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Studies on the Constituents of *Xanthoceras sorbifolia* BUNGE. III. Minor Prosapogenins from the Fruits of *Xanthoceras sorbifolia* BUNGE[1)]

YINGJIE CHEN,[a] TADAHIRO TAKEDA,[b] and YUKIO OGIHARA*,[b]

Pharmaceutical College of Shenyang,[a] 2-7, Culture-road, Shenyang, China and
Faculty of Pharmaceutical Sciences, Nagoya City University,[b]
Tanabe-dori, Mizuho-ku, Nagoya, Japan

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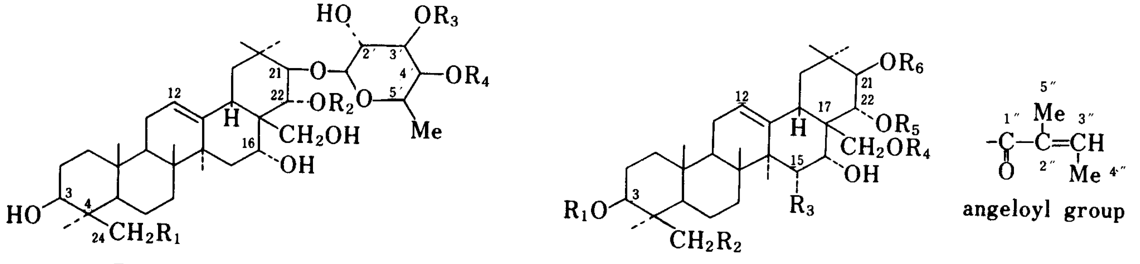
The prosapogenins from the fruits of *Xanthoceras sorbifolia* BUNGE (Sapindaceae) were examined. On the basis of chemical and spectral analyses, the structures of three minor prosapogenins, obtained by acid hydrolysis of crude saponin fraction, were characterized as 21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosyl theasapogenol B (**1**), 21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl theasapogenol B (**2**) and 21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl-22-*O*-acetyl protoaescigenin (**3**), respectively.

Keywords——*Xanthoceras sorbifolia*; Sapindaceae; prosapogenin; theasapogenol B; protoaescigenin; 21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosyl theasapogenol B; 21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl theasapogenol B; 21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl-22-*O*-acetyl protoaescigenin

In the previous paper, we reported the isolation and structure elucidation of the major sapogenol and a prosapogenin from the fruits of *Xanthoceras sorbifolia* BUNGE.[1)] This paper deals with the structure elucidation of the minor prosapogenins.

On acid hydrolysis, the crude saponin fraction, obtained from a methanolic extract of fruits of *Xanthoceras sorbifolia* BUNGE by droplet counter current chromatography (d.c.c.), afforded a mixture of sapogenols and prosapogenins. The mixture was chromatographed on silica gel to give compounds A, B, C, D, E and F. Compounds A (**10**) and E (**4**) were characterized as 21,22-di-*O*-angeloyl R_1-barrigenol and napoleogenin B,[1)] respectively.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) signal assignments (Table I) were carried out by means of known chemical shift rules,[2)] such as hydroxyl substitution shifts, acetylation shifts and glycosidation shifts,[3,4)] as well as proton off resonance decoupling, selective proton decoupling and also by comparison with the data for known compounds.[5)]



	R_1	R_2	R_3	R_4
1	H	H	angeloyl	angeloyl
2	H	H	angeloyl	Ac
3	OH	Ac	angeloyl	Ac
4	OH	H	angeloyl	angeloyl
5	OH	H	H	H

	R_1	R_2	R_3	R_4	R_5	R_6
6	H	H	H	H	H	H
7	Ac	H	H	Ac	Ac	Ac
8	H	OH	H	H	H	H
9	Ac	OAc	H	Ac	Ac	Ac
10	H	H	OH	H	angeloyl	angeloyl

Chart 1

Table I. Carbon-13 Chemical Shifts (δ) in Pyridine-d_5

Carbon	**1**	**2**	**3**	**4**	**5**	**8**	**10**
C-1	39.2	39.1	38.9	38.9	38.9	39.0	39.4
C-2	28.7	28.7	28.4	28.5	28.5	28.5	28.7
C-3	78.0	78.0	80.1	80.1	80.1	80.2	78.0
C-4	39.4	39.4	43.2	43.2	43.2	43.2	39.4
C-5	55.8	55.8	56.4	56.4	56.4	56.4	55.6
C-6	18.8	18.8	19.1	19.1	19.1	19.2	19.1
C-7	33.2	33.1	33.5	33.5	33.5	33.6	36.4
C-8	40.1	40.1	40.1	40.2	40.1	40.1	41.1
C-9	47.2	47.2	47.2	47.2	47.2	47.3	47.3
C-10	37.3	37.2	37.0 (a)	37.2 (a)	37.2 (a)	37.0 (a)	37.4 (a)
C-11	24.0	23.9	24.1	24.2	24.1	24.2	24.0
C-12	123.8	123.8	123.8	123.7	123.8	123.8	125.5
C-13	143.8	143.8	143.1	143.8	143.9	144.0	143.8
C-14	41.9	41.9	41.6	41.9	42.0	42.1	47.1
C-15	34.5	34.5	34.8	34.5	34.5	34.4	67.6
C-16	68.0	68.1	68.8	68.0	68.0	67.9	73.4
C-17	48.2	48.2	47.8	48.2	47.9	47.4	48.4
C-18	40.5	40.5	40.1	40.5	40.6	41.3	41.5
C-19	47.9	48.0	47.8	47.9	48.3	48.3	47.8
C-20	37.3	37.2	37.8 (a)	37.0 (a)	37.0 (a)	36.5 (a)	36.8 (a)
C-21	92.1	92.6	85.1	92.1	92.2	78.7	78.6
C-22	73.5	73.5	74.2	73.6	74.0	77.3	73.6
C-23	28.1	28.1	23.6	23.5	23.5	23.6	28.2
C-24	16.6 (b)	16.7 (b)	64.6	64.6	64.6	64.6	16.6 (b)
C-25	16.0 (b)	16.2 (b)	16.2 (b)	16.2 (b)	16.2 (b)	16.3 (b)	15.9 (b)
C-26	17.0 (b)	17.0 (b)	16.9 (b)	16.8 (b)	16.8 (b)	16.9 (b)	17.7 (b)
C-27	27.5	27.5	27.5	27.5	27.5	27.3	21.2
C-28	66.9	66.7	64.0	66.9	67.4	68.4	63.2
C-29	30.0	29.9	30.2	29.9	30.0	30.6	29.5
C-30	20.4	20.3	20.1	20.3	20.4	19.5	20.2
C-1′	106.1	106.2	105.2	106.1	106.7		
C-2′	69.8	69.8	70.0	70.0	72.5		
C-3′	74.3	74.2	74.2	74.3	75.3		
C-4′	71.3	71.3	71.6	71.1	72.7		
C-5′	69.3	69.3	68.8	69.5	71.6		
C-6′	16.4	16.6	16.7	16.4	17.0		
C-1″	167.3	167.3	167.4	167.3			168.1
	167.3			167.3			167.7
C-2″	128.1	128.0	128.2	128.1			129.1
	127.9			127.9			128.9
C-3″	138.8			138.8			137.4
	138.3	138.3	138.0	138.3			135.8
C-4″	20.8			20.8			21.1
	20.5	20.6	20.6	20.5			20.6
C-5″	15.9			16.0			15.9
	15.9	15.8	15.9	15.9			15.7
O$\underline{\text{C}}$OMe		170.6	171.9				
			170.6				
OCO$\underline{\text{Me}}$		20.6	21.9				
			20.5				

(a), (b) assignments may be reversed in each column.
The signals for carbons bearing OH were assigned by means of selective proton decoupling experiments.

The ^{13}C-NMR data for compound B (**1**) were very close to those for **4**. In particular, the signals for carbons of the C, D and E rings and the fucose moiety of **1** were fully in agreement with those of **4**, the structure of which was established by X-ray diffraction analysis,[1] indicating the presence of a 21*β*-(3,4-di-*O*-angeloyl-*β*-D-fucopyranosyl)oxy-16*α*,22*α*,28-trihydroxyolean-12-ene moiety in **1**.

The differences between **1** and **4** are as follows: there are seven methyls in **1**, instead of six methyls in **4**, based on the ^{1}H-NMR analysis of **1**. On the other hand, one carbinol group is missing in **1**, as compared with **4**, according to the ^{13}C-NMR data. Furthermore, a comparison of the ^{13}C-NMR data for **1** with those for **10** showed that the signals for C-3, C-4, C-5, C-23 and C-24 of **1** were in agreement with those of **10**. This suggests the absence of the C-4 carbinol group in **1**. An upfield shift of the C-24 signal by 48 ppm (from 64.6 in **4** to 16.6 ppm in **1**) suggested the absence of the OH group at C-24 in **1**, according to Roberts's hydroxyl substitution shift rule.[2] This was further confirmed by acid hydrolysis of **1** to give fucose and an aglycone (**6**) which, upon usual acetylation, gave a tetraacetate (**7**) identical with the tetraacetate of theasapogenol B.[6]

Based on these observations, the structure of **1** was characterized as 21-*O*-(3,4-di-*O*-angeloyl)-*β*-D-fucopyranosyltheasapogenol B. This structure was further supported by mass spectral (MS) analysis (Table II and Chart 2). In the MS of **1**, the peaks at *m*/*z* 189 and 207, characteristic fragment ions derived from retro Diels–Alder cleavage of olean-12-ene derivatives,[7] showed that one hydroxyl group was present on the AB rings of **1**, indicating the absence of the C-24 hydroxyl group, compared to **4**. The fragment ions at *m*/*z* 55, 83 (base peak), 111, 211, 311 in the MS of **1** and the ions at *m*/*z* 55, 83, 153, 253, 353 of the tetraacetate of **1** indicated the existence of a diangeloyl-fucosyl moiety in **1**.[8]

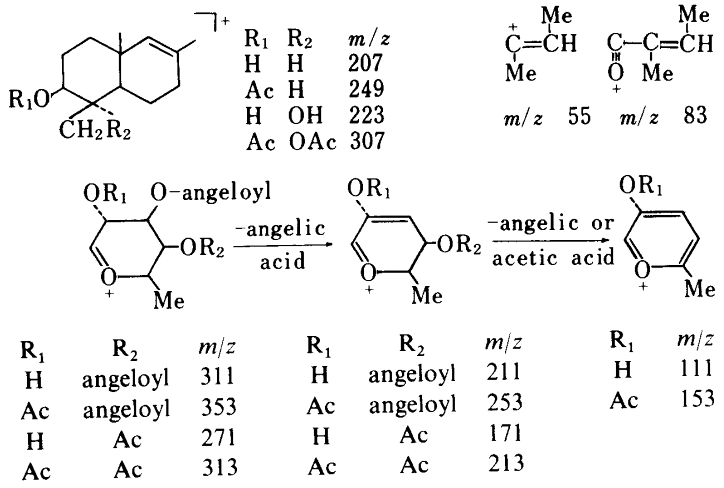
Compound C (**2**), on acid hydrolysis, gave a fucose and compound **6**. On usual acetylation, **6** afforded a tetraacetate (**7**), which was identical with the tetraacetate of theasapogenol B.

The ^{13}C-NMR data for **2**, which are very close to those for **1**, show that **2** is a theasapogenol B linkd to an angeloyl fucose moiety. The C-21 signal (92.6 ppm) in **2**, which is almost the same as in **1** (92.1 ppm), **4** (92.1 ppm) and **5** (92.2 ppm), shows that the fucose moiety should be at C-21 of the aglycone. The differences between **1** and **2** were as follows: in the ^{1}H-NMR spectrum of **2**, the peak at 1.89 (3H, br s, *α*-Me), 1.92 (3H, d, $J=7$ Hz, *β*-Me) and 6.00 ppm (1H, q, $J=7$ Hz, *β*-H) suggested the presence of one angeloyl group[9] in **2**, instead of two groups in **1**. This was supported by ^{13}C-NMR data for **2** based on the existence of signals due to one angeloyl group (Table I). The peaks at 170.6 ppm, assigned to an ester carbonyl, and 20.6 ppm, attributed to a methyl, showed the presence of an additional acetyl group in **2**, and this was supported by the ^{1}H-NMR spectrum (2.03, 3H, s, –OCOMe) of **2**.

Table II. MS Data for Compounds **1**, **2** and **3** and Their Peracetates in *m*/*z* (Intensity %)

	1	Tetraacetate of **1**	**2**	Tetraacetate of **2**	**3**	Tetraacetate of **3**
Angeloyl group	55 (48)	55 (63)	55 (73)	55 (35)	55 (90)	55 (45)
	83 (100)	83 (100)	83 (100)	83 (100)	83 (100)	83 (100)
Sugar moiety	111 (12)	153 (8)	111 (10)	153 (28)	111 (11)	153 (67)
	211 (7)	253 (5)	171 (9)	213 (24)	171 (16)	213 (38)
	311 (7)	353 (1)	271 (3)	313 (40)	271 (17)	313 (52)
AB rings	189 (8)	189 (22)	189 (15)	189 (15)	205 (8)	205 (3)
	207 (10)	249 (7)	207 (13)	249 (6)	223 (3)	307 (5)

The positions of the acetyl and angeloyl groups were considered to be in the sugar moiety, because no acetylation shift was observed for C-3, C-16, C-22 and C-28 in **2**. This was supported by MS analysis based on the presence of a set of fragment ions at *m/z* 153, 213 and 313 (Table II, Chart 2) in tetraacetate of **2**, instead of 153, 253 and 353 in the tetraacetate of **1** and pentaacetate of napoleogenin B, which has two angeloyl groups in the fucose moiety.[8)]



R_1	R_2	m/z
H	H	207
Ac	H	249
H	OH	223
Ac	OAc	307

R_1	R_2	m/z
H	angeloyl	311
Ac	angeloyl	353
H	Ac	271
Ac	Ac	313

R_1	R_2	m/z
H	angeloyl	211
Ac	angeloyl	253
H	Ac	171
Ac	Ac	213

R_1	m/z
H	111
Ac	153

Chart 2

The acetyl group should not be at C-2′ of fucose, because the C-1′ signal would shift upfield to about 101.7 ppm, as in the pentaacetate of napoleogenin B, if the acetyl was at C-2′.[8)] Therefore, the position of the acetyl group should be at C-3′ or C-4′ of the fucose. The fragment ions at *m/z* 171 and 184 (Chart 3) in the MS of the tetraacetate of **2** suggest the acetyl group to be located at C-4′ and the angeloyl at C-3′ of fucose, according to the rule for fragmentation of acetylated sugars.[10)]

Based on these observations, the structure of compound **2** was established to be 21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-*β*-D-fucopyranosyl theasapogenol B.

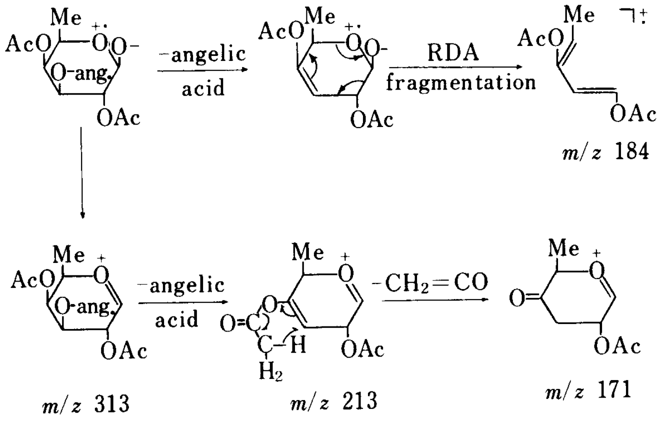


Chart 3

Compound F (**3**), on acid hydrolysis, furnished fucose and an aglycone (**8**) which, on usual acetylation, gave a pentaacetate (**9**) identical with the pentaacetate of protoaescigenin.[11)] In the ^1H-NMR spectrum of **3**, the peaks at 1.91 (3H, br s, α-Me), 2.00 (3H, d, $J=7$ Hz, *β*-Me) and 5.93 (1H, q, $J=7$ Hz, *β*-H) revealed the presence of an angeloyl group. The ^{13}C-NMR data for **3**, which are very similar to those for **4**, support the view that **3** is a protoaescigenin linked with a fucose and an angeloyl group. The differences between **3** and **4** were as follows: there are only one angeloyl group in **3** based on the ^{13}C-NMR data (Table I). In addition, the peaks at 170.6 and 171.9 ppm, assigned to two ester carbonyls, and at 20.5 and 21.9 ppm, attributed to two acetyl methyls, revealed the existence of two additional acetyl groups in **3**. The presence of two acetyl groups was also supported by the ^{1}H-NMR spectrum (2.03, 2.36, 3H, each s, –OCOMe).

The positions of acetyl groups were determined by ^{13}C-NMR and MS analyses. A downfield shift of the C-22 signal to 74.2 ppm and an upfield shift of the C-21 signal to 85.1 ppm, compared with **4**, showed that one acetyl group should be at C-22, according to the acetylation shift rule.[12] This was confirmed by comparison of the C-21 signal of **3** (85.1 ppm) with that of hexaacetate of napoleogenin (84.3 ppm) and that of the pentaacetate of napoleogenin B (85.9 ppm).[8,13] On acetylation of 22-OH of napoleogenin, the C-21 signal shifted upfield by 6.9 ppm (from 91.2 to 84.3 ppm).[13] A similar result was obtained in **3** with an upfield shift of 7.0 ppm (from 92.1 ppm in **4** to 85.1 ppm in **3**), indicating the presence of OAc at C-22.

The second acetyl group may be at either C-16, with a downfield shift of 0.8 ppm compared to **4**, or in the fucose moiety, because no downfield shift caused by acetylation was observed at C-3, C-24 or C-28. If the acetyl was at C-16, the signal for C-15 would have an upfield shift of at least 1 ppm, according to the acetylation shift rule.[12] No such upfield shift was observed for C-15 signal, suggesting that the second acetyl should be in the fucose moiety. The existence of an acetyl and an angeloyl group in the fucose moeity of **3** was confirmed by MS analysis based on the observation of fragment ions at *m/z* 111, 171 and 271 in **3** and at *m/z* 153, 213 and 313 in the tetraacetate of **3** (Table II, Chart 2). The fragment ions at *m/z* 171 and 184 (Chart 3) in the MS of the tetraacetate of **3** suggested that the angeloyl group was at C-3′ and the acetyl at C-4′ of the fucose moiety. Furthermore, comparison of the ^{13}C-NMR data for **3** with those for **2** showed that the signals due to carbons of the fucose and angeloyl groups were all very similar, supporting the presence of a 4-*O*-acetyl-3-*O*-angeloyl-fucopyranosyl moiety in **3**. Based on these observations, **3** is characterized as 21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-*β*-D-fucopyranosyl-22-*O*-acetyl protoaescigenin.

The proposed structure was confirmed by alkaline hydrolysis of **3** to give **5**, which revealed that the sugar moiety is attached at C-21 of protoaescigenin. The signals at 106.7, 72.5, 75.3, 72.7, 71.6 and 17.0, assigned to C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′ of fucose, were coincident with those for methyl *β*-D-fucopyranoside.[4]

Experimental

All melting points were measured on a Yanagimoto microscope hot plate and are uncorrected. Ultraviolet (UV) spectra were taken with a Shimadzu UV-240 spectrometer. MS were recorded on a JEOL DX-300 mass spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on JNM MH-100 spectrometer and ^{13}C-NMR spectra were measured on a JEOL FX-100 spectrometer using tetramethylsilane (TMS) as an internal standard; chemical shifts are given in δ (ppm). Gas-liquid chromatography (GLC) was performed on a Shimadzu GC-6A gas chromatography apparatus, using 2% OV-17 on Chromosorb VAN-DMCS (3 mm × 2 m column) for analysis of TMS-sugars.

Isolation of Saponin from Fruits of *Xanthoceras sorbifolia* Bunge——Fruits (2.5 kg) were extracted with MeOH (5 l × 6) at room temperature. The combined extracts (193 g) were partitioned between *n*-BuOH (1 l) and water (1 l). The BuOH-soluble portion was fractionated by d.c.c. using a $CHCl_3$: MeOH : H_2O (35 : 65 : 40) solvent system (upper layer as the mobile phase, lower layer as the stationary phase). The saponin fractions were collected and combined. Removal of the solvents by evaporation gave the crude saponins (6 g).

Isolation of Prosapogenins——A solution of crude saponin (600 mg) in EtOH (15 ml), H_2O (15 ml) and conc. HCl (7.5 ml) was refluxed for 3 h. The precipitate produced was collected and washed with water to give a crude mixture of sapogenols and prosapogenins (250 mg). The crude mixture was chromatographed on silica gel (20 g) and eluted in a stepwise manner with $CHCl_3$–MeOH mixtures [200 : 1 (600 ml), 100 : 1 (250 ml), 50 : 1 (500 ml), 25 : 1 (250 ml), 7 : 3

(100 ml)] to give the following compounds:

1) $CHCl_3$–MeOH (200 : 1): compound A (**10**)[1] (21, 22-di-*O*-angeloyl R_1-barrigenol 30 mg).

2) $CHCl_3$–MeOH (100 : 1): compound B (**1**) (20 mg).

3) $CHCl_3$–MeOH (50 : 1): a) compound C (**2**) (10 mg); b) compound D (15 mg); c) compound E (napoleogenin B, 24 mg).[1]

4) $CHCl_3$–MeOH (25 : 1): compound F (**3**) (19 mg).

21-*O*-(3,4-Di-*O*-angeloyl)-*β*-D-fucopyranosyltheasapogenol B (1)——A white powder (MeOH), mp 236—238 °C, $[\alpha]_D^{19}$ +62.9 ° (c=0.28, MeOH), *Anal.* Calcd for $C_{46}H_{72}O_{11}\cdot 1/2H_2O$: C, 68.23; H, 9.05. Found: C, 68.17; H, 9.29. UV λ_{max}^{MeOH} nm (log ε): 215 (4.16). Infrared (IR) ν_{max}^{KBr} cm^{-1}: 3430, 2930, 2870, 1720, 1645, 1458, 1384, 1355, 1233, 1155, 1110, 1064, 1045, 980, 935, 845. 754. ^{1}H-NMR (pyridine-d_5) δ: 0.98 (6H, s), 1.07, 1.27, 1.36, 1.49, 1.86 (3H, each s, $\geq$C–Me), 1.15 (3H, d, J=7 Hz, Me of fucose), 1.88, 1.94 (3H, each br s, α-Me of angeloyl), 1.96 (6H, d,[13] J=7 Hz, β-Me of angeloyl), 5.95 (2H, q, J=7 Hz, β-H of angeloyl). ^{13}C-NMR data are given in Table I. MS m/z (%): 55 (48), 57 (20), 83 (100), 111 (12), 189 (8), 207 (10), 211 (7), 311 (7), 437 (5).

Acetylation of 1——A solution of **1** (2 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temperature for 24 h and treated in the usual manner. The crude product obtained was purified by thin-layer chromatography (TLC) with benzene–acetone (10 : 1) to give the tetraacetate of **1** (1 mg). Colorless needles (MeOH), mp 155—157 °C. UV λ_{max}^{MeOH} nm: 215. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, s), 0.92, 0.96, 1.26, 1.38 (3H, each s, $\geq$C–Me), 1.22 (3H, d, J=7 Hz, Me of fucose), 1.77 (6H, br s, α-Me of angeloyl), 1.99 (6H, d, J=7 Hz, β-Me of angeloyl), 2.03 (9H, s), 2.04 (3H, s, -OCOMe), 6.06 (2H, q, J=7 Hz, β-H of angeloyl). MS m/z (%): 55 (63), 57 (30), 69 (26), 83 (100), 153 (8), 189 (22), 215 (11), 231 (5), 249 (7), 253 (5), 353 (1), 355 (11), 478 (3), 524 (2), 636 (1), 695 (1), 737 (1), 914 (1).

Acid Hydrolysis of 1——A solution of **1** (5 mg) in 2 N H_2SO_4–dioxane–H_2O (2 : 2 : 1) mixture (1 ml) was heated on a water bath for 3 h and then diluted with water (5 ml) and extracted with EtOAc. The water layer was trimethylsilylated and analyzed by GC, and the TMS-fucose was identified by comparison with an authentic sample (GC: retention times 3.26 and 3.95 min). The EtOAc extract was chromatographed on a silica gel (5 g) column with $CHCl_3$–MeOH (30 : 1) to give theasapogenol B (**6**) (2 mg) together with two trace compounds. **6** was obtained as colorless needles (MeOH), mp 279—283 °C. $[\alpha]_D^{19}$ +19 ° (c=0.1, pyridine).

Acetylation of 6——A solution of **6** (2 mg) in Ac_2O (1 ml) and pyridine (1 ml) was left at room temperature for 30 h and then the solvents were removed to yield a residue, which was purified on a silica gel TLC plate to give the tetraacetate of theasapogenol B (**7**) (2 mg), a white powder (MeOH), mp 227—229 °C. $[\alpha]_D^{19}$ +18 ° (c=0.1, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.87, 0.89, 0.96, 1.05 (3H, each s), 1.26 (6H, s), 1.44 (3H, s, $\geq$C–Me), 2.01, 2.02, 2.06, 2.07 (3H, each s, -OCOMe), 3.60 (2H, br s, H_2-28), 4.20 (1H, m, H-16), 4.50 (1H, t-like, H-3), 5.37 (1H, m, H-12), 5.40 (1H, d, J=10 Hz, H-22), 5.54 (1H, d, J=10 Hz, H-21).

21-*O*-(4-*O*-Acetyl-3-*O*-angeloyl)-*β*-D-fucopyranosyltheasapogenol B (2)——A white powder (MeOH), mp 239—241 °C. $[\alpha]_D^{19}$ +57.9 ° (c=0.19, MeOH). *Anal.* Calcd for $C_{43}H_{68}O_{11}\cdot 1/2H_2O$: C, 67.10; H, 8.97. Found: C, 67.25; H, 8.93. UV λ_{max}^{MeOH} nm (log ε): 213 (4.19). IR ν_{max}^{KBr} cm^{-1}: 3430, 2930, 2870, 1745, 1720, 1643, 1460, 1384, 1365, 1240, 1155, 1110, 1070, 1035, 980, 932, 855, 752. ^{1}H-NMR (pyridine-d_5) δ: 0.99 (6H, s), 1.08, 1.25, 1.37, 1.51, 1.87 (3H, each s, $\geq$C–Me), 1.15 (3H, d, J=7 Hz, Me of fucose), 1.89 (3H, br s, α-Me of angeloyl), 1.93 (3H, d, J=7 Hz, β-Me of angeloyl), 2.14 (3H, s, -OCOMe), 3.48 (1H, t-like, H-3), 3.72, 4.00 (2H, ABq, J=11 Hz, H_2-28), 5.48 (1H, m, H-12), 6.00 (1H, q, β-H of angeloyl). ^{13}C-NMR data are given in Table I. MS m/z (%): 55 (73), 83 (100), 91 (14), 111 (10), 171 (9), 189 (15), 207 (13), 215 (12), 271 (3), 437 (3), 455 (2), 521 (1), 552 (2), 670 (0.2).

Acetylation of 2——A solution of **2** (2 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temperature for 24 h and treated in the usual way. The crude product obtained was purified by silica gel TLC with benzene–acetone (5 : 1) to give the tetraacetate of **2**. Colorless needles (MeOH–H_2O), mp 153—155 °C. UV λ_{max}^{MeOH} nm: 215. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (9H, s), 0.94, 0.98, 1.28, 1.40 (3H, each s, $\geq$C–Me), 1.24 (3H, d, J=7 Hz, Me of fucose), 1.81 (3H, br s, α-Me of angeloyl), 1.96 (3H, d, J=7 Hz, β-Me of angeloyl), 2.05 (3H, s), 2.08 (9H, s), 2.17 (3H, s, -OCOMe), 6.13 (1H, q, J=7 Hz, β-H of angeloyl). MS m/z (%): 55 (35), 83 (100), 111 (25), 153 (28), 171 (15), 184 (13), 189 (15), 213 (24), 249 (6), 313 (40), 496 (5), 617 (4), 809 (4).

Acid Hydrolysis of 2——A solution of **2** (5 mg) in HCl–H_2O–EtOH (1 : 1 : 3) mixture (10 ml) was refluxed for 6 h and then the solution was neutralized and evaporated to dryness. The residue was partitioned between water (20 ml) and EtOAc (20 ml). The water layer was dried and reacted with TMS reagent. The TMS-sugar was analyzed by GC, and the retention times (3.25, 3.96 min) were in agreement with those of authentic fucose. The EtOAc extract was chromatographed on a column of silica gel (5 g) with $CHCl_3$–MeOH (30 : 1) mixture to give compound **6** (2 mg) together with two trace compounds. **6** was obtained as colorless needles (MeOH), mp 278—282 °C. $[\alpha]_D^{19}$ +19 ° (c=0.1, pyridine). On usual acetylation, **6** gave a tetraacetate (**7**). The mp, $[\alpha]_D$ and ^{1}H-NMR data were in agreement with those of **7** obtained from **1** by acid hydrolysis.

21-*O*-(4-*O*-Acetyl-3-*O*-angeloyl)-*β*-D-fucopyranosyl-22-*O*-acetylprotoaescigenin (3)——A white powder (MeOH), mp 245—247 °C. $[\alpha]_D^{19}$ +35.9 ° (c=0.2, MeOH). *Anal.* Calcd for $C_{45}H_{70}O_{13}\cdot 1/2H_2O$: C, 65.29; H. 8.59, Found: C, 65.15; H, 8.52. UV λ_{max}^{MeOH} nm (log ε): 216 (4.08). IR ν_{max}^{KBr} cm^{-1}: 3430, 2930, 2870, 1745, 1720, 1705, 1645, 1460, 1382, 1370, 1250, 1155, 1110, 1070, 1040, 980, 930, 895, 855, 750. ^{1}H-NMR (pyridine-d_5) δ: 0.93, 0.95, 1.38, 1.43, 1.58, 1.84 (3H, each s, $\geq$C–Me), 1.19 (3H, d, J=7 Hz, Me of fucose), 1.91 (3H, br s, α-Me of angeloyl), 2.00 (3H, d,[15] J=7 Hz,

β-Me of angeloyl), 2.03, 2.36 (3H, each s, –OCOMe), 3.48 (1H, t-like, H-3), 5.93 (1H, q, $J=7$ Hz, β-H of angeloyl), 6.20 (1H, d, $J=10$ Hz, H-22), ^{13}C-NMR data are given in Table I. MS m/z (%): 55 (90), 71 (20), 83 (100), 111 (11), 171 (16), 205 (8), 223 (3), 271 (17), 512 (2), 596 (0.3), 654 (0.1).

Acetylation of 3——A solution of **3** (2 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temperature for 24 h. The solvents were removed and the product was purified by silica gel TLC with benzene–acetone (5 : 1) to give the tetraacetate of **3** (1 mg). Colorless needles (MeOH), mp 156—158 °C. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 215. ^{1}H-NMR ($CDCl_3$) δ: 0.92, 0.94, 0.99 (3H each, s), 1.05 (6H, s), 1.40 (3H, s, $>$C–Me), 1.26 (3H, d, $J=7$ Hz, Me of fucose), 1.80 (3H, br s, α-Me of angeloyl), 1.99 (3H, d, $J=7$ Hz, β-Me of angeloyl), 2.02, 2.04, 2.07, 2.10 (3H each, s), 2.06 (6H, s, –OCOMe), 6.14 (1H, q, $J=7$ Hz, β-H of angeloyl). MS m/z (%): 55 (45), 83 (100), 111 (43), 113 (3), 156 (67), 171 (21), 184 (6), 205 (3), 213 (38), 272 (2), 307 (5), 313 (52), 600 (0.3), 640 (1), 657 (0.5), 700 (0.5), 868 (0.1), 926 (0.4), 970 (0.1).

Acid Hydrolysis of 3——A solution of **3** (9 mg) in EtOH–H_2O–HCl (3 : 1 : 1) mixture was refluxed for 6 h then the solvents were removed by evaporation *in vacuo*. The residue was partitioned between water (10 ml) and EtOAc (10 ml). The water layer was used for identification of the sugar. The EtOAc extract was chromatographed on a column of silica gel (10 g) with $CHCl_3$–MeOH (15 : 1) to give protoaescigenin (**8**) as a major product. **8** was obtained as a white powder (MeOH), mp over 300 °C. $[\alpha]_D^{20}$ +21.7° ($c=0.2$, pyridine). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3430, 2930, 2870, 1635, 1443, 1385, 1375, 1260, 1030, 703. ^{1}H-NMR (pyridine-d_5) δ: 0.95 (6H, s), 1.36, 1.42, 1.57, 1.87 (3H, each s, $>$C–Me), 3.68 (1H, t-like, H-3), 3.73, 4.06 (2H, ABq, $J=11$ Hz, H_2-24), 3.70, 4.54 (2H, ABq, $J=11$ Hz, H_2-28), 4.65 (1H, d, $J=10$ Hz, H-22[15]), 4.82 (1H, d,[14] $J=$ Hz, H-21), 5.06 (1H, m, H-16), 5.46 (1H, m, H-12). ^{13}C-NMR data are given in Table I.

The water layer was dried and analyzed by GC. The fucose was identified by comparison with an authentic sample (GC: retention times, 3.26 and 3.96 min).

Acetylation of 8——Treatment of **8** (3 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temperature for 24 h gave a crude product, which was chromatographed on a column of silica gel (5 g) to give the pentaacetate of protoaescigenin (**9**) (3 mg), mp 139—144 °C. $[\alpha]_D^{19}$ +23° ($c=0.15$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.88, 0.91, 0.96, 1.01, 1.12, 1.47 (3H, each s, $>$C–Me), 2.02, 2.03, 2.04, 2.06, 2.07 (3H, each s, –OCOMe), 3.66 (2H, br s, H_2-28), 4.14, 4.35 (2H, ABq, $J=12$ Hz, H_2-24), 4.20 (1H, m, H-16), 4.60 (1H, t-like, H-3), 5.38 (1H, m, H-12), 5.41 (1H, d, $J=10$ Hz, H-22), 5.54 (1H, d, $J=10$ Hz, H-21).

Alkaline Hydrolysis of 3——A solution of **3** (8 mg) in 5% KOH/MeOH (2 ml) was refluxed for 2 h, then diluted with water (10 ml) and neutralized with hydrochloric acid. The aqueous layer was extracted with EtOAc. The EtOAc extract obtained was chromatographed on a silica gel column with $CHCl_3$–MeOH (25 : 1) to give **5** (4 mg) as colorless needles (pyridine–water). mp 297—300 °C. $[\alpha]_D^{20}$ +9.4° ($c=0.2$, pyridine). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3420, 2970, 2930, 2870, 1635, 1443, 1385, 1375, 1175, 1075, 1030, 990, 900, 855, 750, 705. ^{1}H-NMR (pyridine-d_5) δ: 0.95 (6H, s), 1.37 (3H, s), 1.57 (6H, s), 1.88 (3H, s, $>$C–Me), 3.67 (1H, m, H-3), 3.70, 4.52 (2H, ABq, $J=11$ Hz,[14] H_2-28), 3.72, 4.04 (2H, ABq, $J=11$ Hz,[14] H_2-24), 4.62 (1H, d, $J=10$ Hz,[14] H-22), 4.80 (1H, d, $J=10$ Hz, H-21), 5.06 (1H, m, H-16), 5.48 (1H, m, H-12). ^{13}C-NMR data are in Table I.

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