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Studies on the Constituents of Asclepiadaceae Plants. LXVI. The Structures of Three New Glycosides, Cynapanosides A, B, and C, from the Chinese Drug "Xu-Chang-Qing," *Cynanchum paniculatum* KITAGAWA

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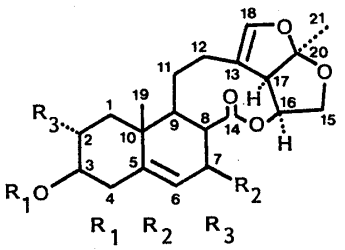
Three new glycosides named cynapanosides A (**1**), B (**2**), and C (**3**) which contain a new aglycone, glaucogenin D (**10**), were isolated from *Cynanchum paniculatum*, in addition to the known compounds, cynatratoside B (**5**) and 3β,14-dihydroxy-14β-pregn-5-en-20-one (**13**). Their structures were elucidated by spectroscopic and chemical methods. The results led us to propose a structure revision of glaucogenin B (**11**).

Keywords——cynapanoside A; cynapanoside B; cynapanoside C; glaucogenin B; glaucogenin D; "Xu-Chang-Qing"; *Cynanchum paniculatum*; Asclepiadaceae

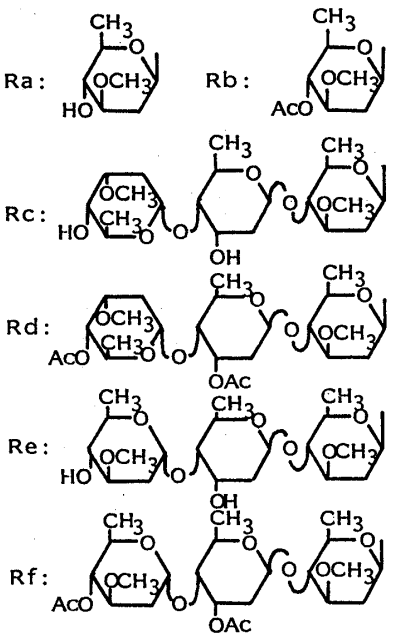
The Chinese crude drug "Xu-Chang-Qing",[1] dried whole plant of *Cynanchum paniculatum* (BUNGE) KITAGAWA (Asclepiadaceae), has been used mainly as an anodyne and for the therapy of chronic tracheitis in China, and is known to contain paeonol.[2] Two other Chinese crude drugs, the roots of "Pai-Ch'ien" (*C. glaucescens*) and "Pai-Wei" (*C. atratum*) which belong to the same genus *Cynanchum*, are closely related to each other, but the therapeutic use of these drugs is different. There is some confusion not only in the therapeutic use but also in the nomenclature between these drugs, and in any case, many different drugs which have the same name exist in different parts of China.[3] The crude drug "Xu-Chang-Qing" is used as a substitute for "Pai-Wei" mainly in the northern part of China.

Our previous studies showed that both "Pai-Ch'ien" and "Pai-Wei" contain glycosides which have a novel 13,14:14,15-disecopregnane-type skeleton as the aglycone.[4,5] In this paper, we wish to report the isolation of three new glycosides with the same skeleton from *C. paniculatum*, in addition to the known compounds, cynatratoside B (**5**) and 3β,14-dihydroxy-14β-pregn-5-en-20-one (**13**).[6] During these structure determinations, it was concluded that the structure of glaucogenin B (**11**)[4a] should be revised to have not a 11α- but a 7β-hydroxyl group. It was found that the new glycosides correspond to the 7β-hydroxy derivatives of cynatratosides A (**4**), B (**5**), and C (**6**) isolated from *C. atratum*,[5] and they were named cynapanosides A (**1**), B (**2**), and C (**3**), respectively. Their common aglycone was named glaucogenin D (**10**).

Cynapanoside A (**1**) has the molecular formula $C_{28}H_{40}O_9$ as determined by high resolution electron impact mass spectrometry (HR-EI-MS), and contains one more oxygen atom than cynatratoside A (**4**).[5] In the EI-MS of **1**, the molecular ion peak at *m/z* 520 and a fragment ion peak at *m/z* 376 ($M^+ - 2$, 6-dideoxy 3-*O*-methylhexose) were observed. On hydrolysis, **1** gave oleandrose and two spots on thin layer chromatography (TLC) which are considered to be derived from the aglycone under the acidic conditions. However, no information on these aglycones could be obtained because of the small amount of the sample



	R_1	R_2	R_3
1	Ra	OH	H
2	Rc	OH	H
3	Re	OH	H
4	Ra	H	H
5	Rc	H	H
6	Re	H	H
7	Rb	OAc	H
8	Rd	OAc	H
9	Rf	OAc	H
10	H	OH	H
11	H	OH	OH
12	Ac	OAc	OAc



available. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** was nearly identical with that of **4** except for the following: (a) the hydroxy methine proton appears as a broad doublet at δ 4.62 (J=9.3 Hz), (b) the multiplicity of the C-6 vinyl proton changed a broad doublet (J=5.4 Hz) in the ^{1}H-NMR spectrum of **4** into a broad singlet in that of **1** at the same chemical shift (δ 5.40). In the two-dimensional proton–proton chemical shift correlation (COSY) spectrum[7] of **1**, cross peaks indicated that the broad doublet signal at δ 4.62 assignable to the C-7 hydroxy methine proton was coupled with the C-4β proton as well as the C-6 vinyl proton and C-8 proton. The small coupling constant between 7-CH and 6-CH (<1 Hz), and the coupling constant between 7-CH and 8-CH (9.3 Hz) indicated that the hydroxyl group at C-7 is β-oriented. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** was nearly identical with that of **4**, except that signals at δ 120.5 and 30.0 in the

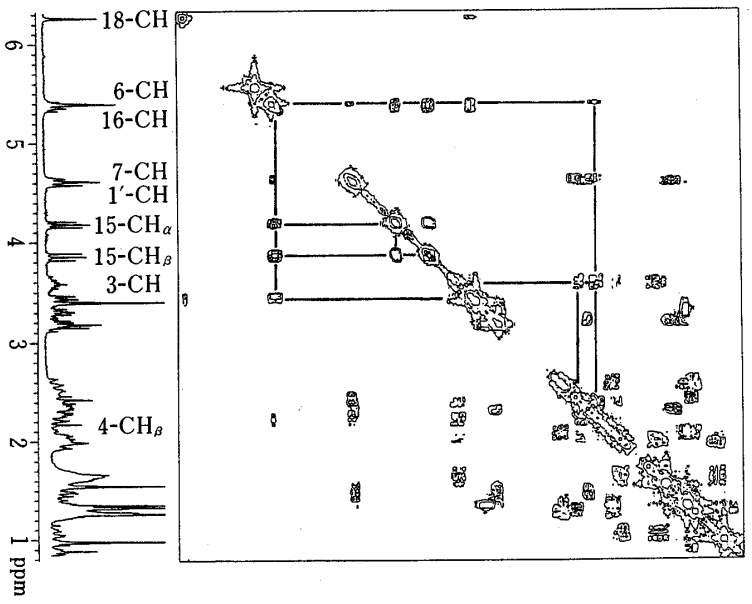


Fig. 1. The COSY Spectrum of Cynapanoside A (**1**)

spectrum of **4** assignable to C-6 and C-7 were shifted to 127.7 and 67.9, respectively in the spectrum of **1**. The signals for the sugar moiety of **1** were identical with those of **4**, and the absolute configuration of oleandrose was assigned tentatively as D-type. Because of the small amount of the sample, however, the optical rotation of the sugar could not be measured. From these data, the structure of **1** is that in which the β-proton at C-7 in the aglycone of **4** is replaced by the hydroxyl group, and this aglycone was named glaucogenin D (**10**). Therefore, **1** was deduced to be glaucogenin D 3-*O*-β-D-oleandropyranoside.

Cynapanoside B (**2**) has the molecular formula $C_{41}H_{62}O_{15}$ as determined by elemental analysis, indicating that **2** has one more oxygen atom than cynatratoside B (**5**).[5] The field desorption (FD)-MS of **2** showed essentially the same fragmentation pattern as that of **5**. On hydrolysis, **2** gave oleandrose, digitoxose, cymarose, and two spots derived from an aglycone similar to **1** on TLC. In the ^{1}H-NMR spectrum of **2**, the signals were nearly identical with those of **1** except for the signals due to the sugar moiety. The signal of the C-7 α proton was observed at δ 4.62 (J=9.8, 1.8 Hz) as a double triplet and the C-6 vinyl proton was observed at δ 5.38 as a triplet (J=1.8 Hz). The anomeric proton signals at δ 4.53 (1H, dd, J=9.8, 1.8 Hz), 4.91 (1H, br d, J=3.6 Hz), and 5.00 (1H, dd, J=9.8, 1.8 Hz) observed in **2** were similar to those of **5**. In the ^{13}C-NMR spectrum of **2**, the signals of the aglycone and the sugar moieties resembled those of **1** and **5**, respectively. Therefore, the aglycone of **2** is the same as that of **1**, and the sugar sequence of **2** is the same as that of **5**. It was suggested that the absolute configurations of the sugars in the sequence of **2** were the same as those of **5** on the basis of the ^{13}C-NMR results. Thus, **2** was deduced to be glaucogenin D 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-oleandropyranoside.

Cynapanoside C (**3**) has the molecular formula $C_{41}H_{62}O_{15}$ as determined by elemental analysis, and contains one more oxygen atom than cynatratoside C (**6**).[5] Hydrolysis of **3** gave oleandrose and digitoxose on TLC, but the spots due to the aglycone could not be observed

TABLE I. ^{13}C-NMR Chemical Shifts for the Aglycone Moieties of **1**—**3** and **5** (δ in C_5D_5N)

	1	2	3	5
C-1	36.3	36.3	36.3	36.6
C-2	30.0	30.1	30.1	30.2
C-3	77.4	77.4	77.4	77.5
C-4	38.8	38.8	38.8	39.2
C-5	141.4	141.4	141.4	140.7
C-6	127.7	127.3	127.3	120.5
C-7	67.9	67.9	67.8	30.0
C-8	51.4	51.3	51.3	53.3
C-9	50.7	50.7	50.7	40.8
C-10	38.8	38.8	38.8	38.7
C-11	23.7	23.7	23.7	24.0
C-12	30.2	30.1	30.1	28.5
C-13	118.6	118.6	118.6	118.5
C-14	174.9	174.9	174.9	175.5
C-15	67.8	67.8	67.8	67.8
C-16	75.8	75.8	75.8	75.6
C-17	56.3	56.3	56.3	56.2
C-18	144.0	144.0	144.0	143.9
C-19	17.8	17.8	17.8	18.0
C-20	114.4	114.4	114.4	114.4
C-21	24.8	24.8	24.8	24.8

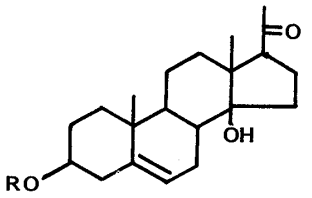
TABLE II. ^{13}C-NMR Chemical Shifts for the Sugar Moieties of **1**—**3** and **5** (δ in C_5D_5N)

	1	2	3	5
C′-1	98.3	98.2	98.2	98.2
C′-2	37.5	37.9	38.0	38.0
C′-3	81.6	79.2	79.2	79.3
C′-4	76.4	83.1	83.2	83.1
C′-5	72.9	71.7	71.7	71.7
C′-6	18.7	18.8	18.8	18.9
3′-OCH_3	56.9	57.4	57.5	57.4
C″-1		98.5	98.5	98.5
C″-2		38.5	38.8	38.5
C″-3		67.9	67.8	67.9
C″-4		80.8	82.3	80.8
C″-5		69.2	69.5	69.2
C″-6		18.4	18.5	18.5
C‴-1		98.4	100.2	98.4
C‴-2		32.2	35.8	32.2
C‴-3		76.6	78.8	76.6
C‴-4		72.8	76.9	72.8
C‴-5		67.1	69.0	67.1
C‴-6		18.5	18.7	18.6
3‴-OCH_3		56.8	57.0	56.7

because of the small amount of the sample available. The FD-MS of **3** gave essentially the same fragmentation pattern as that of **6**. The ^{1}H- and ^{13}C-NMR spectra of **3** suggested that the aglycone of **3** is the same as that of **1**, and the sugar sequence of **3** is the same as that of **6** on the basis of arguments similar to those used in the case of **2**. Thus, **3** was deduced to be glaucogenin D 3-*O*-α-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-oleandropyranoside.

Cynatratoside B (**5**) which has the molecular formula $C_{41}H_{62}O_{14}$ as determined by elemental analysis was identical with an authentic specimen isolated from *C. atratum*[5] on the basis of physical and spectral evidence.

Compound **13** has the molecular formula $C_{21}H_{32}O_3$ as determined by HR-EI-MS, and contains one more oxygen atom than pregnenolone. The infrared (IR) spectrum of **13** showed the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1690 cm^{-1}) groups. In the ^{1}H-NMR spectrum of **13**, the signals of the 18- and 19-methyl protons were superimposed at δ 1.00 as a singlet, the signal of 21-methyl proton was observed at δ 2.25 as a singlet, and the single carbinyl proton signal was observed as a multiplet at δ 3.49 corresponding to the C-3α proton. As the singlet signal at δ 4.44 disappeared on addition of D_2O, the presence of a tertiary hydroxyl was indicated. The physical and spectral properties of **13** were identical with those of the compound isolated from *Isoplexis isabelliana*.[6] The ^{13}C-NMR data of **13** also supported the structure. It is interesting that **13**, which was suggested to be a precursor of the highly oxygenated glaucogenin-type compounds, coexisted with these compounds.



13 : R=H
14 : R=Ac

During this investigation, it was found that the spectral data of the aglycone moiety of cynapanosides are similar to those of glaucogenin B (**11**).[4a] The COSY spectrum of glaucogenin B triacetate (**12**) (Fig. 2) indicated that the double doublet (J=10.3, 2.2 Hz)

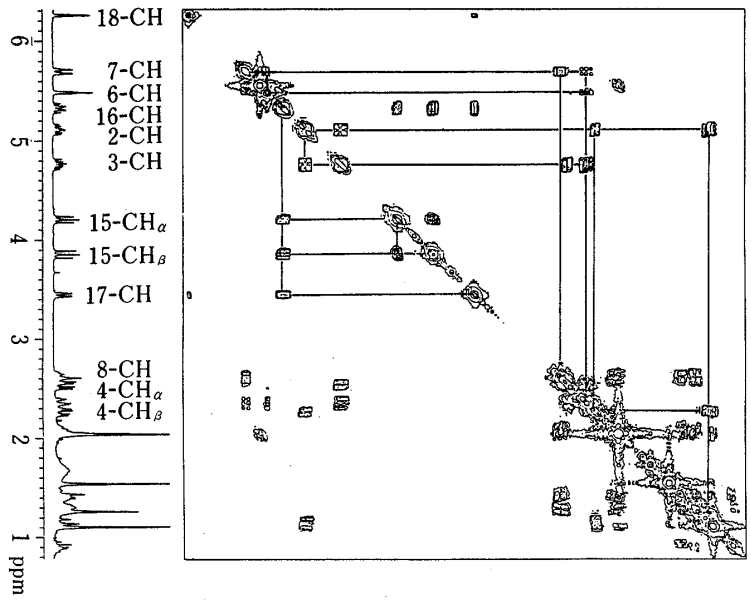


Fig. 2. The COSY Spectrum of Glucogenin B Triacetate (**12**)

signal at δ 5.69 was clearly assignable to the C-7 carbinyl proton. Therefore, the structure of glaucogenin B should be revised to have not a 11α- but a 7β-hydroxyl group.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. IR spectra were recorded on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on a JEOL JNM FX-100 (100 MHz), GX-270 (270 MHz), FX-400 (400 MHz), or GX-500 (500 MHz) spectrometer in $CDCl_3$ or C_5D_5N solution and ^{13}C-NMR spectra on a JEOL FX-90Q (22.5 MHz), FX-200 (50 MHz), or GX-270 (67.5 MHz) spectrometer in $CDCl_3$ or C_5D_5N solution with tetramethylsilane (TMS) as an internal standard. Electron impact mass spectrometry (EI-MS) was done with a JEOL JMS-D-300 machine and field desorption mass spectrometry (FD-MS) with a JEOL JMS-01SG-2 machine. TLC was performed on Merck precoated plates, Kieselgel 60 F_{254} or RP-18 F_{254}. Column chromatography was carried out on Wakogel C-100 (100 mesh), Wakogel C-200 (200 mesh), MCI GEL CHP 20P or RP-18 (Merck).

Extraction from "Xu-Chang-Qing"——The Chinese crude drug "Xu-Chang-Qing," the dried whole plants of *Cynanchum paniculatum* (BUNGE) KITAGAWA, obtained from a market in Taiwan, was powdered (4.6 kg), and extracted with MeOH (25.3 l). The total MeOH extract was filtered and concentrated *in vacuo*. The crude extract (755 g) was dissolved in $CHCl_3$–MeOH (2 : 1). The soluble portion (254 g) was partitioned between petroleum ether and aq. MeOH (MeOH : H_2O = 9 : 1). The lower layer was concentrated (188 g) and dissolved in hexane–benzene (1 : 1); the soluble and insoluble portions were collected (56 and 132 g, respectively).

Isolation of Compounds——The hexane–benzene insoluble portion (127 g) was applied to a column of silica gel (750 g of Wakogel C-100) and the material was eluted stepwise with a mixed solvent of increasing polarity from $CHCl_3$ to MeOH, to give ten fractions. Fraction 3 (14.67 g), eluted with $CHCl_3$–MeOH (95 : 5), was separated repeatedly on a silica gel (Wakogel C-200, 150 g) column with the same solvent, to give nine fractions (fractions A to I). Fraction E (2.35 g), eluted with $CHCl_3$–MeOH (100 : 5), contained **1** and was subjected to rechromatography with $CHCl_3$–MeOH (100 : 3); the product was further rechromatographed with hexane–acetone (3 : 1). The resulting fraction (284.5 mg) was chromatographed on a column of MCI GEL CHP 20P with MeOH–H_2O (9 : 1) to afford a fraction (50.2 mg) which contained mainly **1**. This fraction was further chromatographed on a column of silica gel with hexane–acetone (3 : 1) to yield **1** (10.6 mg). Fraction F (5.54 g), eluted with $CHCl_3$–MeOH (100 : 10), contained **3** and was subjected to rechromatography with $CHCl_3$–MeOH (100 : 3) to give a product (1.21 g), which was further rechromatographed with hexane–acetone (1 : 1). The resulting fraction (204.4 mg) was chromatographed on a column of MCI GEL CHP 20P with MeOH–H_2O (7 : 3) to give **3** (49.6 mg).

The hexane–benzene soluble portion (56 g) was applied to a column of silica gel (Wakogel C-200, 350 g) and eluted stepwise with a mixed solvent of increasing polarity from $CHCl_3$ to 20% MeOH–$CHCl_3$ to give twelve fractions. Fraction 5 (3.87 g), eluted with $CHCl_3$–MeOH (100 : 2), was further purified by silica gel column chromatography, and repeated elution with several solvent systems [hexane–acetone (3 : 1), $CHCl_3$–MeOH (100 : 3), hexane–EtOAc (1 : 1) and hexane–EtOAc (7 : 3)] yielded 55 mg of **13**. Fraction 6 (4.97 g), obtained by further elution with the same solvent system, was also further purified by silica gel column chromatography, and repeated elution with several solvent systems [$CHCl_3$–MeOH (100 : 2), $CHCl_3$–MeOH (100 : 1), hexane–acetone (3 : 1) and hexane–EtOAc (2 : 3)] yielded 55 mg of **5**. Fraction 7 (1.34 g), obtained by further elution with the same solvent system, was also further purified by silica gel column chromatography, and repeated elution with hexane–acetone (3 : 1) and EtOAc gave a fraction (71.5 mg), which was further chromatographed on an RP-18 column with MeOH–H_2O (7 : 3) to yield **2** (26.8 mg).

Cynapanoside A (1)——Amorphous powder, mp 114—117 °C, $[\alpha]_D^{23}$ +21.3° (c = 0.14, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450, 1720, 1650, 1440, 1380, 1300, 1160, 1100, 1070, 985, 890. EI-MS m/z: 520 (M^+), 491, 474, 458, 376 ($M^+ - 144$), 358 (376 − H_2O), 312, 294, 145 (base peak), 137, 113, 87, 43. HR-EI-MS m/z: 520.26649 (M^+, Calcd for $C_{28}H_{40}O_9$ 520.2672). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.98 (3H, s, 19-CH_3), 1.05 (1H, ddd, J = 13.7, 13.7, 3.9 Hz, 1-CH_α), 1.34 (3H, d, J = 6.4 Hz, 6′-CH_3), 1.47 (1H, ddd, J = 13.0, 11.9, 9.8 Hz, 2′-CH_β), 1.54 (3H, s, 21-CH_3), 1.96 (1H, dt, J = 13.7, 3.9 Hz, 1-CH_β), 2.02 (1H, ddddd, J = 13.2, 5.7, 3.9, 3.9, 2.0 Hz, 2-CH_α), 2.07 (1H, ddd, J = 13.2, 13.2, 3.9 Hz, 11-CH_a or -CH_b), 2.20 (1H, ddt, J = 18.6, 11.9, 2.0 Hz, 4-CH_β), 2.31 (1H, ddd, J = 13.0, 4.8, 2.0 Hz, 2′-CH_α), 2.38 (1H, ddd, J = 18.6, 5.7, 2.0 Hz, 4-CH_α), 2.42 (1H, dd, J = 10.4, 9.3 Hz, 8-CH), 2.58 (1H, ddd, J = 13.2, 12.0, 3.9 Hz, 11-CH_a or -CH_b), 3.16 (1H, t, J = 8.7 Hz, 4′-CH), 3.20 (1H, ddd, J = 11.9, 8.7, 4.8 Hz, 3′-CH), 3.31 (1H, dq, J = 8.7, 6.4 Hz, 5′-CH), 3.39 (3H, s, 3′-OCH_3), 3.45 (1H, dd, J = 7.8, 1.0 Hz, 17-CH), 3.58 (1H, tt, J = 11.9, 5.7 Hz, 3-CH), 3.85 (1H, t, J = 9.3 Hz, 15-CH_β), 4.18 (1H, dd, J = 9.3, 7.6 Hz, 15-CH_α), 4.59 (1H, dd, J = 9.8, 2.0 Hz, 1′-CH), 4.62 (1H, br d, J = 9.3 Hz, 7-CH), 5.37 (1H, ddd, J = 9.3, 7.8, 7.6 Hz, 16-CH), 5.39 (1H, br s, 6-CH), 6.26 (1H, br s, 18-CH). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Tables I and II.

Acetylation of 1——**1** (3.7 mg) was dissolved in 1 ml of pyridine, then Ac_2O (0.7 ml) was added, and the mixture was left to stand at room temperature overnight. H_2O (10 ml) was added, and the resulting mixture was extracted with Et_2O (60 ml). The ether layer was washed with 2 N HCl, sat. $NaHCO_3$ aq. and sat. NaCl aq. successively, then dried

over Na_2SO_4. Removal of the solvent followed by silica gel column chromatography (1% MeOH–$CHCl_3$) of the residue gave an amorphous powder, **7** (6.7 mg). mp 75—80 °C, $[\alpha]_D^{24}$ +31.8° (c=0.27, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1370, 1230, 1160. EI-MS m/z: 604 (M^+), 575, 544, 515, 512, 498, 444, 418, 401, 358, 340, 312, 294, 187, 147, 95 (base peak), 43. HR-EI-MS m/z: 604.29131 (M^+, Calcd for $C_{32}H_{44}O_{11}$ 604.28841). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.99 (3H, s, 19-CH_3), 1.20 (3H, d, J=6.2 Hz, 6′-CH_3), 1.53 (3H, s, 21-CH_3), 2.04 and 2.10 (each 3H, s, –O(C=O)CH_3), 2.62 (1H, dd, J=10.3, 9.8 Hz, 8-CH), 3.33 (3H, s, 3′-OCH_3), 3.36 (2H, m, 3′- and 5′-CH), 3.44 (1H, dd, J=7.5, 1.8 Hz, 17-CH), 3.57 (1H, tt, J=11.1, 3.9 Hz, 3-CH), 3.85 (1H, dd, J=9.7, 8.8 Hz, 15-CH_β), 4.20 (1H, dd, J=8.8, 7.0 Hz, 15-CH_α), 4.56 (1H, dd, J=9.5, 1.8 Hz, 1′-CH), 4.66 (1H, t, J=9.5 Hz, 4′-CH), 5.33 (1H, ddd, J=9.7, 7.5, 7.0 Hz, 16-CH), 5.39 (1H, t, J=1.3 Hz, 6-CH), 5.65 (1H, dt, J=10.3, 1.8 Hz, 7-CH), 6.26 (1H, br s, 18-CH).

Acidic Hydrolysis of 1——A solution of 4.0 mg of **1** in 1 ml of MeOH was treated with 2 ml of 0.1 N H_2SO_4 and the mixture was kept at 50 °C for 40 min, then diluted with 2 ml of water and concentrated to half the initial volume. The concentrate was kept at 60 °C for a further 30 min, then neutralized with sat. $Ba(OH)_2$ aq., and the precipitate was filtered off. The filtrate was concentrated and analyzed by TLC with three solvent systems: solvent A, $CHCl_3$–MeOH (9 : 1); solvent B, CH_2Cl_2–EtOH (9 : 1); and solvent C, benzene–acetone (5 : 3). When **1** was hydrolyzed, two spots derived from the aglycone **10** and oleandrose were identified with solvent A; *Rf*, 0.55, 0.47, and 0.43: solvent B; *Rf*, 0.62, 0.55, and 0.44: solvent C; *Rf* 0.37, 0.33, and 0.27, respectively.

Cynapanoside B (2)——Amorphous powder, mp 125—126.5 °C, $[\alpha]_D^{19}$ −39.4° (c=1.37, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{62}O_{15}$: C, 61.94; H, 7.86. Found: C, 61.75; H, 8.13. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1720, 1650, 1450, 1370, 1310, 1150. FD-MS m/z: 795 (M^++H), 794 (M^+), 650 (M^+−144), 520 (650−130), 376 (520−144), 145. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.97 (3H, s, 19-CH_3), 1.25 and 1.26 (each 3H, d, J=5.1, 6.2 Hz, 6″- and 6‴-CH_3), 1.30 (3H, d, J=6.2 Hz, 6′-CH_3), 1.54 (3H, s, 21-CH_3), 3.63 (1H, ddd, J=3.6, 3.6, 3.6 Hz, 3‴-CH), 3.41 and 3.42 (each 3H, s, 3′- and 3‴-OCH_3), 3.50 (1H, m, 3-CH), 3.85 (1H, dd, J=9.3, 8.6 Hz, 15-CH_β), 4.06 (1H, ddd, J=3.4, 3.4, 3.4 Hz, 3″-CH), 4.12 (1H, dd, J=8.6, 7.3 Hz, 15-CH_α), 4.53 (1H, dd, J=9.8, 1.8 Hz, 1′-CH), 4.62 (1H, dt, J=9.8, 1.8 Hz, 7-CH), 4.91 (1H, br d, J=3.6 Hz, 1‴-CH), 5.00 (1H, dd, J=9.8, 1.8 Hz, 1″-CH), 5.37 (1H, dt, J=9.3, 7.3 Hz, 16-CH), 5.38 (1H, t, J=1.8 Hz, 6-CH), 6.25 (1H, br s, 18-CH). ^{13}C-NMR (67.5 MHz, C_5D_5N): see Tables I and II.

Acetylation of 2——**2** (5.3 mg) was dissolved in 1 ml of pyridine, then Ac_2O (0.6 ml) and *N*,*N*-dimethylaminopyridine (DMAP) (a catalyst) were added, and the mixture was allowed to stand at 30 °C for 2 d. Usual work-up and purification by silica gel column chromatography (1% MeOH–$CHCl_3$) gave an amorphous powder, **8** (2.5 mg). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.98 (3H, s, 19-CH_3), 1.13 and 1.27 (3H and 6H, d, J=6.8, 4.3 Hz, 6′-, 6″- and 6‴-CH_3), 1.53 (3H, s, 21-CH_3), 2.03, 2.10 and 2.13 (each 3H, s, –OC(=O)CH_3), 3.13 (1H, t, J=9.0 Hz, 4-CH of sugar moiety), 3.34 and 3.41 (each 3H, s, 3′- and 3‴-OCH_3), 3.56 (1H, m, 3-CH), 3.69 (1H, m, 3-CH of sugar moiety), 3.85 (1H, dd, J=10.0, 8.8 Hz, 15-CH_β), 3.91 (1H, m, 5-CH of sugar moiety), 4.20 (2H, m, 5-CH of sugar moiety and 15-CH_α), 4.50 (1H, dd, J=9.7, 1.4 Hz, 1′-CH), 4.60 (1H, dd, J=9.3, 3.2 Hz, 4‴-CH), 4.83 (1H, br d, J=4.1 Hz, 1‴-CH), 4.92 (1H, dd, J=10.0, 1.8 Hz, 1″-CH), 5.31 (2H, m, 3″-CH and 16-CH), 5.38 (1H, t, J=1.8 Hz, 6-CH), 5.65 (1H, dt, J=10.2, 1.8 Hz, 7-CH), 6.26 (1H, br s, 18-CH).

Acidic Hydrolysis of 2——A solution of **2** (1.2 mg) in 1 ml of MeOH was treated with 2 ml of 0.1 N H_2SO_4 and the mixture was kept at 40 °C for 40 min, then treated as described for **1**. The products were analyzed by TLC with solvents A, B, and C in comparison with authentic samples. The *Rf* values of oleandrose, digitoxose, cymarose, and the two spots derived from aglycone were 0.44, 0.26, 0.49, 0.62, and 0.51 with solvent A, 0.45, 0.29, 0.54, 0.67, and 0.61 with solvent B, and 0.32, 0.15, 0.36, 0.48, and 0.42 with solvent C, respectively.

Cynapanoside C (3)——Amorphous powder, mp 136—138 °C, $[\alpha]_D^{30}$ −11.2° (c=0.32, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{62}O_{15}$: C, 61.94; H, 7.86. Found: C, 61.92; H, 8.12. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3450, 1720, 1655, 1450, 1370, 1300, 980, 890. FD-MS m/z: 795 (M^++H), 794 (M^+), 650 (M^+−144), 521 (650−130+H), 376 (521−144−H), 145 (base peak). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.97 (3H, s, 19-CH_3), 1.26, 1.29, and 1.30 (each 3H, d, J=6.4, 6.4, 5.9 Hz, 6′-, 6″- and 6‴-CH_3), 1.54 (3H, s, 21-CH_3), 2.42 (1H, dd, J=11.2, 8.8 Hz, 8-CH), 3.17 and 3.19 (each 1H, t, J=8.8 Hz, 4′- and 4‴-CH), 3.29 (1H, dd, J=9.3, 2.9 Hz, 4″-CH), 3.29 (1H, m, 5-CH of sugar moiety), 3.37 (1H, ddd, J=11.7, 8.8, 5.4 Hz, 3-CH of sugar moiety), 3.406 and 3.408 (each 3H, s, 3′- and 3‴-OCH_3), 3.45 (1H, br d, J=7.3 Hz, 17-CH), 3.55 (1H, tt, J=11.2, 4.4 Hz, 3-CH), 3.69 (1H, dq, J=8.8, 6.4 Hz, 5-CH of sugar moiety), 3.85 (1H, dd, J=9.4, 8.9 Hz, 15-CH_β), 3.85 (1H, m, 5-CH of sugar moiety), 4.11 (1H, ddd, J=3.5, 3.1, 3.1 Hz, 3″-CH), 4.18 (1H, dd, J=8.8, 7.3 Hz, 15-CH_α), 4.53 (1H, dd, J=9.8, 1.5 Hz, 1′-CH), 4.62 (1H, br d, J=8.8 Hz, 7-CH), 5.03 (1H, br d, J=4.4 Hz, 1‴-CH), 5.03 (1H, dd, J=9.3, 2.4 Hz, 1″-CH), 5.36 (1H, dt, J=9.4, 7.3 Hz, 16-CH), 5.38 (1H, br s, 6-CH), 6.25 (1H, br s, 18-CH). ^{13}C-NMR (50 MHz, C_5D_5N): see Tables I and II.

Acetylation of 3——**3** (7.3 mg) was dissolved in 1 ml of pyridine, then Ac_2O (0.7 ml) and DMAP (a catalyst) were added, and the mixture was allowed to stand at 28 °C for 1 d. Usual work-up and purification by silica gel column chromatography (1% MeOH–$CHCl_3$) gave an amorphous powder, **9** (6.1 mg). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.98 (3H, s, 19-CH_3), 1.11, 1.27 and 1.29 (each 3H, d, J=6.2, 5.9, and 6.2 Hz, 6′-, 6″- and 6‴-CH_3), 1.53 (3H, s, 21-CH_3), 2.05, 2.10 and 2.15 (each 3H, s, –OC(=O)CH_3), 3.14 (1H, t, J=8.4 Hz, 4-CH of sugar moiety), 3.33 and 3.41 (each 3H, s, 3′- and 3‴-OCH_3), 3.72 (1H, m, 5-CH of sugar moiety), 3.85 (1H, dd, J=9.8, 8.9 Hz, 15-CH_β), 3.90 (1H, m, 5-CH of sugar moiety), 4.20 (1H, dd, J=8.9, 6.9 Hz, 15-CH_α), 4.51 (1H, dd, J=9.6, 1.7 Hz, 1′-CH), 4.63 (1H, t, J=9.2 Hz, 4‴-CH), 4.93 (1H, dd, J=10.1, 1.8 Hz, 1″-CH), 4.96 (1H, br d, J=3.8 Hz, 1‴-CH), 5.29 (1H, m, 3″-CH),

5.32 (1H, m, 16-CH), 5.38 (1H, t, J=1.7 Hz, 6-CH), 5.65 (1H, dt, J=10.4, 1.7 Hz, 7-CH), 6.26 (1H, br s, 18-CH).

Acidic Hydrolysis of 3——A solution of **3** (1.3 mg) in 1 ml of MeOH was treated with 2 ml of 0.1 N H_2SO_4 and the mixture was kept at 60 °C for 30 min, then treated as described for **1**. The products were analyzed by TLC with solvents A, B, and C in comparison with authentic samples. The *Rf* values of oleandrose and digitoxose were in the order of 0.33 and 0.18 with solvent A, 0.41 and 0.27 with solvent B, and 0.25 and 0.18 with solvent C, respectively.

Cynatratoside B (5)——Amorphous powder, mp 104—106 °C, $[\alpha]_D^{24}$ −46.6° (c=0.24, $CHCl_3$), $[\alpha]_D^{26}$ −37.0° (c=0.45, MeOH). *Anal.* Calcd for $C_{41}H_{62}O_{14}\cdot 1/2H_2O$: C, 62.50; H, 8.06. Found: C, 62.36; H, 8.15. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1720, 1650, 1450, 1370, 1310, 1150. FD-MS m/z: 779 (M^++H), 778 (M^+), 634 (M^+−144), 504 (634−130), 360 (504−144), 145 (base peak). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.92 (3H, s, 19-CH_3), 1.25 and 1.27 (each 3H, d, J=5.9, 4.4 Hz, 6″- and 6‴-CH_3), 1.30 (3H, d, J=6.4 Hz, 6′-CH_3), 1.53 (3H, s, 21-CH_3), 3.19 (1H, t, J=8.8 Hz, 4′-CH), 3.25 (1H, dd, J=9.3, 2.9 Hz, 4″-CH), 3.29 (1H, dq, J=9.3, 6.4 Hz, 5-CH of sugar moiety), 3.37 (1H, ddd, J=11.2, 8.3, 4.9 Hz, 3′-CH), 3.40 and 3.42 (each 3H, s, 3′- and 3‴-OCH_3), 3.54 (1H, tt, J=10.7, 4.4 Hz, 3-CH), 3.63 (1H, ddd, J=3.4, 3.4, 3.4 Hz, 3‴-CH), 3.77 (1H, dq, J=9.3, 6.4 Hz, 5-CH of sugar moiety), 3.84 (1H, dq, J=9.0, 5.9 Hz, 5-CH of sugar moiety), 3.85 (1H, dd, J=9.8, 8.8 Hz, 15-CH_β), 4.06 (1H, ddd, J=3.4, 3.4, 3.4 Hz, 3″-CH), 4.16 (1H, dd, J=8.8, 6.8 Hz, 15-CH_α), 4.54 (1H, dd, J=9.8, 2.0 Hz, 1′-CH), 4.91 (1H, br d, J=3.9 Hz, 1‴-CH), 5.00 (1H, dd, J=9.8, 2.0 Hz, 1″-CH), 5.30 (1H, dt, J=9.8, 6.8 Hz, 16-CH), 5.39 (1H, br d, J=5.4 Hz, 6-CH), 6.25 (1H, br s, 18-CH). ^{13}C-NMR (50 MHz, C_5D_5N): see Tables I and II.

Acidic Hydrolysis of 5——**5** (1.3 mg) was subjected to acidic hydrolysis in the same manner as described for **3**. The products were analyzed by TLC with solvents A, B, and C in comparison with authentic samples. The *Rf* values of oleandrose, digitoxose and cymarose were in the order of 0.33, 0.18 and 0.42 with solvent A, 0.41, 0.27 and 0.50 with solvent B, and 0.25, 0.18 and 0.34 with solvent C, respectively.

Glaucogenin B 2α,3β,7β-*O*-triacetate (12)——^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.10 (3H, s, 19-CH_3), 1.13 (1H, t, J=12.5 Hz, 1-CH_α), 1.53 (3H, s, 21-CH_3), 2.032 and 2.035 (6H and 3H, each s, −OC(=O)CH_3), 2.25 (1H, dd, J=12.5, 4.9 Hz, 1-CH_β), 2.34 (1H, ddt, J=13.7, 12.0, 2.2 Hz, 4-CH_β), 2.53 (1H, dd, J=13.7, 5.4 Hz, 4-CH_α), 2.60 (1H, t, J=10.3 Hz, 8-CH), 3.44 (1H, br d, J=8.3 Hz, 17-CH), 3.85 (1H, dd, J=9.1, 8.8 Hz, 15-CH_β), 4.20 (1H, dd, J=9.1, 7.3 Hz, 15-CH_α), 4.76 (1H, ddd, J=12.0, 9.8, 5.4 Hz, 3-CH), 5.11 (1H, ddd, J=12.5, 9.8, 4.9 Hz, 2-CH), 5.32 (1H, ddd, J=8.8, 8.3, 7.3 Hz, 16-CH), 5.48 (1H, t, J=2.2 Hz, 6-CH), 5.69 (1H, dt, J=10.3, 2.2 Hz, 7-CH), 6.26 (1H, br s, 18-CH). ^{1}H-NMR (270 MHz, C_5D_5N) δ: 1.04 (3H, s, 19-CH_3), 1.19 (1H, t, J=12.1 Hz, 1-CH_α), 1.54 (3H, s, 21-CH_3), 2.00, 2.07 and 2.11 (each 3H, s, −OC(=O)CH_3), 2.37 (1H, dd, J=12.1, 4.8 Hz, 1-CH_β), 2.48 (1H, ddt, J=13.8, 11.8, 1.8 Hz, 4-CH_β), 2.64 (1H, dd, J=13.8, 5.7 Hz, 4-CH_α), 2.87 (1H, t, J=10.3 Hz, 8-CH), 3.57 (1H, br d, J=8.1 Hz, 17-CH), 3.93 (1H, dd, J=9.7, 8.4 Hz, 15-CH_β), 4.15 (1H, dd, J=8.4, 7.0 Hz, 15-CH_α), 5.08 (1H, ddd, J=9.7, 8.1, 7.0 Hz, 16-CH), 5.45 (1H, ddd, J=12.1, 10.0, 4.8 Hz, 2-CH), 5.51 (1H, ddd, J=11.8, 10.0, 5.7 Hz, 3-CH), 5.63 (1H, t, J=1.8 Hz, 6-CH), 6.09 (1H, dt, J=10.7, 1.8 Hz, 7-CH), 6.57 (1H, br s, 18-CH).

3β,14-Dihydroxy-14β-pregn-5-en-20-one (13)——Colorless needles (EtOAc), mp 215—217.5 °C (lit. mp 190—210 °C[6]), $[\alpha]_D^{20}$ +27.7° (c=0.47, MeOH) (lit. $[\alpha]_D^{23}$ +12.3° (MeOH)[6]). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1690 (C=O). EI-MS m/z: 332 (M^+), 314 (M^+-H_2O), 304, 296, 281, 271 (314−43), 253, 228, 43 (base peak). HR-EI-MS m/z: 332.23381 (M^+, Calcd for $C_{21}H_{32}O_3$ 332.23501). ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.00 (6H, s, 18- and 19-CH_3), 2.25 (3H, s, 21-CH_3), 2.95 (1H, t, J=5.6 Hz, 17-CH), 3.49 (1H, m, 3-CH), 4.44 (1H, s, 14-COH, quenched by addition of D_2O), 5.43 (1H, m, 6-CH). ^{13}C-NMR (22.5 MHz, C_5D_5N) δ: 37.9 (C-1), 32.4 (C-2), 71.2 (C-3), 43.3 (C-4), 140.6 (C-5), 121.9 (C-6), 27.8 (C-7), 37.2 (C-8), 46.4 (C-9), 37.4 (C-10), 21.1 (C-11), 38.8 (C-12), 49.4 (C-13), 85.1 (C-14), 34.6 (C-15), 24.5 (C-16), 63.2 (C-17), 15.5 (C-18), 19.7 (C-19), 216.5 (C-20), 32.6 (C-21). CD (c=6.6×10^{-4}, MeOH) $\Delta\varepsilon$ (nm): +0.55 (295) (pos. max.).

Acetylation of 13——Acetylation of 8.7 mg of **13** with Ac_2O–pyridine for 1 d at room temperature followed by the usual work-up and purification by column chromatography on silica gel (0.5% MeOH–$CHCl_3$) gave an acetate, **14** (8.1 mg). Fine needles (hexane–EtOAc), mp 184—187 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1710 (C=O), 1690 (C=O). EI-MS m/z: 356 (M^+-H_2O), 314 (M^+−60), 304, 296 (314−H_2O), 281, 253, 191, 158, 145, 43 (base peak). ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.00 (6H, s, 18- and 19-CH_3), 2.03 (3H, s, −OC(=O)CH_3), 2.25 (3H, s, 21-CH_3), 2.96 (1H, t, J=5.6 Hz, 17-CH), 4.44 (1H, s, 14-COH), 4.60 (1H, m, 3-CH), 5.42 (1H, m, 6-CH).

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