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Strophanthidin Glycosides from the Roots of *Apocynum venetum* var. *basikurumon* (Studies on *Apocynum.* II)[1]

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Five new strophanthidin glycosides, strophanthidin glucos-3-ulosyl-cymaroside, glucosyl-digitalosyl-cymaroside, cellobiosyl-cymaroside, digitaloside, and glucosyl-digitaloside, were obtained from the roots of *Apocynum venetum* L. var. *basikurumon* HARA, along with free strophanthidin and the common glycosides in *Apocynum* species, cymarin and k-strophanthidin-β. Strophanthidin glucoside was also obtained.

Keywords——Apocynaceae; *Apocynum*; cardenolide; strophanthidin glycoside; glucos-3-ulosyl-cymarin; glucosyl-digitalosyl-cymarin; cellobiosyl-cymarin; basikuloside; apobasinoside; cellostrophanthoside

In the preceding paper of this series,[1] we described the isolation of pregnanes and pregnane glycosides, including neridienone A,[2,3] 6,7-didehydrocortexone and bisdesmosidic glycosides of teikagenin,[4] from the roots of *Apocynum venetum* L. var. *basikurumon* HARA. Isolation of cardiac glycosides from the roots was attempted by Imai and Ikeda, but identification of the glycosides was unsuccessful.[5] This paper deals with five new strophanthidin glycosides and three known glycosides.

When the methanol percolate was partitioned with benzene, $CHCl_3$ and BuOH, pregnanes were obtained from the benzene and $CHCl_3$ fractions.[1] Eight cardiac glycosides were obtained along with strophanthidin from the benzene, $CHCl_3$ and BuOH layers, and tentatively designated as compounds **1**—**8**. Among them, **8** was the main glycoside, and **7** and **8** were identified as cymarin and k-strophanthin-β, respectively.

Compound **1** showed less polar behavior than **8** on thin layer chromatography (TLC), and was isolated as a solid in a small amount. The fast atom bombardment (FAB)-mass spectrum (MS) afforded a molecular peak at *m/z* 731.327 $(M+Na)^+$, suggesting the molecular formula to be $C_{36}H_{52}O_{14}$, 2H smaller than **8**. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, a characteristic signal due to the formyl proton at C-19 (δ 10.42) of strophanthidin, as well as those due to C-18 methyl protons, C-21 methylene protons and the C-22 olefinic proton, was observed besides two anomeric proton signals (δ 5.14, dd, $J=10$, 2 Hz; δ 4.98, d, $J=8$ Hz) and one methoxyl proton signal (δ 3.51) from the sugar moiety. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, signals of two anomeric carbons were observed at δ 97.6 and δ 107.3, and one of the component sugars was identified as D-cymarose based on the signals at δ 97.6, 36.4, 77.6, 83.3, 69.3 and 18.5 (C-1—C-6), and one methoxyl carbon (δ 58.5), as well as the coupling constants of the corresponding protons in the ^{1}H-NMR spectrum. The remaining six signals including one carbonyl carbon at δ 207.5 were identical with those of the glucos-3-uloside which was

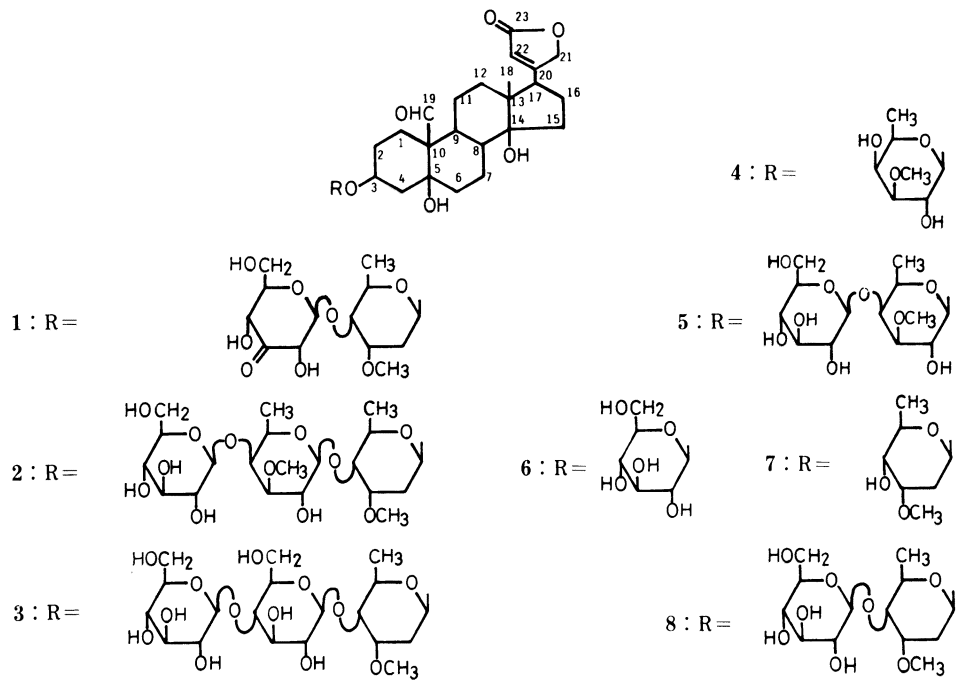

Chart 1

recently isolated from the air-dried leaves of *Cerbera manghas* as 17α-digitoxigenin β-D-glucos-3-ulosyl-α-L-thevetoside.[6] Whereas couplings between H-1″/H-2″, H-4″/H-5″ and H-5″/H-6″ are present, no cross peak was observed between H-2″/H-3″, and H-3″/H-4″ in the ^{1}H–^{1}H COSY spectrum.

In order to confirm the structure, $NaBH_4$ reduction of **1** was carried out and one of the two products was identified as strophanthidol β-D-glucosyl-β-D-cymaroside by comparison with an authentic sample on TLC and high-performance liquid chromatography (HPLC). The other product was considered to be strophanthidol β-D-allosyl-β-D-cymaroside from a comparison of the ^{1}H-NMR spectrum with that of 17α-digitoxigenin β-D-allopyranosyl-α-L-thevetoside.[6] Compound **1** was therefore determined to be strophanthidin 3-*O*-β-D-glucos-3-ulosyl-β-D-cymaroside, and is named basikuloside.

In the ^{1}H-NMR spectrum of **2**, three anomeric proton signals, two methoxyl proton signals and two secondary methyl signals that originated from the 6-methyl groups of 6-deoxyhexoses were observed, besides signals assignable to strophanthidin. One of the three anomeric proton signals at δ 5.12 was observed as a doublet of doublets, and two as doublets, suggesting that the sugar moiety is composed of 1 mol each of a 2,6-dideoxy-3-*O*-methylhexose, a 6-deoxy-3-*O*-methylhexose and a hexose. In the ^{13}C-NMR spectrum, three sugars were identified as cymarose, digitalose and glucose, of which C-4 of cymarose and digitalose were each shifted downfield. Since the fragment peaks in the negative FAB-MS were observed at *m/z* 707 $(M-\text{glucose}-1)^-$ and *m/z* 547 $(M-\text{glucose}-\text{digitalose}-1)^-$, along with those at *m/z* 869 $(M-1)^-$, 407 and 385, the glucose is assigned as the terminal of the sugar chain. Based on the coupling constants in the ^{1}H-NMR spectrum, all the glycosidic linkages were assigned as β, so that the three component sugars are tentatively regarded as D-form. Compound **2** was determined to be strophanthidin β-D-glucosyl-(1→4)-β-D-digitaloside, and is named apobasinoside.

Compound **3** was suggested to be a strophanthidin trioside, based on the ^{13}C-NMR

TABLE I. ^{1}H Chemical Shifts of **1**—**6** and **8**, δ (ppm) from Tetramethylsilane in Pyridine-d_5 (J/Hz in Parentheses)

H	8	1	2	3	4	5	6
H-22	6.13 (br s)	6.13 (br s)	6.12 (br s)	6.13 (br s)	6.13 (br s)	6.13 (br s)	6.12 (br s)
H-21	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)	5.28 (dd, 18, 1) 5.02 (dd, 18, 2)
H-19	10.41	10.42	10.41	10.41	10.38	10.38	10.41
H-18	1.01	1.01	1.01	1.01	1.00	1.01	1.01
H-3α	4.28 (br s)	4.30 (br s)			4.53 (br s)	4.48 (br s)	4.54 (br s)
H-1′	5.11 (dd, 9, 2)	5.14 (dd, 10, 2)	5.12 (dd, 9, 1)	5.12 (dd, 10, 1)	4.98 (d, 8)	4.80 (d, 8)	5.06 (d, 8)
H-2′					4.33 (dd, 8, 9)	4.41 (dd, 8, 9)	4.00 (t, 9) 4.22 (t, 9) 4.24 (t, 9)
H-3′	4.07 (q, 3)	4.09 (q, 3)			3.47 (dd, 9, 3)	3.50 (dd, 9, 3)	
H-4′	3.65 (dd, 9, 3)	3.68 (dd, 9, 3)			4.08 (d, 3)	4.34 (d, 3)	
H-5′	4.20 (m)				3.77 (m)	3.73 (m)	3.90 (m)
H-6′	1.61 (d, 6)	1.59 (d, 6)	1.59 (6H) (d, 6, H-6′′)	1.60 (d, 7)	1.57 (d, 6)	1.56 (d, 6)	4.56 (dd, 12, 2) 4.38 (dd, 12, 5)
3′-OMe	3.47	3.51	3.37	3.46	3.53	3.61	
Others	4.94 (d, 8, H-1′′) 3.99, 4.17 4.23 (t, 9, H-2′′,3′′,4′′) 4.57 (dd, 11, 2, H-6′′a) 4.38 (dd, 11, 5, H-6′′b)	4.98 (d, 8, H-1′′) 4.72 (dd, 8, 1, H-2′′) 4.92 (dd, 10, 1, H-4′′) 4.55 (dd, 11, 2, H-6′′a) 4.45 (dd, 11, 4, H-6′′b)	4.67, 5.08 (d, 8, H-1′′,1′′′) 3.66 (3′′-OMe)	4.88, 5.18 (d, 8, H-1′′,1′′′)		5.15 (d, 8, H-1′′) 3.97, 4.17 4.24 (t, 9, H-2′′,3′′,4′′)	

evidence and the $(M-1)^-$ peak at m/z 871 in the negative FAB-MS. Upon partial hydrolysis with cellulase, **3** afforded **8**. In the ^{13}C-NMR spectrum, the presence of **7** in the structure was also assignable, with a downfield shift of C-4 in the cymarose, while the peaks due to the hexobiosyl moiety were identical with those of β-cellobioside.[7] Compound **3** was therefore determined to be β-cellobiosyl-cymarin, and is named cellostrophanthoside.

Compounds **4**, **5** and **6** were identified as strophanthidin β-D-digitaloside, β-D-glucosyl(1→4)-β-D-digitaloside and β-D-glucoside, respectively, based on the FAB-MS, and a comparison of the ^{1}H-NMR and ^{13}C-NMR spectra with those of known glycosides having the corresponding sugar moieties.[8]

Previously we described the conversion of glucosyl-thevetoside into glucos-3-ulosyl-thevetoside during the air-drying of the leaves of *Cerbera* species.[6] This is the second example of the isolation of a cardenolide glycoside having a glucos-3-ulosyl moiety. Whilst a sugar

TABLE II. ^{13}C Chemical Shifts of **8** and Sugar Moieties of **1**—**6**, from Tetramethylsilane in Pyridine-d_5

C	8	C	8	1	2	3	4	5	6
1	24.7	1′	97.6	97.6	97.6	97.6	101.5	101.3	101.3
2	25.5	2′	36.5[b]	36.4	36.4	36.4	70.3	70.8[a]	75.0
3	75.1[a]	3′	77.9	77.6	77.6	77.8	85.0	85.6	78.8[a]
4	36.6[b]	4′	82.8	83.3	82.9	82.9	68.3	72.2	71.7
5	74.0	5′	69.5	69.3	69.5	69.4	71.5	70.7[a]	78.7[a]
6	36.9	6′	18.5	18.5	18.6	18.5	17.2	17.5	62.7
7	18.5	1″	106.4	107.3	105.6[a]	106.0		105.1	
8	41.9	2″	75.3[a]	78.6[a]	71.3[b]	74.9[a]		75.9	
9	39.6	3″	78.3	207.5	85.2	76.5[b]		78.5[b]	
10	55.3	4″	71.9	73.8	72.7	81.4		71.9	
11	22.6	5″	78.3	78.5[a]	70.6[b]	76.3[b]		78.3[b]	
12	39.6	6″	63.0	62.4	17.6	62.4		63.1	
13	49.8	1‴			106.4[a]	104.9			
14	84.8	2‴			75.9	75.1[a]			
15	32.1	3‴			78.4[c]	78.4[c]			
16	27.2	4‴			71.8	71.5			
17	51.1	5‴			78.3[c]	78.2[c]			
18	16.0	6‴			63.1	62.4			
19	208.5	-OMe	58.6	58.5	58.3	58.6	56.9	58.6	
20	175.5				59.0				
21	73.6								
22	117.8								
23	174.3								

a—*c*) Signal assignments marked *a*), *b*) or *c*) in each column may be reversed.

moiety composed of two different deoxyhexoses is common in pregnane glycosides, this is the first report of the occurrence of the cardiac glycoside having a 6-deoxy-3-*O*-methylhexose and a 2,6-dideoxy-3-*O*-methylhexose. Digitaloside and glucosyl-digitaloside of strophanthidin have not been reported previously, and strophanthidin glucoside is also rare as a natural product.[9] It should be noted that **3** but not k-strophanthoside was found in this plant.

Experimental

Melting points, optical rotations, ^1H- and ^{13}C-NMR spectra and MS were obtained as described in the preceding paper.[1] Column chromatography and TLC were carried out with the following solvent systems: solvent 1, $CHCl_3$–MeOH–H_2O (bottom layer); solvent 2, EtOAc–MeOH–H_2O (top layer). Spots on TLC plate were detected by spraying a) a 1 : 1 mixture of 2% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH (Kedde's reagent) and b) 10% H_2SO_4. When b) was applied, the plate was heated until the spots appeared. HPLC was run with a Waters ALC 200 equipped with a Radial Pack C_{18} column.

Extraction and Isolation——The cardiac glycosides were isolated from the air-dried roots of *Apocynum venetum* L. var. *basikurumon* HARA (2.9 kg), the same plant material used for the isolation of the pregnane glycosides.[1]

From the benzene- and $CHCl_3$-soluble layers (extract: 3 g and 12.7 g, respectively), strophanthidin and **7** were obtained after successive column chromatographies on silica gel columns with solvent 1 (7 : 2 : 2—7 : 2 : 1) and solvent 2 (6 : 1 : 5).

The BuOH-soluble fraction (extract: 130 g) of the MeOH percolate was passed through an MCI-gel column (CHP-20P, Mitsubishi Chem. Ind., Co.) and the column was eluted with MeOH–H_2O, increasing the ratio of MeOH from 0% to 80%. The eluates with 60%—80% MeOH were then chromatographed on silica gel columns with solvent 1 (7 : 2 : 1—7 : 3 : 1.6) and solvent 2 (6 : 1 : 5—4 : 1 : 3), and the fractions containing cardiac glycosides were chromatographed on an ODS column with 20—25% CH_3CN. Each cardiac glycoside was finally isolated by HPLC (20—25% CH_3CN).

Basikuloside (1)——A solid (10 mg), $[\alpha]_D^{26}$ +12.8° (c=0.50, MeOH), FAB-MS m/z: 731.327 (Calcd for $C_{36}H_{52}O_{14}Na$: 731.326). $NaBH_4$ (5 mg) was added to a solution of **1** (5 mg) in EtOH (1 ml), and the mixture was

stirred at room temperature for 30 min. The mixture was diluted with H_2O and extracted with BuOH. The BuOH extract was subjected to HPLC (23% CH_3CN, 0.8 ml/min) and two products were separated (**1a**, 1 mg; **1b**, 1.5 mg). Compound **1a**, FAB-MS *m/z*: 735.356, Calcd for $C_{36}H_{56}O_{14}Na$: 735.357), showed the same retention time on HPLC (23% CH_3CN, 0.7 ml/min, t_R 14.2 min) and the same *Rf* value on TLC (solvent 1, 7:3:1) as strophanthidol β-D-glucosyl-β-D-cymaroside prepared from **8** by $NaBH_4$ reduction.

Compound **1b**: FAB-MS *m/z*: 735.356 (Calcd for $C_{36}H_{56}O_{14}Na$: 735.357), HPLC (23% CH_3CN, 0.7 ml/min, t_R 15.2 min). ^{1}H-NMR δ: 6.12 (1H, br s, H-22), 5.35 (1H, d, J=9 Hz, H-1″), 5.34, 4.90 (1H each, s, –OH), 5.29, 5.02 (1H each, dd, J=18, 1 Hz, H-21), 5.15 (1H, dd, J=10, 1 Hz, H-1′), 4.67 (1H, t, J=3 Hz, H-3′), 4.44 (1H, m, H-5″), 4.36, 4.03 (1H each, d, J=11 Hz, H-19), 4.30 (1H, br s, H-3α), 4.25 (1H, m, H-5″), 4.14 (1H, dd, J=10, 2 Hz, H-4″), 4.10 (1H, q, J=3 Hz, H-3′), 3.92 (1H, dd, J=9, 2 Hz, H-2″), 3.61 (1H, dd, J=9, 3 Hz, H-4′), 3.52 (3H, s, 3′-OMe), 1.55 (3H, d, J=6 Hz, H-6′), 1.04 (3H, s, H-18).

Apobasinoside (2)——A solid (10 mg), $[\alpha]_D^{23}$ +23.1° (*c*=0.065, MeOH), negative FAB-MS *m/z*: 869 $(M-1)^-$, 707, 547, 407, 385.

Cellostrophanthoside (3)——A solid (16 mg), $[\alpha]_D^{26}$ +15.6° (*c*=0.50, MeOH), negative FAB-MS *m/z*: 871 $(M-1)^-$, 709, 675, 657, 423, 385. A solution of **3** (5 mg) in 25% EtOH was treated with 5 mg of cellulase (Sigma Chem. Co.), and the mixture was shaken at 38 °C for 30 min. The mixture was then extracted with BuOH and the BuOH extract was examined by TLC (solvent 1, 7:3:1 and solvent 2, 4:1:0.5) and HPLC (25% CH_3CN, 1 ml/min, t_R 10.6 min), revealing **8**.

Strophanthidin β-D-Digitaloside (4)——A solid (10 mg), $[\alpha]_D^{22}$ +5.21° (*c*=0.23, MeOH), FAB-MS *m/z*: 587.282 (Calcd for $C_{30}H_{44}O_{10}Na$: 587.283).

Strophanthidin β-D-Glucosyl-(1→4)-β-D-digitaloside (5)——A solid (10 mg), $[\alpha]_D^{26}$ +14.3° (*c*=0.30, MeOH), FAB-MS *m/z*: 749.337 (Calcd for $C_{36}H_{54}O_{15}Na$: 749.336), negative FAB-MS *m/z*: 725 $(M-1)^-$, 563, 403, 385.

Known Glycosides——Cymarin (**7**) (solid, 33 mg, $[\alpha]_D^{26}$ +61.1° (*c*=1.52, MeOH)) and k-strophanthin-β (**8**) (790 mg, mp 235—239 °C, $[\alpha]_D^{26}$ +48.1° (*c*=1.58, MeOH)) were identified by comparison with authentic samples (TLC, and ^{1}H- and ^{13}C-NMR spectra).

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