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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, cymaroside and k-strophanthidin- β . Strophanthidin glucoside was also obtained.

Keywords—Apocynaceae; *Apocynum*; cardenolide; strophanthidin glycoside; glucos-3-ulosyl-cymaroside; glucosyl-digitalosyl-cymaroside; cellobiosyl-cymaroside; 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 cymaroside and k-strophanthidin- 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 ($\text{M} + \text{Na}$)⁺, suggesting the molecular formula to be $\text{C}_{36}\text{H}_{52}\text{O}_{14}$, 2H smaller than 8. In the proton nuclear magnetic resonance (¹H-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 (¹³C-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 ¹H-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

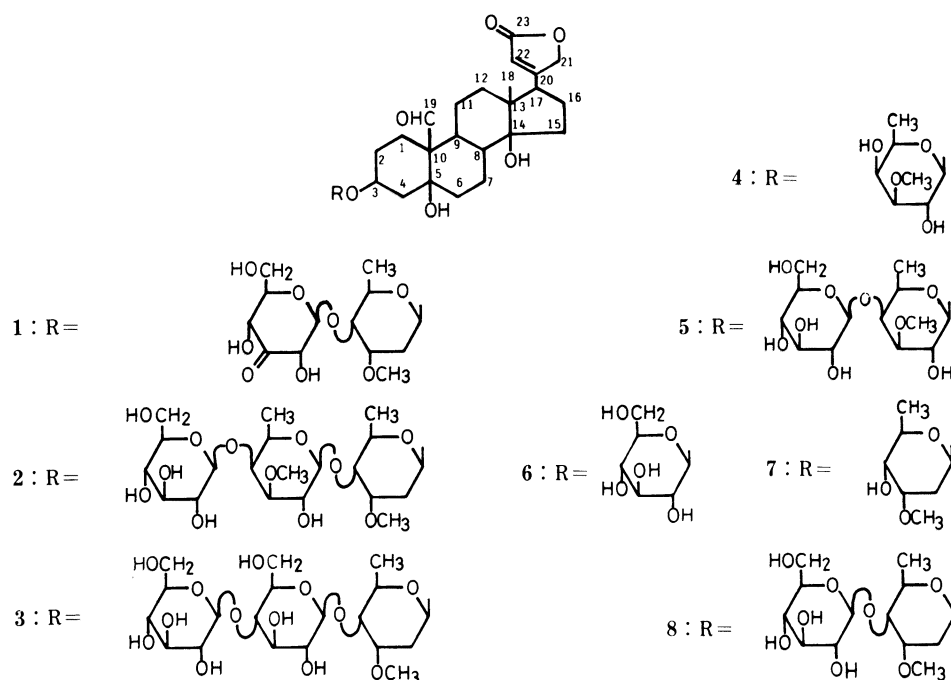


Chart 1

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 ^1H - ^1H 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 ^1H -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 ^1H -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 ($\text{M} - \text{glucose} - 1$)⁻ and m/z 547 ($\text{M} - \text{glucose} - \text{digitalose} - 1$)⁻, along with those at m/z 869 ($\text{M} - 1$)⁻, 407 and 385, the glucose is assigned as the terminal of the sugar chain. Based on the coupling constants in the ^1H -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 $\rightarrow$ 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. ^1H 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)	5.28 (dd, 18, 1)	5.28 (dd, 18, 1)	5.28 (dd, 18, 1)	5.28 (dd, 18, 1)	5.28 (dd, 18, 1)	5.28 (dd, 18, 1)
	5.02 (dd, 18, 2)	5.02 (dd, 18, 2)	5.02 (dd, 18, 2)	5.02 (dd, 18, 2)	5.02 (dd, 18, 2)	5.02 (dd, 18, 2)	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-3 α	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)
H-3'	4.07 (q, 3)	4.09 (q, 3)			3.47 (dd, 9, 3)	3.50 (dd, 9, 3)	4.22 (t, 9)
H-4'	3.65 (dd, 9, 3)	3.68 (dd, 9, 3)			4.08 (d, 3)	4.34 (d, 3)	4.24 (t, 9)
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'')	4.98 (d, 8, H-1'')	4.67, 5.08 (d, 8, H-1'', 1''')	4.88, 5.18 (d, 8, H-1'', 1''')		5.15 (d, 8, H-1'')	
	3.99, 4.17 4.23 (t, 9, H-2'', 3'', 4'')	4.72 (dd, 8, 1, H-2'')	3.66 (3''-OMe)			3.97, 4.17 4.24 (t, 9, H-2'', 3'', 4'')	
	4.57 (dd, 11, 2, H-6''a)	4.92 (dd, 10, 1, H-4'')					
	4.38 (dd, 11, 5, H-6''b)	4.55 (dd, 11, 2, H-6''a)					
		4.45 (dd, 11, 4, H-6''b)					

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-cymaridin, and is named cellostrophanthoside.

Compounds **4**, **5** and **6** were identified as strophanthidin β -D-digitaloside, β -D-glucosyl(1 $\rightarrow$ 4)- β -D-digitaloside and β -D-glucoside, respectively, based on the FAB-MS, and a comparison of the ^1H -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 2N 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), $[\alpha]_{\text{D}}^{26} + 12.8^\circ$ ($c=0.50$, MeOH), FAB-MS m/z : 731.327 (Calcd for $\text{C}_{36}\text{H}_{52}\text{O}_{14}\text{Na}$: 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 H₂O and extracted with BuOH. The BuOH extract was subjected to HPLC (23% CH₃CN, 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 C₃₆H₅₆O₁₄Na: 735.357, showed the same retention time on HPLC (23% CH₃CN, 0.7 ml/min, t_R 14.2 min) and the same R_f value on TLC (solvent 1, 7:3:1) as strophanthidol β -D-glucosyl- β -D-cymaroside prepared from **8** by NaBH₄ reduction.

Compound **1b**: FAB-MS m/z : 735.356 (Calcd for C₃₆H₅₆O₁₄Na: 735.357), HPLC (23% CH₃CN, 0.7 ml/min, t_R 15.2 min). ¹H-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-3 α), 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), $[\alpha]_D^{23} + 23.1^\circ$ ($c=0.065$, MeOH), negative FAB-MS m/z : 869 ($M-1$)⁻, 707, 547, 407, 385.

Cellostrophanthoside (3)—A solid (16 mg), $[\alpha]_D^{26} + 15.6^\circ$ ($c=0.50$, 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% CH₃CN, 1 ml/min, t_R 10.6 min), revealing **8**.

Strophanthidin β -D-Digitaloside (4)—A solid (10 mg), $[\alpha]_D^{22} + 5.21^\circ$ ($c=0.23$, MeOH), FAB-MS m/z : 587.282 (Calcd for C₃₀H₄₄O₁₀Na: 587.283).

Strophanthidin β -D-Glucosyl-(1 $\rightarrow$ 4)- β -D-digitaloside (5)—A solid (10 mg), $[\alpha]_D^{26} + 14.3^\circ$ ($c=0.30$, MeOH), FAB-MS m/z : 749.337 (Calcd for C₃₆H₅₄O₁₅Na: 749.336), negative FAB-MS m/z : 725 ($M-1$)⁻, 563, 403, 385.

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

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