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Studies on the Constituents of *Xanthoceras sorbifolia* BUNGE. V. Major Saponins from the Fruits of *Xanthoceras sorbifolia* BUNGE[1]

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Four new saponins were isolated from the fruits of *Xanthoceras sorbifolia* BUNGE. The structures of these saponins were deduced on the basis of chemical and spectral evidence as 22-*O*-acetyl-21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin (**7**), 22-*O*-acetyl-21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin (**8**), 28-*O*-acetyl-21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin (**9**) and 28-*O*-acetyl-21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin (**10**). The names "bunkankasaponins A, B, C and D" are proposed for **7**, **8**, **9** and **10**, respectively.

Keywords——*Xanthoceras sorbifolia*; Sapindaceae; bunkankasaponin A; bunkankasaponin B; bunkankasaponin C; bunkankasaponin D; protoaescigenin

We have already reported the isolation and structure elucidation of prosapogenins from the fruits of *Xanthoceras sorbifolia* BUNGE.[1] A further investigation on the chemical constituents of the title plant has resulted in the isolation of four new saponins. In this paper we report the evidence which led to the establishment of the structures of these saponins.

The saponin fraction, obtained from a methanolic extract of fruits of *Xanthoceras sorbifolia*, was shown to be a mixture of highly polar saponins, some of which contained glucuronic acid. The total saponins gave a single spot on a silica gel thin-layer chromatography (TLC) plate. In order to separate the glucuronide-type saponins from the non-glucuronide saponins, the crude saponins were methylated with diazomethane and four kinds of glucuronide-type saponins were obtained in methyl ester form by means of repeated silica gel and reversed phase column chromatography of methylated crude saponins.

Compound M-A (**11**) was a white powder. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **11** showed three anomeric carbon signals of sugar components at 105.2, 105.1 and 104.8 ppm, indicating the existence of three sugars in **11** (Table I). On acid hydrolysis, **11** gave fucose, glucose, glucuronic acid[2] and protoaescigenin (**1**) which, on usual acetylation, afforded a pentaacetate (**2**) identical with a pentaacetate of authentic protoaescigenin.[3] This result shows that **11** is a triterpenoidal saponin composed of protoaescigenin linked with fucose, glucose and glucuronic acid.

Partial acid hydrolysis of **11** furnished **5** and **6**. Compound **5**, on alkaline hydrolysis, gave 21-*O*-β-D-fucopyranosylprotoaescigenin (**3**), showing that the fucose moiety was linked to C-21 of protoaescigenin. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **11**, the peaks at 1.92 (3H, br s, α-Me), 2.00 (3H, d, $J=7$ Hz, β-Me) and 5.92 ppm (1H, q, $J=7$ Hz, β-H) suggest the presence of an angeloyl [(*Z*)-2-methyl-2-butenoyl] group.[4] The signals at 2.03 and 2.39 ppm (3H, each s, OCO<u>Me</u>) indicate the existence of two acetyl groups in **11**.

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

Carbon	**1**	**3**	**4**	**5**	**6**	**11**	**12**	**13**	**14**	**17**
1	39.0	38.9	38.9	38.9	38.9	38.6	38.5	38.5	38.7	38.9
2	28.5	28.5	28.5	28.4	26.9	26.6	26.6	26.7	26.6	26.6
3	80.2	80.1	80.1	80.1	90.1	90.8	90.8	90.8	90.8	90.7
4	43.2	43.2	43.2	43.2	44.0	43.8	43.8	43.8	43.7	43.6
5	56.4	56.4	56.4	56.4	56.1	56.2	56.1	56.1	56.1	56.1
6	19.2	19.1	19.1	19.1	19.1	18.5	18.6	18.6	18.5	18.7
7	33.6	33.5	33.5	33.5	33.2	33.3	33.2	33.2	33.2	33.2
8	40.1	40.1	40.2	40.1	40.0	39.9	40.0	40.0	40.0	40.0
9	47.3	47.2	47.2	47.2	47.0	46.7	46.6	46.7	46.7	46.8
10	37.0[a]	37.2[a]	37.2[a]	37.0[a]	36.5[a]	36.4[a]	36.4[a]	36.4[a]	36.4[a]	36.4[a]
11	24.2	24.1	24.2	24.1	24.1	24.1	24.1	24.2	24.1	24.1
12	123.8	123.8	123.7	123.8	123.8	123.8	123.8	123.7	123.8	123.8
13	144.0	143.9	143.8	143.1	143.0	143.1	143.1	142.9	142.9	143.9
14	42.1	42.0	41.9	41.6	41.6	41.6	41.6	41.8	41.8	41.9
15	34.4	34.5	34.5	34.8	34.7	34.8	34.8	34.7	34.8	34.8
16	67.9	68.0	68.0	68.8	68.7	68.8	68.7	67.9	67.9	68.0
17	47.4	47.9	48.2	47.8	47.8	47.8	47.8	46.6	46.6	47.8
18	41.3	40.6	40.5	40.1	40.0	39.9	40.0	40.5	40.4	40.5
19	48.3	48.3	47.9	47.8	47.8	47.8	47.8	47.8	47.7	47.8
20	36.5[a]	37.0[a]	37.0[a]	37.8[a]	37.8[a]	37.7[a]	37.8[a]	37.1[a]	37.1[a]	37.2[a]
21	78.7	92.2	92.1	85.1	85.1	85.1	85.7	92.3	92.0	92.1
22	77.3	74.0	73.6	74.2	74.2	74.2	74.4	74.2	74.3	74.1
23	23.6	23.5	23.5	23.6	23.3	22.5	22.5	22.5	22.5	22.5
24	64.6	64.6	64.6	64.6	63.3	63.2	63.3	63.3	63.3	63.4
25	16.3[b]	16.2[b]	16.2[b]	16.2[b]	16.1[b]	15.9[b]	16.1[b]	16.2[b]	16.3[b]	15.6[b]
26	16.9[b]	16.8[b]	16.8[b]	16.9[b]	16.7[b]	16.7[b]	16.9[b]	16.8[b]	16.8[b]	16.8[b]
27	27.3	27.5	27.5	27.5	27.5	27.4	27.4	27.5	27.5	27.5
28	68.4	67.4	66.9	64.0	64.0	64.0	63.9	66.4	66.4	67.5
29	30.6	30.0	29.9	30.2	30.2	30.2	30.1	29.8	29.8	30.0
30	19.5	20.4	20.3	20.1	20.1	20.1	20.0	20.2	20.2	20.4
Fuc. 1′		106.7	106.1	105.2	105.2	105.2	105.4	106.2	106.2	106.6
2′		72.5	70.0	70.0	70.0	70.0	70.2	69.8	70.0	72.5
3′		75.3	74.3	74.2	74.2	74.2	74.0	74.2	74.3	75.4
4′		72.7	71.1	71.6	71.6	71.6	71.4	71.4	71.0	72.7
5′		71.6	69.5	68.8	68.8	68.8	68.9	69.3	69.5	71.6
6′		17.0	16.4	16.7	16.7	16.7	16.7	16.8	16.8	17.1
Glc-UA 1″					106.3	105.1	105.1	105.1	105.1	105.0
2″					75.0	81.6	81.5	81.6	81.6	81.6
3″					78.1	78.0	78.0	78.0	78.0	78.0
4″					73.0	72.6	72.6	72.6	72.6	72.6
5″					76.6	77.0	77.0	77.0	77.0	77.0
6″					176.8	170.3	170.3	170.3	170.3	176.6
COOMe						52.1	52.1	52.1	52.1	

This was supported by the ^{13}C-NMR data for **11** (peaks at 170.6 and 171.9 ppm, assigned to two acetyl carbonyl carbons, and peaks at 20.5 and 21.9 ppm, attributed to two acetyl methyls).

In the mass spectrum (MS) of **11**, the abundant ions at m/z 43, 55, 83 and 271 suggest the presence of an acetyl and an angeloyl group in the fucose moiety (Chart 2).[1] The other acetyl group was shown to be located at C-22 based on the observation of an upfield shift of the C-21 signal to 85.1 ppm, as compared with **3** and **4**. This suggestion was supported by comparison of the C-21 signal of **11** (85.1 ppm) with those of the hexaacetate of napoleogenin [21β-(3,4-

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TABLE I. (continued)

	Carbon	1	3	4	5	6	11	12	13	14	17
Glc.	1'''						104.8	104.8	104.8	104.8	104.3
	2'''						75.8	75.7	75.7	75.7	75.8
	3'''						78.5	78.5	78.5	78.5	78.5
	4'''						69.9	69.9	69.8	69.9	69.9
	5'''						78.2	78.2	78.3	78.2	78.2
	6'''						61.5	61.6	61.7	61.6	61.5
Ang.	1			167.3	167.4	167.4	167.4	167.4	167.3	167.3	
				167.3				167.4		167.3	
	2			127.9	128.2	128.2	128.2	127.9	128.0	127.8	
				128.1				128.2		128.0	
	3			138.3	138.0	138.0	138.0	138.1	138.4	138.4	
				138.8				138.9		138.8	
	4			20.5	20.6	20.6	20.6	20.6	20.6	20.5	
				20.8				20.8		20.7	
	5			15.9	15.9	15.6	15.6	15.5	15.8	15.6	
				16.0				15.9		15.9	
Ac.	<u>C</u>OMe				170.6	170.6	170.6	171.6	170.6	170.6	
					171.9	171.9	171.9		170.7		
	CO<u>Me</u>				20.5	20.5	20.5	22.0	20.6	20.7	
					21.9	21.9	21.9		20.8		

a, b) Assignments may be reversed in each column. The carbons bearing an OH group were assigned by means of selective proton decoupling.

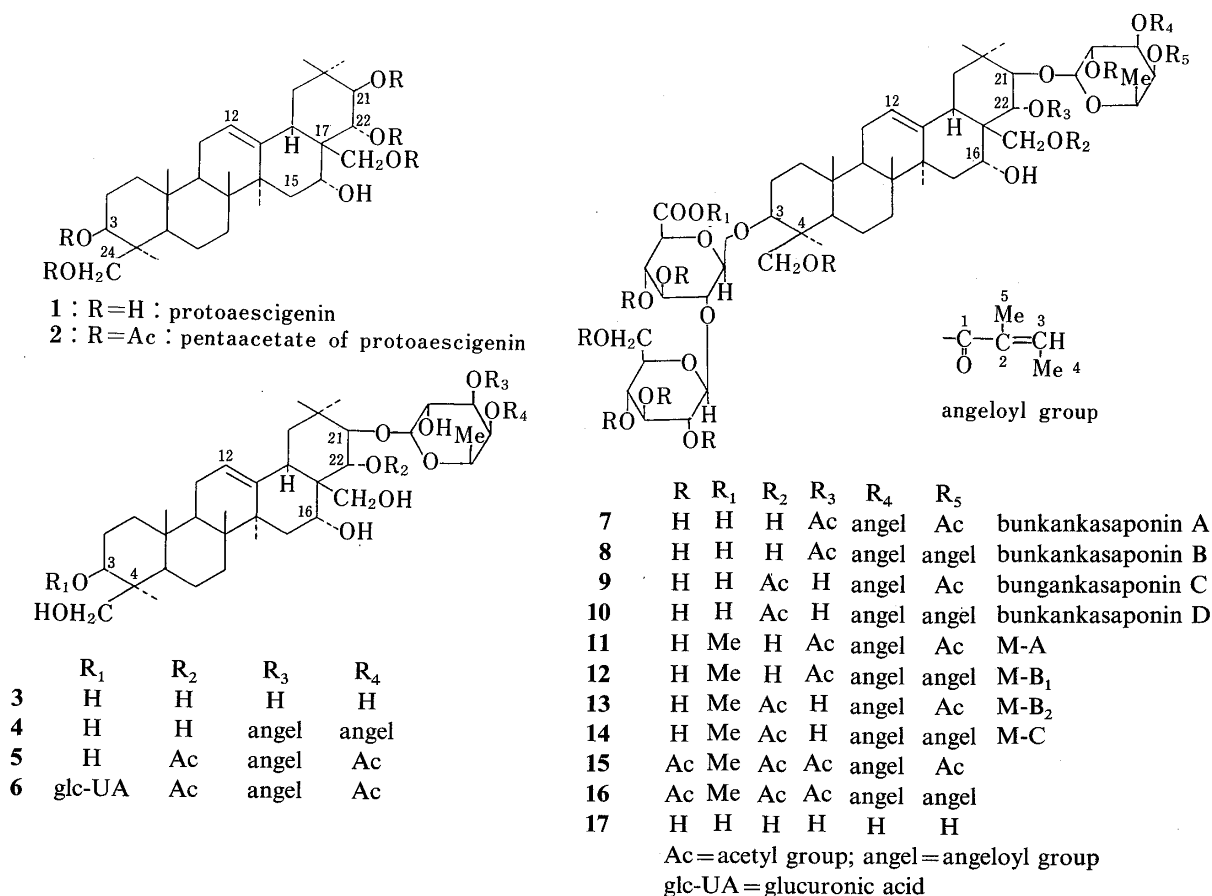

Chart 1

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diangeloyl-6-deoxy-β-glucopyranosyl)oxy-3β,16α,22α,24,28-pentahydroxyolean-12-ene][5a] (84.3 ppm) and the pentaacetate of napoleogenin B [21β-(3,4-diangeloyl-6-deoxy-β-galactopyranosyl)oxy-3β,16α,22α,24,28-pentahydroxyolean-12-ene][5b] (85.9 ppm). In the case of napoleogenin, the C-21 signal shifted upfield by 6.9 ppm (from 91.2 to 84.3 ppm) when the OH at C-22 was acetylated. A similar result was observed in **11** with an upfield shift of 7.1 ppm (from 92.2 ppm in **3** to 85.1 ppm in **11**), indicative of the presence of an acetoxyl group at C-22. This was further confirmed by identifying **5**, one of the products obtained from partial acid hydrolysis of **11**, as 22-*O*-acetyl-21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosylprotoaescigenin by comparison with an authentic sample.

The other product (**6**) obtained from partial acid hydrolysis of **11** revealed the presence of glucuronic acid in addition to a substituted fucosyl moiety at C-21 and an acetoxyl group at C-22, on the basis of the ^{13}C-NMR data (Table I). This result shows that **6** is a glucuronide of **5**. Comparison of the ^{13}C-NMR data of **6** with those of **5** showed that the C-2 signal is shifted 1.5 ppm upfield and the C-3 signal is shifted 10 ppm downfield on going from **5** to **6** (Table I), suggesting that glucuronic acid is linked to C-3, according to the glycosidation shift rule.[6]

Methylation analysis of the permethylate of **11** showed that the terminal sugars should be glucose and fucose, on the basis of the identification of methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 2,3,4-tri-*O*-methylfucopyranoside. A comparison of the ^{13}C-NMR spectrum of **11** with that of **6** showed a set of signals assignable to a terminal glucosyl unit in the spectrum of **11**. The C-1′′ of glucuronic acid is shifted 1.2 ppm upfield, while C-2′′ is shifted 6.6 ppm downfield on going from **6** to **11**. These shifts can be regarded as glycosidation shifts[6] and therefore the location of glucose is considered to be C-2′′ of glucuronic acid.

Measurements of nuclear Overhauser effect (NOE) in the gated decoupling mode, which gave $^{1}J_{CH}$ of the three sugars as 153.8 (peak at 105.2 ppm) and 158.7 Hz (peaks at 105.1 and 104.8 ppm), showed that all three pyranoses have an axial anomeric proton, indicative of β-configuration.[7]

Based on these observations, compound M-A (**11**) was established as 22-*O*-acetyl-21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosylprotoaescigenin-3-yl-[β-D-glucopyranosyl-

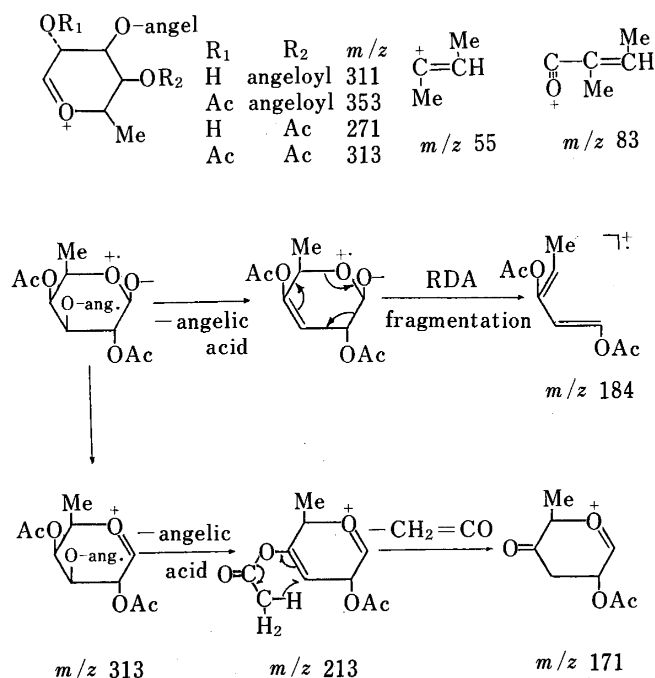

Chart 2

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(1→2)]-methyl- (β-D- glucopyranosid)uronate. The proposed structure was further supported by MS analysis of the peracetate of **11** (**15**) and the results of alkaline hydrolysis. In the MS of **15**, the fragment ions at *m/z* 83 (angeloyl) and 313 (Chart 2) showed the presence of an angeloyl-fucosyl moiety. On alkaline hydrolysis, **11** furnished **17**, supporting the attachment of the β-D-fucopyranosyl moiety at C-21 and the β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl moiety at C-3 of protoaescigenin.

M-B_1 (**12**), on acid hydrolysis, gave fucose, glucose, glucuronic acid and an aglycone (**1**) identical with protoaescigenin. On alkaline hydrolysis, **12** afforded **17**, which was identified as 21-*O*-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin. This result shows that the fucose moiety is linked to C-21 and the glucosyl-(1→2)-glucuronosyl moiety to C-3 of protoaescigenin.

The ^{1}H-NMR spectrum suggested the presence of two angeloyl groups in **12** based on the observation of signals due to two angeloyls at 1.87, 1.97 (3H, each br s, α-Me), 1.98, 2.04 (3H, each d, $J=7$ Hz, β-Me), 5.91 and 5.97 ppm (1H, each q, $J=7$ Hz, β-H).[4] This conclusion was supported by the ^{13}C-NMR data, which showed two sets of signals of angeloyl groups (Table I). The fragment ions at *m/z* 311 in the MS of **12** and 353 (Chart 2) in that of the peracetate of **12** (**16**) indicate that both angeloyl groups are located on the fucose moiety. The possible positions for the two angeloyl groups are C-3′ and C-4′, because no upfield shift is observed for C-1′ of fucose, compared with **11**, showing that no acyl group is present at C-2′, according to the acylation shift rule.[8]

The ^{13}C-NMR (171.6 ppm, –O$\underline{C}$OMe and 22.0 ppm, –OCO$\underline{Me}$) and ^{1}H-NMR (2.34 ppm, 3H, s, –OCO$\underline{Me}$) spectra indicated the presence of an acetyl group in **12**. A downfield shift of the C-22 signal to 74.4 ppm and an upfield shift of the C-21 signal to 85.7 ppm, compared with **4**, show the acetyl group to be located at C-22, according to the acetylation shift rule.[9] This conclusion was supported by comparing the C-21 signal of **12** (85.7 ppm) with those of **11** (85.1 ppm) and the pentaacetate of napoleogenin B (85.9 ppm), which have an acetoxyl group at C-22.[5]

Based on these analyses, **12** was established to be 22-*O*-acetyl-21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosylprotoaescigenin-3-yl-[β-D-glucopyranosyl-(1→2)]-methyl-(β-D-glucopyranosid)uronate.

M-B_2 (**13**), on acid hydrolysis, gave fucose, glucose, glucuronic acid and protoaescigenin. Alkaline hydrolysis of **13** afforded **17** which was identified as 21-*O*-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin.

The ^{1}H-NMR spectrum suggests the existence of an angeloyl and two acetyl groups in **13** based on the observation of signals at 1.89 (3H, br s, α-Me of angeloyl), 1.99 (3H, d, $J=7$ Hz, β-Me of angeloyl), 5.92 (1H, q, $J=7$ Hz, β-H of angeloyl) and 2.06, 2.11 ppm (3H, each s, –OCO$\underline{Me}$). This conclusion is further supported by the ^{13}C-NMR data, which show a set of signals for one angeloyl (Table I) and two acetyls at 170.6, 170.7 ppm (O$\underline{C}$OMe) and 20.6, 20.8 ppm (OCO$\underline{Me}$). In the MS of **13**, the fragment ion at *m/z* 271 shows that an acetyl and an angeloyl group are present in the fucose moiety (Chart 2). The ions at *m/z* 171, 184 in the MS of the peracetate of **13** (**15**) suggest that an acetyl group is present at C-4′, and the angeloyl group at C-3′ of fucose (Chart 2) according to the fragmentation rule for acetylated sugars.[10] The other acetyl group was shown to be located at C-28 based on the observation of acetylation shifts of the signals for C-28 (downfield shift by 2.4 ppm) and C-17 (upfield shift by 1.2 ppm) compared to those of **11**.

Based on these observations, **13** is characterized as 28-*O*-acetyl-21-*O*-(4-*O*-acetyl-3-*O*-angeloyl)-β-D-fucopyranosylprotoaescigenin-3-yl-[β-D-glucopyranosyl-(1→2)]-methyl-(β-D-glucopyranosid)uronate. The proposed structure was further supported by acetylation of **13** to give the undecaacetate (**15**), which was identical with the undecaacetate (**15**) obtained by acetylation of **11**.

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M-C (**14**), on acid hydrolysis, gave fucose, glucose, glucuronic acid and protoaescigenin. Alkaline hydrolysis of **14** afforded **17**, which was identified as 21-*O*-β-D-fucopyranosyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]protoaescigenin. The ^{1}H-NMR spectrum of **14** suggests the presence of an acetyl and two angeloyl groups based on the observation of signals at 1.97 (3H, s, OCOMe), 1.87 (6H, br s, α-Me of angeloyl), 2.00 (6H, d, $J=7$ Hz, β-Me of angeloyl) and 5.94 (2H, q, $J=7$ Hz, β-H of angeloyl). This was also supported by the ^{13}C-NMR data, with signals at 170.6 (OCOMe), 20.7 ppm (OCOMe) and the signals of two angeloyl groups (Table I). In the MS of the peracetate of **14** (**16**), the abundant ions at m/z 55, 83, 353 (Chart 2) suggest that both angeloyl groups are in the fucose moiety.[5] No upfield shift was observed for C-1′ of fucose, indicating that the two angeloyl groups are at C-3′ and C-4′ of the fucose moiety. This was supported by partial acid hydrolysis of **14** to give **4**, which was identified as napoleogenin B by comparison with an authentic sample.

A comparison of the ^{13}C-NMR data of **14** with those of **13** shows that the signals of C-17 and C-28 of **14** are fully coincident with those of **13**, suggesting that the acetoxyl group is at C-28.

Based on these observations, **14** was established to be 28-*O*-acetyl-21-*O*-(3,4-di-*O*-angeloyl)-β-D-fucopyranosylprotoaescigenin-3-yl-[β-D-glucopyranosyl-(1→2)]-methyl-(β-D-glucopyranosid)uronate. The proposed structure was further supported by acetylation of **14** to give a decaacetate (**16**) identical with the decaacetate obtained by acetylation of **12**.

Since the compounds M-A (**11**), M-B_1 (**12**), M-B_2 (**13**) and M-C (**14**) were obtained by methylation of the saponins with diazomethane, the structures of original saponins in the title plant should be assigned as **7**, **8**, **9** and **10**, respectively. The names "bunkankasaponins A, B, C and D" are proposed for **7**, **8**, **9** and **10**, respectively.

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. Electron impact (EI)-MS were recorded on a JEOL DX-300 mass spectrometer at 70 eV by direct insertion. ^{1}H-NMR spectra were measured on a 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) were performed on a Shimadzu GC-6A gas chromatograph, using 2% OV-17 on Chromosorb VAN-DMCS (3 mm × 2 m column) for analysis of TMS-sugars; 10% DEGS on Chromosorb W (3 mm × 2 m column) for analysis of methylated sugars.

Isolation of Saponins 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 BuOH (1 l) and water (1 l). The BuOH soluble portion was fractionated by droplet counter-current chromatography (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). The saponin mixture gave a single spot on a silica gel TLC plate with *Rf* value = 0.11 [solvent system: the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)].

The crude saponins (1 g) were methylated with a solution of diazomethane (*ca.* 300 mg) in ether and the products obtained were chromatographed on a column of silica gel (100 g) and eluted in a stepwise manner with $CHCl_3$–MeOH mixtures [15 : 1 (400 ml), 10 : 1 (1600 ml), 5 : 1 (200 ml), 2 : 1 (700 ml)] to give four fractions: M-1 (30 mg); M-2 (238 mg); M-3 (40 mg) and M-4 (440 mg). Fraction M-2 was chromatographed on a reversed-phase column of Silica gel 60 silanized (40 g) with MeOH–H_2O (5 : 4) mixture to give M-A (**11**) (35 mg), M-B (77 mg) and M-C (**14**) (39 mg). M-B was further chromatographed on a LiChroprep RP-18 Lobar column with MeOH–H_2O (7 : 3) mixture to give M-B_1 (**12**) (25 mg) and M-B_2 (**13**) (21 mg).

M-A (11)——A white powder (MeOH), mp 261—264 °C. $[\alpha]_D^{20}$ −14.2° (c = 0.9, pyridine). *Anal.* Calcd for $C_{58}H_{90}O_{24}\cdot 5/2H_2O$: C, 57.28; H, 7.82. Found: C, 57.34; H, 7.88. UV λ_{max}^{MeOH} nm: 215. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2920, 2870, 1745, 1720, 1635, 1460, 1440, 1383, 1255, 1160, 1075, 1045, 1025, 970, 800. ^{1}H-NMR (pyridine-d_5) δ: 0.71, 0.81 (3H, each s), 1.37 (9H, s), 1.84 (3H, s, Me), 1.20 (3H, d, $J=7$ Hz, Me of fucose), 2.03, 2.39 (3H, each s, OCOMe), 3.79 (3H, s, COOMe), 1.92 (3H, br s, α-Me of angeloyl), 2.00 (3H, d, $J=7$ Hz, β-Me of angeloyl), 5.92 (1H, q, $J=7$ Hz, β-H of angeloyl), 5.40 (1H, m, H-12). ^{13}C-NMR data are given in Table I. MS m/z (%): 43 (44.0), 55 (55.0), 83 (100.0), 111 (13.0), 169 (83.0), 171 (10.7), 271 (11.2), 335 (13.3), 453 (1.6), 501 (3.0), 667 (0.1).

M-B_1 (12)——A white powder (MeOH), mp 262—265 °C. $[\alpha]_D^{19}$ −0.41° (c = 1.2, pyridine). *Anal.* Calcd for

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$C_{61}H_{94}O_{24}\cdot 5/2H_2O$: C, 58.32; H, 7.89. Found: C, 58.11; H, 7.82. UV λ_{max}^{MeOH} nm: 215. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2920, 2850, 1745, 1720, 1640, 1460, 1382, 1260, 1160, 1070, 1040, 978, 855. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.79 (3H, each s), 1.39 (9H, s), 1.88 (3H, s, Me), 1.19 (3H, d, J=7 Hz, Me of fucose), 2.34 (3H, s, OCOMe), 3.78 (3H, s, COOMe), 1.87, 1.97 (3H, each br s, α-Me of angeloyl), 1.98, 2.04 (3H, each d, J=7 Hz, β-Me of angeloyl), 5.91, 5.97 (1H, each q, J=7 Hz, β-H of angeloyl), 5.40 (1H, m, H-12). ^{13}C-NMR data are given in Table I. MS m/z (%): 43 (84.4), 55 (49.2), 83 (100), 111 (53.1), 191 (7.8), 205 (13.2), 211 (66.0), 311 (75.0), 353 (10), 482 (1.8), 493 (1.5), 495 (4.2), 508 (1.6), 513 (2.5), 637 (0.1).

M-B$_2$ (13)——A white powder (MeOH), mp 260—263 °C. $[\alpha]_D^{19}$ $-0.20°$ (c=0.9, pyridine). *Anal.* Calcd for $C_{58}H_{90}O_{24}\cdot 5/2H_2O$: C, 57.28; H, 7.82. Found: C, 57.34; H, 7.88. UV λ_{max}^{MeOH} nm: 215. IR ν_{max}^{KBr} cm^{-1}: 3450, 2920, 2860, 1743, 1720, 1630, 1450, 1383, 1240, 1160, 1070, 1040, 977, 855. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.93 (3H, each s), 1.36 (6H, s), 1.49, 1.83 (3H, each s, Me), 1.12 (3H, d, J=7 Hz, Me of fucose), 2.06, 2.11 (3H, each s, OCOMe), 3.79 (3H, s, COOMe), 5.45 (1H, m, H-12), 1.89 (3H, br s, α-Me of angeloyl), 1.99 (3H, d, J=7 Hz, β-Me of angeloyl), 5.92 (1H, q, J=7 Hz, β-H of angeloyl). ^{13}C-NMR data are given in Table I. MS m/z (%): 43 (80.1), 55 (48.0), 83 (100.0), 111 (57.4), 169 (71.9), 171 (34.8), 271 (30.4), 335 (17.2), 353 (1.5), 435 (5.6), 453 (5.2), 479 (2.2), 496 (2.2), 501 (3.6), 521 (0.9), 599 (0.1).

M-C (14)——A white powder (MeOH), mp 253—256 °C. $[\alpha]_D^{20}$ $+5.2°$ (c=1.7, pyridine). *Anal.* Calcd for $C_{61}H_{94}O_{24}\cdot 5/2H_2O$: C, 58.32; H, 7.89. Found: C, 58.21; H, 7.86. UV λ_{max}^{MeOH} nm: 215. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2920, 2850, 1740, 1720, 1640, 1460, 1440, 1384, 1365, 1235, 1155, 1065, 1042, 978, 848, 752. ^{1}H-NMR (pyridine-d_5) δ: 0.72, 0.96 (3H, each s), 1.34 (6H, s), 1.52, 1.88 (3H, each s, Me), 1.16 (3H, d, J=7 Hz, Me of fucose), 1.97 (3H, s, OCOMe), 3.78 (3H, s, COOMe), 5.44 (1H, m, H-12), 1.87 (6H, br s, α-Me of angeloyl), 2.00 (6H, d, J=7 Hz, β-Me of angeloyl), 5.94 (2H, q, J=7 Hz, β-H of angeloyl). ^{13}C-NMR data are given in Table I. MS m/z (%): 43 (45.2), 55 (51.5), 83 (100.0), 111 (13.9), 169 (77.6), 171 (36.8), 211 (78.2), 311 (70.8), 353 (3.8), 435 (10.7), 453 (9.6), 479 (3.9), 495 (2.9), 513 (2.2), 522 (1.4), 596 (0.1), 733 (0.1).

Acid Hydrolysis of 11, 12, 13 and 14——The sample was hydrolyzed by refluxing with a mixture of HCl–H_2O–EtOH (2 : 1 : 2) on a water bath for 30 min. The hydrolysate was partitioned between EtOAc and H_2O. The EtOAc extract was chromatographed on silica gel to give the aglycone. All four aglycones obtained from **11**—**14** were the same and were identical with authentic protoaescigenin.

Usual acetylation of **1** gave a pentaacetate (**2**) identical with pentaacetate of protoaescigenin.

The water extract was thoroughly dried and divided into two parts. Trimethylsilylation of one of the two parts followed by GC showed the presence of fucose and glucose in a ratio of 1 : 1. The retention times were 3.26, 3.95 min (TMS-fucose) and 8.39, 12.85 min (TMS-glucose).

The other part was treated according to Hulyalkar's method[2] and showed the existence of glucuronic acid. The retention time was 10.41 min (2,3,5,6-tetra-O-trimethylsilyl-L-gul-gulono-1,4-lactone).

All of four saponins gave the same sugars: fucose, glucose and glucuronic acid.

Partial Acid Hydrolysis of 11 and 14——A solution of **11** (15 mg) in 1% HCl–MeOH (10 ml) was refluxed for 1 h and the solution was neutralized and evaporated to give a residue which was chromatographed on a column of silica gel (3 g) with $CHCl_3$–MeOH mixture in a stepwise manner. Compounds **5** (2 mg) and **6** (5 mg) were obtained from the eluates of $CHCl_3$–MeOH (20 : 1) and (5 : 1), respectively.

5 was identified as 22-O-acetyl-21-O-(4-O-acetyl-3-O-angeloyl)-β-D-fucopyranosylprotoaescigenin by comparison with an authentic sample.

6 is a white powder, mp 272—274 °C. UV λ_{max}^{MeOH} nm: 215. IR ν_{max}^{KBr} cm^{-1}: 3420, 2920, 2860, 1740, 1720, 1695, 1635, 1460, 1385, 1255, 1140, 1065, 1040, 980. ^{1}H-NMR (pyridine-d_5) δ: 0.85, 1.26 (6H, each s), 1.41, 1.88 (3H, each s, Me), 1.20 (3H, d, J=7 Hz, Me of fucose), 2.03, 2.36 (3H, each s, OCOMe), 1.95 (3H, br s, α-Me of angeloyl), 2.00 (3H, d, J=7 Hz, β-Me of angeloyl), 5.93 (1H, q, J=7 Hz, β-H of angeloyl). ^{13}C-NMR data are given in Table I.

Partial acid hydrolysis of **14** (15 mg) with HCl–H_2O–EtOH (1 : 2 : 2) afforded **4** (3 mg), which was identical with authentic napoleogenin B.

Methylation Analysis of 11——The permethylate of **11** was prepared according to Hakomori's method.[11] The methylated product (2 mg) was methanolyzed with 5% HCl–MeOH (2 ml) in a sealed ampoule at 100 °C for 5 h. The resulting methylglycosides were analyzed by GC. Methyl 2,3,4,6-tetra-O-methylglucopyranoside (4.33, 5.80 min) and methyl 2,3,4-tri-O-methylfucopyranoside (3.44 min) were identified by comparison with authentic samples.

Acetylation of 11, 12, 13 and 14——A solution of a sample (4 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 chromatography on a silica gel column with benzene–acetone (7 : 1) mixture. Acetylation of **11** and **13** gave the same undecaacetate (**15**), colorless needles, mp 170—173 °C. UV λ_{max}^{MeOH} nm: 215. IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1750, 1720, 1635, 1435, 1370, 1240, 1172, 1070, 1043, 983, 905, 800. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s), 0.98 (6H, s), 1.03, 1.22, 1.41 (3H, each s, Me), 1.25 (3H, d, J=7 Hz, Me of fucose), 2.02 (6H, s), 2.05 (9H, s), 2.10, 2.12 (6H, each s), 2.14, 2.17 (3H, each s, OCOMe), 3.78 (3H, s, COOMe), 1.81 (3H, br s, α-Me of angeloyl), 1.96 (3H, d, J=7 Hz, β-Me of angeloyl), 6.12 (1H, q, J=7 Hz, β-H of angeloyl). MS m/z (%): 43 (100.0), 55 (83.6), 83 (99.0), 111 (39.2), 153 (34.5), 171 (59.9), 184 (7.2), 205 (9.0), 213 (66.1), 313 (63.5), 331 (2.2), 335 (7.4), 477 (4.7), 537 (1.9), 598 (0.1), 866 (0.1).

Acetylation of **12** and **14** gave the same decaacetate (**16**), colorless needles, mp 168—171 °C. UV λ_{max}^{MeOH} nm: 215.

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IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2870, 1755, 1735, 1720, 1645, 1460, 1438, 1370, 1230, 1155, 1067, 1040, 980, 900, 850, 752. ^{1}C-NMR ($CDCl_3$) δ: 0.92 (6H, s), 0.98, 1.22, 1.28, 1.39 (3H, each s, Me), 1.25 (3H, d, $J=7$ Hz, Me of fucose), 2.03 (6H, s), 2.06 (9H, s), 2.08, 2.10, 2.12, 2.13, 2.14 (3H, each s, –OCOMe), 3.77 (3H, s, COOMe), 1.78 (6H, br s, α-Me of angeloyl), 1.97 (6H, d, $J=7$ Hz, β-Me of angeloyl), 6.10 (2H, q, $J=7$ Hz, β-H of angeloyl). MS m/z (%): 43 (100.0), 55 (80.2), 83 (98.7), 111 (53.6), 153 (38.0), 205 (13.7), 253 (31.9), 353 (39.2), 356 (3.5), 377 (3.5), 391 (3.1), 405 (4.4), 421 (3.3), 435 (4.7), 464 (2.1), 465 (2.3), 477 (6.1), 537 (2.6), 598 (1.3), 775 (0.1), 907 (0.1).

Alkaline Hydrolysis of 5, 11, 12, 13 and 14——A solution of a sample (8 mg) in 5% KOH–MeOH (2 ml) was refluxed for 2 h and neutralized with hydrochloric acid. Removal of the solvents gave a residue, which was purified by chromatography on a column of silica gel (5 g) with $CHCl_3$–MeOH–H_2O (7 : 6 : 1.5) mixture to give compound **17** as a white powder, mp 245—250 °C. IR ν_{max}^{KBr} cm^{-1}: 3200, 2930, 2870, 1610, 1410, 1380, 1170, 1070, 1040, 980, 890, 855, 755. $\nu_{max}^{KBr+Et_3N}$ cm^{-1}: 3400, 2970, 2930, 2870, 2490, 1610, 1443, 1400, 1295, 1255, 1170, 1075, 1040, 900, 855, 805, 760. ^{13}C-NMR data are given in Table I. The four products from the alkaline hydrolyzates of **11**—**14** were all identical.

The alkaline hydrolysis of **5** by the same procedure as described above gave **3**, which was identical with authentic 21-*O*-β-D-fucopyranosylprotoaescigenin.

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