.0 Hz)	4.94 (d, *J*=7.5 Hz)	4.92 (d, *J*=7.7 Hz)
2′	4.11[a)]	4.16[a)]	4.16[a)]
3′	4.14[a)]	4.33[a)]	4.32[a)]
4′	4.15[a)]	4.18[a)]	4.17[a)]
5′	3.75 (m)	3.93 (m)	3.92 (m)
6′	4.33 (dd, *J*=4.5, 11.0 Hz)	4.36 (dd, *J*=5.1, 12.0 Hz)	4.36[a)]
	4.45 (br d, *J*=11.0 Hz)	4.54 (br d, *J*=12.0 Hz)	4.54 (br d, *J*=11.5 Hz)
GlcA			
1″	5.38 (d, *J*=7.5 Hz)	5.40 (d, *J*=7.7 Hz)	5.40 (d, *J*=7.7 Hz)
2″	4.23[a)]	4.25 (t, *J*=7.7 Hz)	4.21[a)]
3″	4.27[a)]	4.30[a)]	4.29[a)]
4″	4.59[a)]	4.62[a)]	4.61[a)]
5″	4.58[a)]	4.61[a)]	4.60[a)]
C-28-Glc			
1			6.32 (d, *J*=8.1 Hz)
2			4.18[a)]
3			4.25[a)]
4			4.35[a)]
5			4.00 (m)
6			4.39[a)]
			4.45 (br d, *J*=11.5 Hz)

a) Chemical shifts of these signals were determined by ^{1}H-^{1}H COSY and HMQC.

Table 2. ^{13}C-NMR Spectral Data of Conpounds **1**—**3** in C_5D_5N

Position	**1**[a]	**2**[b]	**3**[b]
1	38.6	38.8	39.1
2	26.2	26.8	27.0
3	81.8	89.2	89.5
4	43.5	39.6	39.9
5	47.4	55.9	56.2
6	18.0	18.5	18.9
7	32.7	33.3	33.5
8	39.6	39.8	40.3
9	47.9	48.0	48.3
10	36.7	37.0	37.3
11	23.7	23.8	23.8
12	122.4	122.5	123.3
13	144.7	144.7	144.5
14	41.8	42.2	42.5
15	28.2	28.3	28.6
16	23.5	23.9	24.0
17	46.5	46.8	47.4
18	42.0	42.1	42.1
19	46.3	46.5	46.6
20	30.8	31.1	31.2
21	34.1	34.3	34.4
22	33.1	33.3	32.9
23	63.8	28.4	28.6
24	13.6	17.1	17.3
25	15.9	15.6	15.9
26	17.3	17.5	17.8
27	26.1	26.3	26.5
28	180.1	180.0	176.9
29	33.1	33.4	33.5
30	23.6	23.9	24.1
1′	104.1	104.8	105.2
2′	85.3	85.1	85.4
3′	78.3	78.6	78.9
4′	71.1	71.5	71.8
5′	77.8	78.1	78.5
6′	62.5	62.9	63.1
1″	106.9	107.0	107.3
2″	76.6	76.9	77.2
3″	77.6	77.7	78.0
4″	73.1	73.4	73.7
5″	78.3	78.5	78.8
6″	172.2	172.0	172.5
1‴			96.1
2‴			74.5
3‴			79.3
4‴			71.4
5‴			79.8
6‴			62.5

a) 125 MHz. b) 75 MHz.

moiety. This was further confirmed by considering the glycosylation shifts at C-2 (−1.68 ppm) and C-3 (+8.5 ppm) of the aglycone moiety, when compared with the spectral data of hederagenin. In addition, the significant fragment ions observed at *m/z* 633 ([M−glcA−H]$^-$) and 471 ([M−glcA−glc−H]$^-$) in the negative ion API-MS, suggested glucuronic acid as the terminal sugar unit. The anomeric configuration of the sugar moieties was determined to be *β* for both on the basis of the J_{H-H} values (7.0 and 7.5 Hz, respectively) of their anomeric proton signals. From these findings, the structure of **1** was determined as 3-*O*-[*β*-D-glucuronopyranosyl-(1→2)-*β*-D-glucopyranosyl]hederagenin.

Compound **3** (abrus-saponin II) showed an $[M+Na]^+$ ion peak at *m/z* 979 in the positive ion API-MS and an $[M-H]^-$ ion peak at *m/z* 955 in the negative ion API-MS. On acid hydrolysis, **3** yielded glucose and glucuronic acid as sugars. The IR spectrum of **3** showed an ester carbonyl absorbance at 1740 cm^{-1} besides a carboxylic acid absorbance at 1680 cm^{-1}. In its ^{1}H-NMR spectrum, **3** exhibited signals for three anomeric protons at *δ* 4.92 (d, *J*=7.7 Hz), 5.40 (d, *J*=7.7 Hz) and 6.32 (d, *J*=8.1 Hz). Detailed analysis of the 2D NMR (^{1}H–^{1}H COSY, HMQC, HMBC, HOHAHA) revealed that its aglycone and two sugars were similar to those of **2**. Long-range correlations observed in the HMBC spectrum between the anomeric proton signal at *δ* 6.32 and the carbonyl signal at *δ* 176.9 (C-28) of the aglycone suggested the third sugar moiety to be an ester-linked one at C-28. This was confirmed by comparing the ^{13}C-NMR spectrum of **3** with that of **2**, in which an esterification shift at C-28 (−3.1 ppm) of the aglycone moiety was observed. Moreover, significant fragment ions at *m/z* 955 ([M−H]$^-$), 793 ([M−glc−H]$^-$), 779 ([M−glcA−H]$^-$), 617 ([M−glcA−glc−H]$^-$) and 455 ([M−glcA−2glc−H]$^-$) in the negative API-MS agreed well with the arrangement of the sugars. The ester-linked sugar was determined to be D-glucose by alkaline hydrolysis of **3** followed by GC analysis of its sugar derivative (see Experimental). The prosapogenin obtained was found to be identical with that of **2** by direct spectroscopic and chromatographic comparisons. The anomeric configurations of the sugars were all determined to be *β* from the J_{H-H} values of the respective anomeric proton signals. From the above evidence, the structure of **3** was concluded to be 3-*O*-[*β*-D-glucuronopyranosyl-(1→2)-*β*-D-glucopyranosyl]oleanolic acid 28-*β*-D-glucopyranosyl ester.

Compound **9** (precatorin I), yellow needles, gave a quasi-molecular ion peak $[M+H]^+$ at *m/z* 477 in the positive FAB-MS. Its physical properties, chromatographic behavior and spectral data were similar to those of **12**. On treatment with boiling 2 N HCl for 2 h, about 1/3 of **9** was rearranged to **12**, while the remaining 2/3 was recovered. This suggested that **9** was a 6-*C*-glucosyl isomer of **12**, produced by Wessely–Moser rearrangement in acid.[13] However, the spectra of **9** were more complicated than those of **12**. In the ^{1}H- and ^{13}C-NMR spectra, double signals were observed for the A ring, C-4 and the glucose in **9**. A similar effect in the NMR spectra of some flavonoid 6-*C*-*β*-D-glucosides has been reported by Davoust *et al.*[14] and Tanaka *et al.*[15] The unusual spectral behavior was considered to be due to the presence of two isomeric forms, in which the interconversion is slow at low temperature but rapid at high temperature. As expected, when **9** was measured at 100 °C, its ^{1}H-NMR spectrum became normal. At this temperature, the double signals of H-3 at *δ* 6.94 (6.92) became a single one at *δ* 6.79. Double signals of OCH_3 at *δ* 3.94 (3.90) and 3.91 (3.93) became single signals at *δ* 3.96 and 3.95, respectively. Furthermore, double signals of 5-OH at *δ* 13.30 (13.21) converted to a single one at *δ* 13.10, and those of glc-1″ at *δ* 4.59 (4.46) converted to a single one at *δ* 4.62. The attached position of the glucose moiety was confirmed by the HMBC experiment, in which long-range correlations were observed between the anomeric proton of glucose at

Table 3. ^{1}H-NMR Spectral Data of Compounds **9**—**11** (DMSO-d_6, 500 MHz)

Position	**9** (23 °C)	**9** (100 °C)	**10** (23 °C)	**10** (100 °C)	**11** (23 °C)	**11** (100 °C)
3	6.94 (6.92) (s)	6.79 (s)	6.91 (s, br)	6.79 (s)	6.86 (6.86) (s)	6.76 (s)
8					6.83 (6.82) (s)	6.73 (s)
2′,6′	7.97 (d, J=8.5 Hz)	7.92 (d, J=8.5 Hz)	7.97 (d, J=8.5 Hz)	7.93 (d, J=8.5 Hz)	7.98 (d, J=8.8 Hz)	7.92 (d, J=9.0 Hz)
3′,4′	6.96 (d, J=8.5 Hz)	6.99 (d, J=8.5 Hz)	6.97 (d, J=8.5 Hz)	6.99 (d, J=8.5 Hz)	6.93 (d, J=8.8 Hz)	6.95 (d, J=9.0 Hz)
7-OCH$_3$	3.94 (3.90) (s)	3.96 (s)	3.94 (3.91) (s)	3.97 (s)	3.90 (3.85) (s)	3.90 (s)
8-OCH$_3$	3.91 (3.93) (s)	3.95 (s)	3.90 (3.92) (s)	3.94 (s)		
5-OH	13.30 (13.21)	13.10	13.20 (13.21)	13.10	13.52 (13.43)	13.25
4′-OH	10.40	—	10.40	—	10.45	—
Glc-1″	4.59 (4.46) (d, J=9.0 Hz)	4.62 (d, J=9.0 Hz)	4.62 (4.52) (d, J=9.9 Hz)	4.79 (d, J=9.9 Hz)	4.62 (4.61) (d, J=9.8 Hz)	4.70 (d, J=10.0 Hz)
Api-1‴			5.20 (s, br)	5.20 (d, J=1.5 Hz)	5.17 (s, br)	5.18 (s, br)

Table 4. ^{13}C-NMR Spectral Data of Flavonoids Isolated from *Abrus precatorius* (DMSO-d_6)

Position		**9**[a]	**10**[b]	**11**[a]
Flavone	2	164.0 (163.9)	164.1 (164.9)	163.7 (163.6)
	3	103.1	103.2 (102.7)	103.1
	4	182.6 (182.3)	182.4 (182.8)	182.1 (181.8)
	5	154.6 (155.7)	154.4 (156.2)	160.5 (159.1)
	6	115.7 (115.1)	115.5 (114.6)	109.5 (109.4)
	7	159.0 (157.9)	159.5 (157.7)	165.1 (163.1)
	8	133.4 (132.6)	133.6 (132.4)	90.1 (91.1)
	9	149.0 (148.7)	149.3 (149.0)	156.7 (156.9)
	10	106.1 (106.7)	106.2 (106.5)	104.5 (104.1)
	1′	120.9	121.1	120.9
	2′,6′	128.6	128.7	128.6
	3′,5′	116.2	116.3	116.0
	4′	161.5	161.6	161.3
Glucose	1″	72.7 (74.3)	71.0 (72.7)	71.1 (71.2)
	2″	71.3 (69.5)	75.1 (73.9)	74.2 (74.0)
	3″	79.1 (78.8)	79.2 (79.1)[d]	79.1 (79.0)
	4″	70.8	71.0	70.9
	5″	81.7 (82.0)	81.6 (81.9)	81.8 (81.5)
	6″	61.9[c]	61.7 (62.1)[e]	61.8
Apiose	1‴		109.0 (109.2)	109.0 (108.9)
	2‴		75.7 (76.5)	75.6 (75.7)
	3‴		79.2 (79.5)[d]	79.5
	4‴		73.7 (73.0)	73.6 (73.4)
	5‴		64.7 (63.6)	64.5
OMe		61.6 (61.7)[c]	61.5 (60.0)[e]	56.5 (56.4)
		61.9 (62.1)[c]	61.9 (62.0)[e]	

a) 75 MHz. *b*) 125 MHz. *c*—*e*) Assignments bearing the same superscript may be reversed.

δ 4.59 (4.46) and C-5, C-6 at δ 154.6 (155.7), 115.7 (115.1), respectively. The assignment of C-5 and C-6 was confirmed by their correlations with a proton signal at δ 13.30 (13.21) [5-OH, the most down-field signal when the compound was measured in dimethyl sulfoxide-d_6 (DMSO-d_6)]. Compound **9** was, therefore, determined to be 6-*C*-β-D-glucopyranosyl-4′,5-dihydroxy-7,8-dimethoxyflavone. This compound has been isolated from some species of *Siphonoglossa*, but not yet been fully characterized due to insufficient sample being available for NMR analysis.[16]

Compound **10** (precatorin II), a yellow powder, showed a quasi-molecular ion peak $[M+H]^+$ at m/z 609 in the positive FAB-MS, and its elemental analysis revealed the formula $C_{28}H_{32}O_{15}$. Like **9**, compound **10** exhibited double signals for its ring-A and sugar components in its NMR spectra. When the compound was measured at 100 °C, its ^{1}H-NMR spectrum became simpler. On acid hydrolysis, **10** afforded apiose, **9** and a trace of **12**. The attached position of the glucose moiety was determined by HMBC in the same manner as for **9**. The linked site of apiose to glucose was also determined by the HMBC experiment where a long-range correlation was observed between api-H-1 at δ 5.20 and glc-C-2 at δ 75.1 (73.9). By comparing the NMR spectra of **10** with those of **13** and detailed analysis of its 2D-NMR, the ^{1}H- and ^{13}C-NMR signals were assigned as shown in Tables 3 and 4. The structure of **10** was, therefore, determined as 6-*C*-[β-D-apiofuranosyl (1→2)-β-D-glucopyranosyl]-4′,5-dihydroxy-7,8-dimethoxyflavone.

Compound **11** (precatorin III), a yellow powder, showed a quasi-molecular ion peak $[M+H]^+$ at m/z 579 in the positive API-MS and $[M-H]^-$ at m/z 577 in the negative API-MS, thus, its molecular weight was 30 less than that of **10**. The NMR spectrum of **11** was similar to that of **10**, with doubling signals for its ring-A and sugar parts. Its ^{1}H-NMR spectrum became normal at 100 °C. The attached position of the sugars was established in the same manner as in **9** and **10**. The difference in ^{1}H-NMR spectra between **10** and **11** was that **11** had an additional aromatic proton signal at δ 6.83 (6.82) and only one methoxy signal at δ 3.90 (3.85). The signals of ring B were the same as in **10** and **11**, therefore, compound **11** contains only one methoxy group in its ring A rather than two methoxy groups in the case of **10**. The position of this methoxy in **11** was determined by the HMBC experiment, in which a long-range correlation was observed between this methoxy proton at δ 3.90 (3.85) and C-7 at δ 165.1 (163.1), while the assignment of C-7 was confirmed by its long-range correlation with the anomeric proton of glucose linked at C-6 at δ 4.62 (4.61). All these findings were further confirmed by the significant long-range correlations between H-8 at δ 6.83 (6.82) and C-6 at δ 109.5 (109.4), between H-8 and C-10 at δ 104.5 (104.1). From these data, the structure of **11** was determined as 6-*C*-[β-D-apiofuranosyl (1→2)-β-D-glucopyranosyl]-4′,5-dihydroxy-7-methoxyflavone.

The effects of the compounds isolated on HIV-protease and HIV-induced cytopathogenicity are now under investigation.

Experimental

General Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus without correction. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. UV

spectra were measured with a SHIMADZU UV-2200 UV-VIS recording spectrophotometer. IR spectra were measured with a JASCO FT/IR-230 infrared spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with Varian GEMINI 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or Varian UNITY 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometers, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. Electron impact (EI) MS were measured with a JEOL JMS-AX 505 HAD mass spectrometer at an ionization voltage of 70 eV. FAB-MS were obtained with a JEOL JMS-DX 300L spectrometer using glycerol as a matrix. API-MS were measured with a Perkin–Elmer SCIEX API III Biomolecular Mass Analyzer.

Column chromatography was carried out on Silica-gel 60 (silica gel) (Merck, 70—230 mesh), Silica-gel 60 silanised (RP-2) (Merck, 70—230 mesh), Diaion HP-20 (Mitsubishi Chemical Co.), Sephadex LH-20 (Pharmacia), MCI gel CHP20P (Mitsubishi Chemical Co.), Amberlite MB-3 (Organo). Medium pressure liquid chromatography (MPLC) was carried out on a LiChroprep RP-18 (Merck, size A), LiChroprep Si 60 (Merck, size A). Preparative HPLC was carried out on a Gilson instrument with a 231XL injector, a 119 UV/VIS detector and a TSK gel ODS-80T$_M$ column (21.5 × 300 mm, Tosoh Co.). Analytical HPLC was carried out on a TOSOH CCP8020 system. Preparative TLC was carried out on pre-coated Silica-gel 60 F_{254} plates (Merck, 0.5 mm). Analytical TLC was carried out on pre-coated Silica-gel 60 F_{254} plates (Merck, 0.25 mm) and RP-18 F_{254} S plates (Merck, 0.25 mm), cellulose F plates (Merck, 0.1 mm), and spots were detected under UV light and after spraying with anisaldehyde–5% H_2SO_4 or aniline–phthalic acid. GC was performed on a Shimadzu GC-17A instrument.

Isolation of Compounds 1—17 from the Seeds of *A. precatorius* The seeds of *A. precatorius* L. were purchased from Tochimoto Tenkaido Co. (Osaka, Japan). Crushed seeds (3 kg) were extracted three times by refluxing with MeOH (each 6 l) for 2 h. The methanol extracts were filtered and the combined solution was evaporated to give an extract of 180 g. The extract was passed through a Diaion HP-20 column (9 × 55 cm) eluted with H_2O (fraction 1), MeOH–H_2O (1 : 1) (fractions 2 and 3) as well as MeOH (fraction 4). Fraction 4 (21.8 g) was chromatographed on an RP-2 column with MeOH–H_2O (1 : 1) (fraction 4a) and MeOH–H_2O (2 : 1) (fraction 4b). Fraction 4b (4.3 g) was further separated by silica-gel [$CHCl_3$–MeOH–H_2O (14 : 6 : 1—13 : 7 : 1)] and preparative HPLC [MeOH–0.1%TFA/H_2O (8 : 2—10 : 0), 5 ml/min, monitored at 205 nm] to afford **1** (t_R 56 min, 100 mg), **2** (t_R 69 min, 33 mg), **3** (t_R 48 min, 16 mg), **4** (t_R 67 min, 11 mg), **5** (t_R 62 min, 7 mg), **6** (t_R 65 min, 1 g), **7** (t_R 74 min, 4 mg) and **8** (t_R 73 min, 22 mg).

Fraction 4a (7.9 g) was further separated by silica-gel with EtOAc–EtOH–H_2O (20 : 2 : 0.5—12 : 2 : 1) to provide five fractions (4a-1—4a-5). Fraction 4a-1 was applied to Sephadex LH-20 (2.5 × 30 cm) with MeOH and then preparative TLC with $CHCl_3$–MeOH (9.5 : 0.5) to afford **14** (30 mg) and **15** (5 mg). Fraction 4a-2 was purified with MCI gel CHP20P (2.5 × 27 cm) with MeOH–H_2O (1 : 1) to give **12** (140 mg). Fraction 4a-3 was applied to a RP-18 MPLC with MeOH–H_2O (1 : 1) to give **9** (10 mg). Fraction 4a-4 was purified by RP-18 MPLC with MeOH–H_2O (4 : 6) to give **13** (100 mg). Fraction 4a-5 was purified by MCI gel CHP20P with MeOH–H_2O (30 : 70—45 : 55) to give **10** (45 mg).

Meanwhile, fraction 3 (14.9 g) was subjected to chromatography on a silica-gel column (4.5 × 30 cm) with EtOAc–EtOH–H_2O (22 : 2 : 0.3—12 : 2 : 1) to yield **13** (3.2 g) and a mixture of **10** and **11**. The mixture was applied to Si 60 MPLC with EtOAc–EtOH–H_2O (18 : 2 : 1) and then RP-18 MPLC with MeOH–H_2O (4 : 6) to afford **10** (200 mg) and **11** (20 mg).

Fraction 2 (27.7 g) was subjected to RP-2 column chromatography to furnish **16** (1 g) from the MeOH–H_2O (1 : 1) eluate. The MeOH–H_2O (1 : 9) eluate from this RP-2 column was further purified by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (225 : 75 : 10) to obtain **17** (500 mg).

3-*O*-[*β*-D-Glucuronopyranosyl-(1→2)-*β*-D-glucopyranosyl]-hederagenin (Abrus-saponin I, 1) Colorless amorphous powder, $[\alpha]_D^{28}$ +25.1° (c = 1.0, pyridine). Positive ion API-MS m/z (% intensity): 855 ($[M+2Na-H]^+$, 100), 849 ($[M+K]^+$, 25), 833 ($[M+Na]^+$, 50), 657 ($[M+Na-glcA]^+$, 45). Negative ion API-MS m/z (%): 809 ($[M-H]^-$, 100), 633 ($[M-H-glcA]^-$, 20), 471 ($[M-H-glcA-glc]^-$, 40). IR ν_{max}^{KBr} cm^{-1}: 3410, 2950, 1720 sh, 1698, 1080, 1050. ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2. *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot 2.5H_2O$: C, 58.94; H, 8.36. Found: C, 58.86; H, 8.15.

Identification of an Aglycone and a Sugar Part of 1 Compound **1** (9 mg) was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated to dryness to afford hederagenin (2 mg) as an aglycone. The structure was determined by comparison of its spectroscopic data including ^{1}H-NMR, ^{13}C-NMR and MS [EI-MS m/z 472 $[M]^+$, 248 (100%) with that of authentic compound. The water layer was concentrated and checked by TLC [1: silica-gel, $CHCl_3$–MeOH–H_2O (65 : 40 : 10 the lower phase); 2: cellulose, (phenol saturated with H_2O); 3: cellulose, [BuOH–benzene–pyridine–H_2O (5 : 1 : 3 : 3)].

Determination of Sugar Components Determination of the absolute configuration of sugars was performed as reported by Hara *et al.*[12] Compound **1** (1 mg) was methylated with trimethylsilyldiazomethane (TMS–CHN_2) in methanol. After being dried, the residue was dissolved in 0.6 ml MeOH and treated with 5 mg $NaBH_4$ at room temperature for 10 min. The mixture was passed through an MCI gel CHP 20 column with H_2O as mobile phase to eliminate inorganic compounds, then the column was eluted with MeOH to yield 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]hederagenin-28-methyl ester. API-MS (positive) m/z: 833 $[M+Na]^+$; (negative) m/z: 809 $[M-H]^-$, 647 $[M-glc-H]^-$. The product was then refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h, neutralized with 1 N NaOH and washed with $CHCl_3$. The residual water layer was desalted with Amberlite MB-3 and dried. The residue was dissolved in pyridine (0.1 ml), then a pyridine solution (0.2 ml) of L-cysteine methyl ester hydrochloride (0.1 M) was added to the sugar solution. The mixture was kept at 60 °C for 1.5 h, dried *in vacuo*, and trimethylsilylated with hexamethyldisilazane–trimethylchlorosilane (HMDS–TMCS) (0.1 ml) at 60 °C for 1 h. After partition with *n*-hexane (0.3 ml) and water (0.3 ml), the *n*-hexane extract was analyzed by GC [column, DB-1, J & W Scientific, 0.25 mm i.d. × 30 m; column temperature, 50—230 °C, 15 °C/min then 230 °C, 18 min; carrier gas, He]. The sugar derivatives thus obtained showed a retention time of 22.55 min, identical with that of authentic D-glucose. Under the same conditions, a derivative of L-glucose showed a retention time of 23.44 min.

3-*O*-[*β*-D-Glucuronopyranosyl-(1→2)-*β*-D-glucopyranosyl]-oleanolic Acid (2)[5] Colorless amorphous powder, $[\alpha]_D^{28}$ +19.8° (c = 0.30, pyridine). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2. Positive ion API-MS m/z (%): 839 ($[M+2Na-H]^+$, 100), 833 ($[M+K]^+$, 25), 817 ($[M+Na]^+$, 80), 641 ($[M+Na-glcA]^+$, 30). Negative ion API-MS m/z (%): 793 ($[M-H]^-$, 100), 617 ($[M-H-glcA]^-$, 10), 455 ($[M-H-glc-glcA]^-$, 25).

3-*O*-[*β*-D-Glucuronopyranosyl-(1→2)-*β*-D-glucopyranosyl]-oleanolic Acid 28-*β*-D-Glucopyranosyl Ester (Abrus-Saponin II, 3) Colorless amorphous powder, $[\alpha]_D^{28}$ +13.3° (c = 0.61, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1740, 1680, 1640, 1075. ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2. Positive ion API-MS m/z (%): 995 ($[M+K]^+$, 25), 979 ($[M+Na]^+$, 100), 817 ($[M+Na-glc]^+$, 20), 803 ($[M+Na-glcA]^+$, 35), 641 ($[M+Na-glc-glcA]^+$, 10), 439 ($[M+H-glcA-2glc-H_2O]^+$, 17). Negative ion API-MS m/z (%): 955 ($[M-H]^-$, 100), 793 ($[M-H-glc]^-$, 65), 617 ($[M-H-glc-glcA]^-$, 15), 455 ($[M-H-2glc-glcA]^-$, 18).

Identification of the Sugar Moiety of 3 Compound **3** (1 mg) was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 1 ml) for 3 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O. The water layer was concentrated and checked by TLC [1: silica-gel, $CHCl_3$–MeOH–H_2O (65 : 40 : 10 the lower phase); 2: cellulose, (phenol saturated with H_2O); 3: cellulose, BuOH–benzene–pyridine–H_2O (5 : 1 : 3 : 3)].

Identification of the Ester Linked Sugar and Prosapogenin Compound **3** (3 mg) was hydrolyzed with 0.5 N KOH (1 ml) in H_2O–EtOH (1 : 1) at 80 °C for 2 h, neutralized with 1 N HCl and passed through an MCI CHP 20 column. The water eluate was desalted with Amberlite MB-3, treated with L-cysteine methyl ester hydrochloride, trimethylsilylated and analyzed by GC in the same manner as for **1**. D-Glucose was identified as a component of the ester-linked sugar. The MeOH eluate from the MCI CHP 20 column was concentrated to dryness to give a prosapogenin, which was identified as **2** by comparing their API-MS and t_R in HPLC.

Kaikasaponin I (4)[6] Colorless amorphous powder, $[\alpha]_D^{28}$ +15.6° (c = 0.17, pyridine). Positive ion API-MS m/z (%): 825 ($[M+2Na-H]^+$, 100), 803 ($[M+Na]^+$, 60). Negative ion API-MS m/z (%): 779 ($[M-H]^-$, 100).

3-*O*-[*β*-D-Xylopyranosyl-(1→2)-*β*-D-galactopyranosyl (1→2)-*β*-D-glucuronopyranosyl]sophoradiol (5)[7] Colorless amorphous powder, $[\alpha]_D^{28}$ +2.3° (c = 0.24, pyridine). Positive ion API-MS m/z (%): 935 ($[M+Na]^+$, 100). Negative ion API-MS m/z (%): 911 ($[M-H]^-$, 100).

Kaikasaponin III (6)[8] Colorless amorphous powder, $[\alpha]_D^{28}$ −7.9° (c = 1.00, pyridine). Positive ion API-MS m/z (%): 971 ($[M+2Na-H]^+$,

40), 949 ([M+Na]$^+$, 65). Negative ion API-MS m/z (%): 925 ([M−H]$^-$, 100).

Kaikasaponin III Methyl Ester (7)[3] Colorless amorphous powder, $[\alpha]_D^{28}$ +2.7° (c=0.15, pyridine). Positive ion API-MS m/z (%): 963 ([M+Na]$^+$, 100), 817 ([M+Na−rha]$^+$, 10). Negative ion API-MS m/z (%): 975 ([M+Cl]$^-$, 35), 925 ([M−Me]$^-$, 100), 939 ([M−H]$^-$, 60).

Phaseoside IV (8)[8] Colorless amorphous powder, $[\alpha]_D^{28}$ −28.5° (c=0.78, pyridine). Positive ion API-MS m/z (%): 963 ([M+K]$^+$, 100), 947 ([M+Na]$^+$, 100). Negative ion API-MS m/z (%): 923 ([M−H]$^-$, 100).

6-*C*-β-D-Glucopyranosyl-4′,5-dihydroxy-7,8-dimethoxyflavone (Precatorin I, 9) Yellow needles (EtOH), mp 278—280 °C. $[\alpha]_D^{27}$ +4.3° (c=0.44, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3450—3200 br, 1650, 1610, 1580, 1480, 1440, 1350, 1220, 1100, 1040. UV λ_{max}^{MeOH} nm (ε): 275 (19000), 295 (17500), 327 (21900). ^{1}H-NMR: Table 3. ^{13}C-NMR: Table 4. FAB-MS (positive) m/z: 477 [M+H]$^+$, 357, 343, 327, 313.

Isomerization of 9 Compound **9** (1 mg) was refluxed in 1 ml 2 N HCl for 2 h. No sugars were produced. On TLC [1: silica-gel, EtOAc–EtOH–H_2O (12:2:1), 2: RP-18, MeOH–H_2O (3:2)], two spots were identified as **9** and **12** in a ratio of 2:1, respectively.

6-*C*-[β-D-Apiofuranosyl-(1→2)-β-D-glucopyranosyl]-4′,5-dihydroxy-7,8-dimethoxyflavone (Precatorin II, 10) Yellow amorphous powder, $[\alpha]_D^{27}$ −50.6° (c=0.40, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3100 br, 1650, 1610, 1570, 1480, 1450, 1350, 1220, 1100, 1040. UV λ_{max}^{MeOH} nm (ε): 275 (18500), 295 (16700), 327 (22200). ^{1}H-NMR: Table 3. ^{13}C-NMR: Table 4. FAB-MS (positive) m/z: 609 [M+H]$^+$, 477 [M−api+H]$^+$, 357, 343, 327, 313. *Anal.* Calcd for $C_{28}H_{32}O_{15}\cdot 2H_2O$: C, 52.18; H, 5.63. Found: C, 52.00; H, 5.29.

Acid Treatment of 10 Compound **10** (31 mg) was refluxed in 3.0 ml 1 N HCl for 1 h, neutralized with 1 N NaOH and extracted with BuOH. The BuOH layer was concentrated to dryness and applied to RP-18 MPLC using MeOH–H_2O (40:60) as mobile phase to yield **9** (16 mg) and **12** (2 mg), which were identified by TLC and NMR. After being desalted with Amberlite MB-3, one tenth of the water layer was treated and analyzed by GC in the same manner as in **3**. Derivative of apiose[17] t_R: 16 min 55 s.

6-*C*-[β-D-Apiofuranosyl-(1→2)-β-D-glucopyranosyl]-4′,5-dihydroxy-7-methoxyflavone (Precatorin III, 11) Yellow amorphous powder, $[\alpha]_D^{25}$ −55.4° (c=0.92, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3100 br, 1650, 1610, 1570, 1490, 1450, 1350, 1070, 1000. UV λ_{max}^{MeOH} nm (ε): 272 (18200), 332 (20800). ^{1}H-NMR: Table 3. ^{13}C-NMR: Table 4. API-MS (positive) m/z: 617 [M+K]$^+$, 601 [M+Na]$^+$, 579 [M+H]$^+$; (negative) m/z: 577 [M−H]$^-$.

Acid Treatment of 11 Compound **11** (5 mg) was treated in the same manner as **10** to afford apiose.

Abrusin (12)[4] Yellow amorphous powder, $[\alpha]_D^{27}$ −57.8° (c=0.45, MeOH).

Abrusin 2″-*O*-β-D-Apiofuranoside (13)[4] Yellow amorphous powder, $[\alpha]_D^{27}$ −132.9° (c=0.48, MeOH).

4′,5-Dihydroxy-6,7-dimethoxyflavone (14)[9] Yellow needles (MeOH), mp 257—259 °C.

4′,5,7-Trihydroxy-6-methoxyflavone (15)[10] Yellow needles (MeOH), mp 287—290 °C.

Abrine (16)[3] Colorless amorphous powder, $[\alpha]_D^{27}$ +56.1° (c=0.38, 1 N NaOH).

***N,N,N*-Trimethyl Tryptophan (17)**[3] Colorless crystals (MeOH–H_2O), mp 267 °C (dec.), $[\alpha]_D^{27}$ +85.4° (c=0.49, MeOH).

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